Genes affecting triglyceride metabolism: from steatosis to lipodystrophy

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Introduction
Atherosclerosis

Atherosclerosis is the underlying cause of major cardiovascular diseases such as myocardial infarction, peripheral arterial disease and stroke. Atherosclerosis is a chronic and complex process that involves an inflammatory response in the vessel wall induced by lipid particles such as oxidized Low Density Lipoproteins (ox-LDL). The crosstalk between inflammation and lipid metabolism is the hallmark of this process. Atherosclerotic lesions themselves are the best example of this crosstalk. Fatty streaks, that are prevalent in young adults, are focal lesions in the inner layer of the vascular wall, called neo-intima, and contain lipid-laden macrophages. In time, these apparently innocent fatty streaks evolve to the full-blown atherosclerotic lesions that are composed of debris, foam cells, activated smooth muscle cells and many inflammatory cells such as T-cells, dendritic cells and mast cells (1). To understand the exact pathophysiology of atherosclerosis, we need to define how these two processes are coupled. Figure 1 depicts that inflammation is the basis of atherosclerosis and that lipid metabolism is closely linked to these processes.

The role of LDL-cholesterol in atherosclerosis has been extensively studied and has been thoroughly reviewed in a number of perspectives (2-6). The impact of hypertriglyceridemia on atherosclerosis has not been appreciated as much until recently. More and more observational and interventional studies are starting to unravel the fact that high triglyceride (TG) is an independent risk factor for cardiovascular disease (7;8). Here, I will focus on the role of TG metabolism and the genes involved in this process. Activation of nuclear transcription factors such as PPARγ and the subsequent transcription of genes involved in TG metabolism could affect lipid content of tissues as well as plasma lipids. On the other hand, liver steatosis could induce insulin resistance and the subsequent increased Very Low Density Lipoproteins (VLDL) secretion and increased plasma TG levels. In the following paragraphs each of the players in this metabolic triangle will be discussed (figure 1).

**Figure 1.** Atherosclerosis is an inflammatory disease of the vessel wall. The role of lipid metabolism in inflammation and atherosclerosis is depicted. Nuclear transcription factors control the expression of many genes in lipid metabolism and are involved in pathologies like obesity and lipodystrophies. These metabolic pathways in turn affect the inflammatory response and the chronic process of atherosclerosis.
Apolipoproteins and Lipases

To understand the cross-talk between inflammation and lipid metabolism, it is crucial to understand how lipids are formed, transported and metabolized in the body. Lipids are essential for living organisms, not only as a source of energy but they are also involved in many other processes. In summary: inside the cell (i.c. transcription activation, lipid storage and breakdown), at the level of cell membrane (the membrane itself and anchoring the receptors for signal transduction, endocytosis and exocytosis), outside the cells (hormones and inflammatory agents such as leukotriens). The proteins that are associated with the lipid particles in the plasma are called apolipoproteins. The major apolipoproteins are apolipoprotein A1 on High Density Lipoproteins (HDL) particles and apolipoprotein B on LDL particles.

In the fed state, chylomicrons are formed in the gut and are secreted into the plasma and distribute the lipids to the peripheral tissues. After removal of lipids from the core of chylomicrons, the so-called remnant particles are then taken up by the liver and metabolized. In the fasting state, VLDL particles are produced by the liver to ensure supply of lipids to peripheral tissues (figure 2). These particles are metabolized in the plasma and, as they drop their cargo, become smaller and form LDL particles. There is substantial evidence that high LDL-cholesterol is associated with cardiovascular disease, and the causal role of LDL-cholesterol in the pathogenesis of atherosclerosis has been proven beyond doubt (2-6). The common concept is that LDL particles are modified in the circulation and become oxidized. These ox-LDL particles are then taken up by the monocytes and activate these cells. Lipid-laden macrophages (foam cells) are then formed and can recruit more inflammatory cells. The result is an inflamed vascular wall at risk of rupture and complete occlusion. Therefore, LDL particles are called atherogenic particles.

The small and dense HDL particles are produced by the gut and liver. The nascent HDL particles can obtain cholesterol from the peripheral tissue and transport it to other tissues. The transport of HDL-cholesterol to the liver is called “reverse cholesterol transport” and has been suggested to be very important in the removal of cholesterol from the arterial wall and thereby reducing atherosclerotic burden (9). Therefore, HDL particles are thought to be anti-atherogenic. But in the past decade it has become clear that the anti-atherogenic effect of HDL is not only by promoting reverse cholesterol transport but also by inhibiting oxidation of phospholipids within LDL and downregulating associated inflammatory cytokines and vascular cell-adhesion molecules (10). HDL can lose its protective capacity and even become proinflammatory in the setting of systemic inflammation (11). Conditions that have been associated with proinflammatory or dysfunctional HDL include coronary heart disease (CHD) (12), metabolic syndrome (13), chronic kidney disease (14), obstructive sleep apnea (15), infections (16) and some rheumatologic diseases (17). Apolipoproteins associated with HDL are essential in these processes and are shown to be modified in patients with coronary artery disease (CAD). Vaisar et al. elegantly showed that the composition of HDL, isolated from healthy subjects and subjects with CAD, was substantially different (18). To disentangle
the anti-atherogenic properties of HDL particles, we need to understand the function of each of these proteins.

Other proteins that interact with lipoprotein particles are the enzymes that are located at the inner-surface of vessel walls, i.e. on the endothelial cells. Lipoprotein Lipase (LPL) is one the best studied proteins but our knowledge of other lipase such as Hepatic Lipase (HL) and Endothelial Lipase (EL) is growing. LPL is involved in hydrolysis of TGs and as a result, free fatty acids (FFAs) are formed. FFAs are then taken up by other cells and are either used in β-oxidation in peripheral cells (i.e. myocytes) or re-esterified to TGs again in adipocytes. There is a complex fine tuning of this process where LPL could be stimulated by other proteins such as apolipoprotein CII and inhibited by others such as apolipoprotein CIII.

**Figure 2.** The exogenous and endogenous lipoprotein metabolic pathways. The exogenous pathway transports dietary lipids to the periphery and the liver. The endogenous pathway transports hepatic lipids to the periphery.

- **LPL**, lipoprotein lipase;
- **FFA**, free fatty acid;
- **VLDL**, very low density lipoprotein;
- **IDL**, intermediate-density lipoprotein;
- **LDL**, low-density lipoprotein;
- **LDLR**, low-density lipoprotein receptor.
It is known that infection and inflammation induce the acute-phase response, leading to multiple alterations in lipid- and lipoprotein metabolism. Plasma TG levels increase due to increased VLDL secretion, as a result of adipose tissue lipolysis, and decreased VLDL clearance, secondary to decreased lipoprotein lipase activity. In human sepsis, plasma TGs are increased, phospholipids are maintained at near normal levels, while total cholesterol is decreased (19). It is not only the lipids that are altered during inflammation but the protein composition of particles is modified as well. During inflammation, HDL drops dramatically mainly because of a decrease in large apo A1-containing HDL particles, an almost total loss of apoC-I, and an increase in apoE-HDL (20).

Whereas inflammation causes a massive change in lipid metabolism, the same holds true for the opposite direction; lipids and lipoproteins do affect inflammatory responses. Apo A1, which is the major component of HDL, was shown to inhibit the lipopolysaccharide (LPS)-induced release of cytokines by monocytes (21). ApoAI was also reported to bind LPS and to protect mice against LPS-induced mortality (22). Not only apolipoproteins, but also LPL, can modulate inflammatory response. For instance, treatment of endothelial cells by exogenous LPL could attenuate the tumor necrosis factor α (TNF-α) induced expression of VCAM1 on endothelial cells (23).

From the abovementioned details on the crosstalk between inflammation and lipid metabolism, it must be clear that inflammatory responsive apolipoprotein are of interest to resolve the remaining pieces of the puzzles on TG metabolism and inflammation.

**Extracellular TG metabolism**

Triglyceride is an efficient energy storage molecule, because its hydrophobicity allows tight packing into droplets without rigidity. Consequently, the energy to mass ratio of TG is at least 10 times that of hydrated carbohydrates or proteins (24). Whereas the ability to store intracellular TG is conserved from *Saccharomyces cerevisiae* to humans, the genes involved in this delicate system of energy storage have been modified during the course of evolution to adjust to environmental demands (25). Moreover, the ability of secreting TG-rich particles is a phenomenon specific for multicellular organisms to ensure energy supply for the rest of body that is obviously not an issue for a single cell organism. This is also the reason that the superfamily of large lipid transfer proteins (LLTP) has been identified in most animals, in vertebrates as well as invertebrates but not in single cell eukaryotes. The LLTP protein family includes apolipoprotein B, insect apolipoprotein (apolipophorin- II/I), vitellogenin (Vtg), and microsomal triglyceride transfer protein (MTP) (26). These major LLTP genes arose in the earliest animals, suggesting that the creation of this genetic module coincided with animal multicellularity (27).

The pool of TG-rich particles in human plasma are from two separate sources; the so-called endogenous and exogenous pathway (figure 2). The endogenous pathway refers to the continuous production of TG-rich VLDL particles by the liver. This ensures that there is a constant supply of energy to other tissues. The exogenous pathway is the production of
chylomicrons by the gut after a meal. The TGs from the exogenous pathway are mainly used for storage in adipocytes but are also used, although to a lesser extent, by other tissues. The energy balance is dictated by different organs in the body and by the interrelations among them. Food intake, energy storage and energy expenditure is well orchestrated in normal situation. Many genes are involved in this process that influence appetite and food intake, TG breakdown, β-oxidation and ATP formation or for storage in the adipose tissue. Appetite is the first step in setting this interplay in motion. During the past few years many encouraging findings have been reported about the role of hypothalamus as the control center of food intake. The gastrointestinal tract is the organ responsible not only for food digestion but also transmitting signals to the brain through hormones. The role of these hormones in satiety and glucose homeostasis has been extensively reviewed elsewhere (28;29).

In the intestine, TGs are split into glycerol and FFAs by pancreatic lipase. These lipids are taken-up by the enterocytes and the TGs are rebuilt and packaged together with cholesterol and proteins to form chylomicrons. These large particles are excreted from the cells and, via the lymphatic system, transported directly to the heart where they are mixed into the blood and distributed to other organs. TGs are then hydrolyzed and taken up by cells as a source of energy. Lipoprotein lipase (LPL) is the rate limiting enzyme for hydrolysis of TGs in the circulation and is mainly expressed by myocytes and adipocytes. LPL is then translocated to the endothelial luminal surface where it can hydrolyse TG. This ensures that TG is hydrolyzed exactly where it is needed, i.e. muscles for energy expenditure and adipose tissue for storage in lipid-droplets. Due to western life style the balance in this delicate system, that has evolved to store energy for the time of famine, is distorted. Because of excess energy input compared to energy output, TGs are stored in adipose tissue as well as non-adipose tissue resulting in diseases such as obesity and non-alcoholic fatty liver disease (NAFLD). Obesity can be viewed as an energy balance disorder, arising when energy input exceeds energy output (29).

**Intracellular TG metabolism**

Once synthesized from FFAs, intracellular TG is processed in three different ways depending on the cell type and the nutritional state. It is either stored in lipid droplets in specialized cells (adipocytes) or transported into plasma as lipoprotein particles by hepatocytes and enterocytes or hydrolyzed again into FFAs for β-oxidation. The intermediate products of TG hydrolysis are also used for phospholipids synthesis and membrane assembly. Here, I will try to summarize the currently known genes that are generally recognized to be important in these processes.

**Genes involved in TG synthesis**

Unesterified fatty acids are highly toxic to cells and have been implicated in much of the pathology associated with obesity and diabetes. Thus, efficient fatty acid esterification into TG and packaging of the newly synthesized TG are necessary for viability of cells apart from energy storage (24). The acylation of glycerol-3-phosphate represents the first
and committed step in glycerolipid biosynthesis (figure 3). The reaction is catalyzed by acyl-CoA: glycerol-sn-3-phosphate acyltransferase (GPAT), resulting in the production of 1-acyl-sn-glycerol-3-phosphate (lysophosphatidate). Phosphatidate is synthesized de novo from the acylation of lysophosphatidate in a reaction catalyzed by acyl-CoA:1-acylglycerol-sn-3-phosphate acyltransferase (AGPAT). The phosphatidate formed by AGPAT occupies a central branch point in lipid biosynthetic pathways (30). Phosphatidate can be converted to phospholipids or to diacylglycerol (DAG), which serves as the precursor for TG (31). Dacylglycerol:acyl-CoA acyltransferase (DGAT) is the enzyme involved in the conversion of DAG into TG.

![Diagram of TG synthesis in the ER](image)

**Figure 3.** A schematic presentation of intracellular TG synthesis in the ER.

The terminal enzymes for triacylglycerol synthesis residing in the ER are: AGPAT, acylglycerol-P acyltransferase; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; ER, endoplasmic reticulum; FA, fatty acid; G3P, glycerol-3-phosphate; GPAT, glycerol-P acyltransferase; LPA, lysophosphatidic acid; PA, phosphatidic acid; TG, triglyceride; VLDL, very low density lipoprotein.

DGAT activity is highest in organs that have high rates of TG synthesis: adipose tissue, liver, lactating mammary gland, small intestinal mucosa, and adrenal gland. TG synthesis takes place at the surface of endoplasmic reticulum (ER), where the above mentioned enzymes are located. Because TG storage is essential to all eukaryotic cells, the DGAT gene family has been preserved in yeast, plants and animals and there is a clear redundancy. There are multiple pathways to the formation of neutral lipids in eukaryotes. The importance of these reactions is further demonstrated by the fact that they are conserved across many billions of years of evolution and have arisen independently (32). This apparent redundancy in neutral lipid synthesis is obviously advantageous and necessary otherwise these genes presumably would have been mutated or silenced.
Genes involved in TG storage
The exact mechanism behind TG packaging into cytosolic droplets is not well understood. However, the vast majority of TG in mammalian cells is in droplets coated with one or more of the Perilipin–Adipophilin-TIP47 (PAT) family of proteins (33). Mammalian genomes have at least five genes that encode regulatory proteins that coat lipid droplets (24). Perilipin is the major lipid droplet PAT protein of differentiated adipocytes (34). Perilipin is a multifunctional protein, capable of lowering basal lipolysis, promoting lipolysis after PKA activation, and controlling lipid droplet fragmentation through mechanisms that are lipase-dependent and -independent (35). It is generally accepted that lipid droplets emerge from the ER that at some point during expansion buds as a droplet into the cytosol. This model is based on two important observations: i) mature lipid droplets are physically associated and in some cases appear to be continuous with the ER as shown by electron microscopy, ii) many genes involved in TG synthesis have been identified and confirmed to reside in the ER (31). It is not clear when these genes have appeared in the evolution but it has been suggested that TIP47 could be the ancestral gene of PAT protein family because it is ubiquitously expressed and is the only PAT gene that is not PPAR regulated. TIP47 is likely duplicated four times and gave rise to the more specialized PAT proteins that encoded by different chromosomes (24).

Genes involved in TG breakdown
During fasting and exercise TGs are the main source of energy. Therefore, lipolysis and FFA formation is under direct control of glucagon and norepinephrine. Both hormones can activate protein kinase A (PKA) and thereby activate Hormone Sensitive Lipase (HSL) (36). HSL is one of the key regulating enzymes in TG lipolysis that is translocated to lipid droplets during PKA activation (37). Interestingly, insulin reduces HSL activity by reducing PKA activity. This means that PKA functions as a second messenger where its activity related to the level of lipolysis (38). Until the discovery of adipose triglyceride lipase (ATGL), HSL was considered to be the main triglyceride lipase inside the cell. ATGL has strong TG hydrolase activity, but no activity against diacylglyceride or monoacylglyceide substrates. ATGL is highly expressed in adipose tissue of mice and humans. It exhibits high substrate specificity for TG and is associated with lipid droplets (39). Both enzymes likely maintain hepatic lipid homeostasis by mobilizing TG from storage pools to fatty acid (FA) oxidation pathways and possibly also by releasing FFA directly into the circulation (40). The fact that overexpression of these enzymes reduces hepatic TG, without increasing hepatic apoB or TG secretion, may make them attractive therapeutic targets in steatosis.

Genes involved in TG secretion
Once synthesized, TGs are packaged into TG-rich particles and secreted into the plasma. As already mentioned, the major enzymes in the conversion of FFAs into TGs reside in the ER. Apolipoprotein B (apoB) is synthesized in the mammalian small intestine and liver, where it has an essential role in the assembly and secretion of TG-rich lipoproteins. Each particle contains just one apoB molecule (41). Full-length apoB, apoB100, is synthesized in the liver as a 4536
—amino-acid polypeptide. Mutations that lead to the production of truncated forms of apoB of insufficient length for assembly of fully-lipidated lipoproteins lead to hypolipidemia (42). ApoB is a large hydrophobic protein that exists in plasma as apoB48 or apoB100, associated with intestinally derived chylomicrons or the liver-derived VLDL, respectively. These two proteins are formed from the same mRNA through an RNA editing event that converts the Gln2153 codon to a stop codon, leading to a truncated form (apoB48) containing 48% of the protein from the N-terminus. This post-transcriptional modification is performed by an enzyme called Apolipoprotein B mRNA-Editing Enzyme 1 (APOBEC1) that is only expressed in the human intestine but not in the liver. Therefore, human chylomicrons carry apoB48, whereas VLDL and LDL carry apoB100. The C-terminal part of apoB is involved in binding to LDL-receptor (LDLR) that is lacking in apoB48. Thus, chylomicrons depend upon apolipoprotein E to bind to the LDLR (and to other members of the LDLR family) to mediate their clearance from the circulation (43). Interestingly, avian species lack apoE and also do not edit apoB: i.e. their intestines produce apoB100 (44). Thus, the appearance, during evolution, of a form of apoB unable to bind to the LDLR coincided with the appearance of another LDLR ligand.

A major determinant of apoB maturation into VLDL particles is the microsomal triglyceride transfer protein (MTP), an ER protein with lipid-transfer activity that exists as a heterodimer with protein disulfide isomerase (PDI) (45). MTP is physically associated with apoB and transfers TG into the lumen of ER while apoB is being synthesized. The importance of this chaperone protein in lipid enrichment of apoB is well demonstrated by a loss of function mutation in MTP that causes a syndrome called abetalipoproteinemia (46). These patients have steatosis in the gut epithelium and liver whereas the lipid levels in the plasma are very low. ApoB is not secreted in this disorder and is degraded intracellularly, due to lack of maturation into VLDL particles. MTP was known to transfer phospholipids in many species, but has gained TG transfer activity during evolution. The TG transfer activity was absent in Drosophila but in fish, amphibians, and birds was shown to be 27%, 40% and 100%, respectively, of that observed in mammals (47). One might argue that MTP triglyceride-transfer activity first appeared in fish, matured in birds, and remained conserved in mammals. This has made possible that sufficient TG is packaged into lipoprotein particles for distribution of energy to the rest of the body.

TG storage disorders

TG is stored in adipose tissue and can be mobilized during fasting. In Western societies, due to sedentary life style and increased caloric intake, there is an imbalance between storage and mobilization of TGs leading to obesity. In time there is also ectopic TG deposition in other tissues such as liver. It is now recognized that non-alcoholic fatty liver disease (NAFLD) is a manifestation of the metabolic syndrome (48). Obesity is a tremendous health issue in Western societies and the number of affected subjects in the United States is dramatically increasing (49). The prevalence of obesity is also increasing in European countries (50). There is higher incidence of cardiovascular morbidity and mortality in obese subjects (51;52).
mortal analysis of coronary arteries of adults dying of other causes than cardiovascular disease (CVD) showed a clear association between Body Mass Index and the extent of atherosclerosis (53). The association between obesity and CVD could be explained by the metabolic disturbances that go together with obesity as well as by the associated inflammatory burden. Obesity with enlarged fat cells is associated with an increased number of macrophages in the adipose tissue surrounding individual adipocytes (54). TNF-α is an inflammatory cytokine that is mainly produced by macrophages. Adipocytes also express TNF-α and adipose tissue has been shown to be a major source of TNF-α locally and systemically.

The term metabolic syndrome (MetS) has been introduced to describe this cluster of metabolically related cardiovascular risk factors i.e obesity, insulin resistance, dyslipidemia and hypertension. The most important obstacle in identifying genes involved in MetS is the fact that the definition of MetS is not universal and the population is very heterogeneous. Therefore, monogenic disorders that resemble metabolic syndrome are perfect disease models that could help us understand the pathogenesis of MetS. Lipodystrophies represent a heterogeneous group of diseases characterized by an abnormal, subcutaneous fat distribution that are associated with metabolic abnormalities comparable to the metabolic syndrome. Familial partial lipodystrophy type 3 (FPLD3) is one of these monogenic disorders that is caused by mutations in the gene for the Peroxisome Proliferator-Activated Receptors γ (PPARγ). FPLD3 patients have a reduced expression of PPARγ due to haploinsufficiency of the PPARG gene.

**Nuclear transcription factors**

Nuclear receptors orchestrate the expression profile of cells upon activation. They act as intracellular sensors that become activated by biologically active molecules. Liver X receptor (LXR), Farnesoid X receptor (FXR) and Peroxisome Proliferator-Activated Receptors (PPARs) are nuclear receptors that play a major role in lipid metabolism. LXR and FXR are activated by cholesterol derivatives, whereas PPARs become active by FFAs and eicosanoids. Because transcription factors are at the top of a pathway-specific regulatory hierarchy, they have been the target of drug design. One drug can affect not only one gene but an array of genes that are involved in the same pathway. There are also many drugs available for activation of these nuclear receptors that have been tested in clinical settings. I will discuss the key transcription factors in lipid metabolism with emphasis on TG metabolism.

LXR plays a key role in promoting removal of excess cholesterol from cells and is important in the so-called reverse cholesterol transport (RCT). LXR is activated by endogenous oxysterols and upregulates ATP binding cassette transporter (ABC) A1 and ABCG1, promoting cholesterol efflux to apoAI and HDL, respectively. LXR also promotes fecal excretion of cholesterol by upregulating ABCG5 and ABCG8 in the intestine. Hence, LXR agonists are a potential approach to promote cholesterol efflux and RCT and to reduce atherosclerosis. In rodents, but not in man, LXR activation enhances hepatic cholesterol catabolism partly through increased expression of cholesterol 7-α-hydroxylase gene (CYP7A1), the rate-limiting
enzyme in the classic conversion of cholesterol to bile acids (55). Therefore, rats and mice have the unusual capacity to convert cholesterol to bile acids by LXR-mediated stimulation of CYP7A1 transcription, whereas other species do not respond to cholesterol and develop hypercholesterolemia on a high cholesterol diet. Kotokorpi et al. also showed significant differences in the response to GW3965 (a synthetic LXR agonist) between human and rat hepatocytes (56). These data demonstrate that pathophysiology of lipid metabolism in rodents could not be unequivocally extrapolated into humans. Caution is needed when drugs affecting lipid metabolism tested in rodents are going to be used in humans.

FXR plays a major role in protecting the hepatocyte from the toxic effects of excess bile acids (BA). BAs are the major ligand to activate FXR and consequently downregulate BA synthesis and the import of BAs from the plasma. In addition, BAs upregulate their export into the bile. FXR also downregulates the intestinal reabsorption of BAs (57). Taken together, excess BAs activates FXR and thereby promotes the removal of excess BA. Whether FXR activation is a useful therapeutic target is still not clear. Chenodeoxycholic acid (CDCA) is a bile acid and acts as a natural FXR agonist and reduces plasma TG in man (58). The synthetic FXR agonist GW4064 (cholic acid) significantly reduced plasma TG as well as glucose when used in diabetic mice (59). Therefore, FXR seems to be potential drug target for treating dyslipidemia, mainly hypertriglyceridemia. Whether FXR activation could have a role in atherosclerosis is uncertain because FXR deficiency in mice seems to be anti-atherogenic, but only in females (60). Future investigations are needed to define the therapeutic potential of FXR activation in humans.

PPARs, including the three known isotypes PPARα, PPARγ and PPARδ, are members of the steroid hormone nuclear-receptor superfamily (61). Like other nuclear receptor family members, PPARs contain both a ligand-binding domain, and a DNA-binding domain that mediates binding to specific PPAR response elements in the promoter region (62). In response to ligand binding, PPARs undergo a conformational change that facilitates the formation of a hetero-dimeric complex with another ligand-activated nuclear receptor, retinoid X receptor (RXR). This ligand-induced conformational change also facilitates the binding and release of small accessory molecules that are critical for transcription. Natural ligands of PPARs include FA and eicosanoids. The three isotypes of human PPAR (α, δ and γ) have been characterized, showing distinct tissue distributions, physiological roles and ligand specificity (63).

PPARα is predominantly expressed in the liver and is activated by fibrates. Drugs such as cipofibrate or fenofibrate have been successfully prescribed in patients with hypertriglyceridemia, but their role in PPARα activation was demonstrated long after their introduction as a drug (64). PPARα activation by fibrates initiates FA oxidation and thereby reducing the intracellular TG store. Apparently, this also affects VLDL production due to lack of sufficient TG in hepatocytes. It has been shown that fibrates also enhance the catabolism of VLDL particles by activating LPL (65). Eventually, these changes will lead to reduction of TGs in liver (less steatosis) as well as in plasma. The effect of fenofibrate on steatosis was
more pronounced in a mouse model of non-alcoholic fatty liver disease (NAFLD) than in human subjects (66;67). Although their role in dyslipidemia has been extensively studied, their role in atherosclerosis is still under debate. Gemfibrozil, a PPARα agonist, showed 34% reduction in the incidence of coronary heart disease in hyperlipidemic subjects (68), whereas the role of other fibrates in preventing cardiovascular disease is not clearly established yet. PPARγ is classically characterized by its high expression and functional role in adipose tissue, where it was first identified (69). Considerable evidence has established the importance of PPARγ in adipose tissue, including its high level of expression in adipocytes, the lack of white fat in PPARγ-deficient mice, PPARγ regulation of adipokine expression, and the association of a PPARγ deficiency with lipodystrophy (70). In addition to adipogenesis, PPARγ also regulates genes involved in TG metabolism, including LPL, acyl-coenzyme A synthetase, and glucose control such as the glucose transporter GLUT4 (71). The relationship of PPARγ and inflammation is a two way communication. On the one hand, PPARγ ligands have been shown to attenuate the inflammatory response of macrophages by inhibiting the production of inflammatory cytokines such as TNF-α (72), whereas in T lymphocytes it also limits the production of cytokines such as IFNγ and interleukin-1 (73). On the other hand, inflammatory agents like endotoxin, via the increase in TNF-α release, downregulates PPARγ expression in macrophages (74). TNF-α was discovered not only as a soluble protein that induces the death of tumor cells but also as a molecule (cachectin) that causes hypertriglyceridemia and wasting of muscle and fat tissue (75). TNF-α has been shown to affect hepatic lipogenesis due to reducing the expression and activity of different nuclear hormone receptors such as PPARγ (76;77). Adipose tissue macrophages are suspected to be the major source of inflammatory mediators such as TNF-α and IL-6 that interfere with adipocyte function by inhibiting insulin action (78). Taken together, PPARγ appears to be anti-inflammatory and its activation by specific ligands could be used for treating atherosclerosis.

Whereas PPARα and PPARγ have been studied extensively over the past 20 years, relatively little is known with respect to PPARδ. PPARδ has almost ubiquitous tissue expression which suggests that it might be involved in different metabolic pathways. In fact, by analysis of PPARδ null mice (79), it could be demonstrated that PPARδ-deficiency was associated with multiple developmental and metabolic abnormalities, including aberrant growth, diminished adipose tissue mass, demyelination, impaired wound healing and skin abnormalities, which were apparently due to altered inflammatory responses in the skin (80). Selective PPARδ agonists raise plasma HDL-cholesterol levels in rhesus monkeys as well as in human subjects (81;82). This increase in HDL-cholesterol is at least partly due to enhanced ABCA1 expression. In monkey and in man, but not in mice, PPARδ agonists decrease plasma TG levels as well (83). Primates differ in their lipid metabolism when compared to rodents with the most prominent difference being lack of CETP in the latter species (84). This is in line with the differences in lipid metabolism of primates compared to other mammalian species such as rodents. It has been suggested that PPARδ agonists could be used in the whole spectrum of metabolic syndrome because of their effects on HDL-cholesterol, TG and adipose tissue (85).
Outline of this thesis

In chapter 2, I will go into details on inflammatory response and gene expression of different cell types of the vessel wall involved in this process. Special attention has been made to nuclear transcription factors such as Peroxisome proliferator-activated receptor gamma (PPARγ).

In chapter 3, I will characterize the expression profile of the novel TNF-α-responsive Apolipoprotein L gene cluster. Their expression in different species as well as in human atherosclerotic tissues is demonstrated.

In chapter 4 I will present the function of the intracellular apolipoproteins L2 and L3 in TG metabolism.

Chapter 5 describes the expression pattern of a novel gene, Lipase H (LIPH) that is a member of the triglyceride lipase family.

Chapter 6 describes a lipodystrophic patient with metabolic syndrome due to a genetic mutation in PPARG.

In Chapter 7 all types of lipodystrophy and their associated metabolic abnormalities will be discussed.

Finally, Chapter 8 summarizes the findings in this thesis and the role of genes involved in TG metabolism and fat storage in adipose tissue as well as in non-adipose tissue in relation to the introduced common diseases and syndromes.
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