CHAPTER I

General Introduction
**Introduction**

Coronary artery disease (CAD) is a leading cause of death and morbidity in industrialized societies and soon worldwide (Lloyd-Jones, 2010). Atherosclerosis underlies CAD through slowly progressing lesion formation and luminal narrowing of arteries. Atherogenesis involves imbalanced lipid metabolism and a chronic inflammatory status of the arterial wall. Lesion formation occurs predominantly at sites with disturbed laminar flow, such as arterial branch points (Gimbrone, 1999).

**Atherosclerosis: lesion initiation and development**

Atherosclerosis has a complex etiology. There are several known risk factors, such as hypercholesterolemia, diabetes, obesity, smoking, sedentary lifestyle and genetic factors (Assmann, 1999). Morphological and functional studies on atherogenesis, both in man and laboratory animals, indicate that the key initiating step is subendothelial accumulation of lipoproteins (LPs), in particular low density lipoprotein (LDL) (Williams and Tabas, 1995). This process is increased when the levels of circulating LDL are abnormally elevated. The sub-endothelial retention of LDL occurs preferentially at sites where the flow is low (Gimbrone, 1999). Retention of LPs and their subsequent modification (oxidation, lipolysis, aggregation) promotes an inflammatory response of endothelial cells that when activated, secrete chemo-attractants for monocyte recruitment (Glass and Witztum, 2001; Mestas and Ley, 2008). In response to macrophage colony-stimulating factor (M-CSF), and likely to other differentiation factors, most monocytes in early atherosclerotic lesions develop into cells with features of macrophages or dendritic cells (Johnson and Newby, 2009; Paulson, 2010). In atherosclerotic lesions different kinds of macrophages are detected: prominent are macrophages involved in pro-inflammatory processes (often designated as M1), but also present are macrophages involved in resolution and repair, (often referred to as M2) (Johnson and Newby, 2009). In the early atherosclerotic lesion, modified LPs are taken up by macrophages via receptor-mediated endocytosis and pinocytosis. The LDL receptor (LDLR) is normally largely responsible for cellular endocytosis of LDL. Since lipid-laden macrophages are also found in atherosclerotic lesions of patients with familial hypercholesterolemia with genetically impaired LDLR, a new class of receptors binding to modified LDLs was identified (Podrez, 2000). These are called scavenger receptors (SR), notably the type A scavenger receptor (SRA) and a member of the type B family, CD36 (Kunjathoor, 2002). Mice lacking either receptors show less lesion formation (Suzuki, 1997; Febbraio, 2000). In contrast to the expression of LDLR, the expression of SR is not down-regulated.
by increased intracellular levels of cholesterol. As a result, ongoing SR-mediated uptake of modified LPs leads to their continuous internalization and accumulation of excess quantities of lipids by macrophages (Rios, 2011). Upon internalization, lipoproteins are delivered to the lysosome, where cholesterol esters (CEs) are hydrolyzed into free cholesterol (FC) and fatty acids (FAs) (Maxfield and Tabas, 2005). Free cholesterol is transported to the cytosol where it is re-esterified to cholesteryl fatty acid esters, by the enzyme acyl-CoA:cholesterol ester transferase (ACAT) (Brown, 1980). Cholesterol loading in time leads to the formation of lipid-laden macrophages of the plaque's core. The excess lipids are stored in the form of lipid droplets in the cytoplasm, creating a foamy appearance. These so-called “foam cells” are the hallmark of atherosclerosis (Li and Glass, 2002). These cells can produce pro-inflammatory mediators, reactive oxygen species, and tissue factor pro-coagulants that amplify local inflammation (Moore and Tabas, 2011). Although fewer in number than the macrophages, T-cells also enter the intima and produce locally regulatory signals. After antigenspecific activation, T-helper 1 cells secrete interferon-gamma (IFN-γ), which can activate various cells in the vascular wall, and amplify and sustain the inflammatory response in the intima. Lesions also contain regulatory T-cells, producing interleukin-10 and transforming growth factor-β, two cytokines considered to exert anti-inflammatory actions (Hansson and Hermansson, 2011). Macrophages can efflux cholesterol to high density lipoprotein (HDL) (Tall, 2008; Rothblat and Phillips, 2010). Cholesterol efflux is assumed to be crucial for prevention of plaque formation and plaque regression. It is mediated by the two ATP binding cassette(ABC)-transporters, ABCA1- and ABCG1, to apolipoprotein A1 and HDL, respectively. Cholesterol can also passively diffuse to cholesterol-poor HDL (Tall, 2008; Rothblat and Phillips, 2010). Next, HDL particles can exit the tissue and enter the bloodstream, contributing to reverse cholesterol transport from lesional macrophages to the liver (Rosenson, 2012).

During atherogenesis, smooth muscle cells (SMCs) migrate from the media into the intima, and proliferate in response to mediators such as platelet-derived growth factor (Thyberg, 1990). In the intima, the SMCs produce extracellular matrix molecules, including interstitial collagen and elastin, leading to the development of fibrous plaques (Lusis, 2000). The fibrous cap extends along a collection of macrophage-derived foam cells, some of which die (for example, by apoptosis) and release lipids that accumulate extracellularly. The inefficient clearance of dead cells — a process known as efferocytosis — can promote the accumulation of cellular debris and extracellular lipids, forming a lipid-rich pool called the necrotic core of the plaque (Tabas, 2010).

The pathological manifestations originating from plaques are flow-limiting stenosis (leading to tissue ischaemia), or development of thrombi that can inter-
rupt blood flow locally. Thrombi often arise after disruption of the fibrous cap that exposes pro-coagulant material of plaque's core to coagulation proteins in the blood, triggering thrombosis (Fuster, 1996). Vulnerable plaques are prone to rupture and typically have thin, collagen-poor fibrous caps with few SMCs but abundant macrophages (Shah, 2003). The inflammatory cells contribute to plaque disruption by release of enzymes that can degrade collagen, and by generating mediators that provoke the death of SMCs (Libby, 2009).

In summary, atherogenesis can be considered as a “response to injury” from the body to deposited abnormal LPs, a pathological process influenced by several risk factors.

**Cholesterol homeostasis**

Hypercholesterolemia is a well-known risk factor for atherosclerosis. Reduction of circulating cholesterol, particularly LDL-associated sterol, is therefore a valid therapeutic target. Several drugs for this purpose have been developed, targeting cholesterol synthesis and homeostasis. Cholesterol in the body has two different sources. One-third of the cholesterol comes from the diet and is taken up by the small intestine. The small intestine is highly specialized in lipid absorption. Exposed to the lumen are epithelial cells rich in microvilli, increasing the surface for absorption. These small intestinal epithelial cells, named enterocytes are polarized cells with an apical membrane containing many transporters. Cholesterol absorption takes largely place by the transmembrane protein Niemann-Pick C1 Like 1 (NPC1L1) (Altmann, 2004). The subcellular localization of NPC1L1 is regulated by cholesterol and involves recycling of NPC1L1 between the plasma membrane and endocytic compartment (Skov, 2011). NPC1L1 null mice exhibit a strong reduction of intestinal absorption of cholesterol, are resistant to diet-induced hypercholesterolemia, and when crossed with APOE(-/-) mice do not develop atherosclerosis (Davis, 2007). Ezetimibe, a cholesterol absorption inhibitor binding specifically to an extracellular loop of NPC1L1, promotes fecal sterol loss in APOE(-/-) mice and reduces plasma cholesterol (Davis, 2001). In the enterocytes, cholesterol is packed together with triglycerides and other lipids in a particle containing APOB48. This so-called chylomicron is next secreted into the lymphatic system. In the circulation, chylomicrons are modified by lipoprotein lipase (LPL) (Lambert and Parks, 2012), hydrolyzing triglycerides in the particle. The remaining chylomicron-remnant is, after enrichment with APOE molecules, taken up by the liver (Cianflone, 2008).

The other two third of the cholesterol in the body is endogenously produced, in rodents largely via synthesis in hepatocytes. The major precursor for cholesterol synthesis is acetyl-CoA which gives rise to hydroxyl methylglutaryl-CoA
(HMG-CoA). The rate limiting step in the cholesterol biosynthetic pathway is the conversion of HMG-CoA to mevalonic acid by HMG-CoA reductase (HMG-CoAR) (Espenshade and Hughes, 2007). HMG-CoAR is inhibited by statins (Grundy, 1988), a commonly prescribed class of drugs for lowering plasma cholesterol levels (Bhattacharya and Chaturvedi, 2011). In addition to de novo synthesis, cholesterol can also be acquired by hepatocytes through LDLR-mediated endocytosis (Tolleshaug and Goldstein, 1983). Expression of LDLR and other proteins involved in cholesterol metabolism regulation, such as HMG-CoAR, is influenced by the cellular sterol content via a family of transcription factors called sterol regulatory element binding proteins (SREBPs) (Brown and Goldstein, 1997). The SREBP family consists of 3 isoforms: SREBP-1a, SREBP-1c, and SREBP-2. SREBP-1a and 1c have transcriptional activity for genes involved in fatty acid and triglycerides synthesis, while SREBP-2 activates transcription of genes involved in cholesterol synthesis (Shimano, 2001). SREBPs associate with the endoplasmic reticulum (ER) membrane where they remain transcriptionally inactive. In the ER, the C-terminus of SREBP interacts with the cargo protein SCAP (SREBP cleavage activating protein) which functions as a sterol sensor (Espenshade and Hughes, 2007). In sterol-deprived cells, SCAP binds SREBP carrying it to the Golgi apparatus where they are converted proteolytically to active fragments entering the nucleus and inducing the expression of their target genes such as LDLR and HMG-CoAR. At high cellular cholesterol levels, activation of SREBP is prevented by SCAP binding with the insulin induced gene protein (Insig), which retains the SCAP/SREBP complex into the ER (Espenshade and Hughes, 2007). This transcriptional regulation of cholesterol biosynthesis is key for body’s cholesterol homeostasis. Cholesterol produced by the liver is in part secreted and delivered to the peripheral organs following incorporation into the very low density lipoprotein (VLDL). VLDL is produced in hepatocytes similar to chylomicrons in enterocytes, with the exception that APOB100 is the main apolipoprotein in VLDL. After secretion by the hepatocyte, VLDL is modified in the circulation to the heavier low density lipoprotein (LDL) particles. This modification is again mediated by LPL hydrolyzing triglycerides in VLDL particles. The resulting LDL is taken up largely by hepatocytes via LDLR mediated endocytosis (Kendrick, 1998).

Elimination of excessive cholesterol from the body via the feces is a crucial process in cholesterol homeostasis. Two distinct pathways for this so-called reversed cholesterol transport have been described. The first pathway is the canonical route of cholesterol secretion from liver into bile. This pathway requires efflux of cholesterol from peripheral cells to high density lipoprotein (HDL) and the subsequent uptake of HDL-cholesterol by hepatocytes via the scavenger receptor class B type 1 (SR-B1). HDL derived cholesterol is de-esterified and finally secreted as free cholesterol into bile or modified to bile salt. Cholesterol
7α-hydroxylase (CYP7A1), a cytochrome P450 enzyme, catalyzes the first and key step in this pathway (Chiang, 2009). Once produced in the liver, bile salts are transported across the canalicular membrane of the hepatocytes into the bile duct and stored in high concentrations in the gallbladder. Upon a meal, gallbladder bile salts are released into the intestinal tract, reabsorbed in the ileum, and transported back to the liver via portal blood for re-excretion into the bile. In the intestine, bile salts are taken up by the apical sodium-dependent bile salt transporter (ASBT) (Shneider, 1995). This cycling of bile salts is known as the enterohepatic circulation of bile salts (Hofmann, 2009). The biliary excretion of bile salts is the major driving force of bile flow. Several members of the ABC-transporter family are responsible for transporting bile salts across the canalicular membrane against their concentration gradients (Dawson, 2009). The bile salt export pump (BSEP, ABCB11), is mainly responsible for bile salt transport at the canalicular membrane (Stieger, 1992). Phospholipids are excreted via the phospholipid flippase MDR2 (ABCB4) (Smit, 1993). Biliary cholesterol secretion is mediated by ABCG5/G8 transporters (Yu, 2002). Next to the classic functions of bile salts in the secretion of endogenous metabolites and xenobiotics and the intestinal absorption of lipophilic nutrients, bile salts also play important roles in glucose and lipid metabolism (Lefebvre, 2009; Nguyen and Bouscarel, 2008). An additional route for elimination of cholesterol from the body has only been recently recognized, the so-called trans-intestinal cholesterol excretion (TICE). The first indication for an alternative route for fecal cholesterol excretion came from studies in mouse models with extremely low biliary cholesterol secretion rates (ABCG5(-/-); ABCG8(-/-) and MDR2(-/-) mice). In these animals, fecal neutral sterol excretion was unchanged or even increased (Plosch, 2004; van der Velde, 2007). TICE involves direct transport of cholesterol across the intestine, and subsequent secretion of sterol into the feces (van der Velde, 2008). The various molecular components involved in TICE have not yet been identified and are topic of further research (Brufau G, 2011).

The Metabolic Syndrome, lipid-associated pathologies in the vessel wall and liver

Atherosclerosis often occurs not as single pathology in individuals, but in the context of a complex of abnormalities, designated as the metabolic syndrome. The metabolic syndrome (MetS) is a cluster of metabolic aberrations, such as obesity, hypertension, low HDL-cholesterol (HDL-C), high triglycerides, insulin resistance with or without impaired glucose tolerance, and pro-inflammatory and pro-thrombotic states (Liese, 1998; Lakka, 2002). The development of MetS is known to be promoted by various factors such as abdominal obesity, seden-
tary lifestyle and genetic factors (Laaksonen, 2002; Eckel, 2010). The MetS progresses to clinical manifestations like type 2 diabetes and atherosclerosis with associated cardiovascular disease (Ardern and Janssen, 2007; Novo, 2012). Atherosclerosis can be considered as the vessel manifestation of the MetS, while its hepatic manifestation is represented by non-alcoholic fatty liver disease (NAFLD) (Kim and Younossi, 2008). NAFLD includes a mixture of liver perturbations ranging from simple fat accumulation in the parenchymal cells (steatosis) to non-alcoholic steatohepatitis (NASH) including inflammation and different degrees of fibrosis. If hepatic lipid accumulation is generally considered to be benign, more harmful conditions such as steatohepatitis and cirrhosis can ultimately develop (Farrell and Larter, 2006). NAFLD is estimated to affect at least 20% of the general adult population and over 50% of the obese population (Varela-Rey, 2009). It is expected that as the prevalence of obesity and metabolic syndrome rises, NAFLD-associated diseases will be an increasing healthcare concern (Lewis and Mohanty, 2010). Fat accumulation in hepatocytes is the result of an imbalance between synthesis and degradation of triglycerides. A high influx and/or endogenous synthesis of free fatty acids (FFA) may lead to accumulation of triglycerides within the liver when mitochondrial β-oxidation and VLDL production and secretion are not sufficient to balance the FFA load.

Glycosphingolipid metabolism

Sphingolipids constitute a broad family of lipids that are essential structural components of mammalian cell membranes. They are composed of a ceramide moiety, i.e., an N-acylated sphingosine group. Ceramide is the simplest sphingolipid and serves as a precursor for the synthesis of more complex molecules. The biosynthesis and degradation of sphingolipids and derivatives containing sugar, the so-called glycosphingolipids (GSLs) involves numerous enzymes that act at various subcellular locations and which are tightly regulated (Wennekes, 2009). De novo synthesis of ceramide takes place at the cytosolic side of the ER membrane. Firstly, the enzyme serine palmitoyl-CoA transferase (SPT) links L-serine with palmitoyl-CoA. The formed 3-ketosphinganine is modified to sphinganine by 3-ketosphinganine reductase. Next, dihydroceramide is formed by dihydroceramide synthase (CerS), and finally ceramide dihydroceramide desaturase (DES) catalyzes the formation of ceramide (Wennekes, 2009). Modification of ceramide by linkage of a phosphorylcholine moiety from phosphatidylcholine results in sphingomyelin. The simplest GSL, glucosylceramide (GlcCer), is next formed via glucosylation of ceramide by the enzyme glucosylceramide synthase (GCS), transferring a glucose moiety from a UDP-glucose donor to ceramide. This reaction takes place at the cytosolic leaflet of the membranes of the Golgi
apparatus. Next, GlcCer flips to the luminal membrane of the Golgi apparatus where it can be stepwise modified to more complex GSLs by sequential glycosylations catalysed by a variety of glycosyltransferases. From the Golgi apparatus, the GSLs can be transported to the plasma membrane (Futerman, 2006; Futerman and Riezman, 2005; Lahiri, 2007). Alternatively, the newly formed GlcCer at the cytosolic leaflet of the Golgi apparatus may be transported directly to other membranes, via transfer proteins still requiring identification (Wennekes, 2009).

Catabolism of GSLs takes place following their endocytosis to lysosomes. It is thought only GSLs are degraded that are present in luminal membrane vesicles, so-called multivesicular bodies formed in late endosomes (Kolter and Sandhoff, 1998). During lysosomal degradation of GSLs carbohydrate residues of the GSL glycans are sequentially cleaved of by the action of exo-glycosidases. The GSL ultimately formed in this manner, GlcCer, is degraded into ceramide and glucose by the enzyme glucocerebrosidase (GBA1). The existence of this lysosomal beta-glucosidase was first described by de Duve. The molecular features of the enzyme were only characterized in the seventies and eighties by several researchers (Ho, 1973; Pentchev, 1973). The gene encoding GBA1 was cloned independently by the groups of Beutler and Barranger (Sorge, 1985; Tsuji, 1986). The importance of efficient glycosphingolipid catabolism is illustrated by the existence of sphingolipid storage disorders caused by inherited deficiencies in lysosomal glycosidases and activator proteins (Futerman and Van Meer, 2004). The most common of the sphingolipidoses is Gaucher disease (GD), caused by deficiency of GBA1 (Brady, 1965; Patrick, 1982). Interestingly, although GBA1 activity is reduced in all cell types of GD patients, the lysosomal storage of glucosylceramide is, in most patients, restricted to cells of the monocyte/macrophages lineage (Bussink, 2006). Characteristically, tissue macrophages in GD patients are transformed to lipid-laden “Gaucher cells” with massive deposits of GlcCer in tubules (Bussink, 2006). These storage cells are present predominantly in the bone marrow, spleen and liver (where they are responsible for the characteristic organomegalia). Macrophages are thought to be particularly prone to become storage cells in GD patients since their lysosomal apparatus is confronted with high quantities of GSLs for degradation following phagocytosis of GSL-rich senescent and dead blood cells (Bussink, 2006). Other cell types of GD patients show hardly GlcCer accumulation. This may be due to the existence of an alternative pathway for degradation of GlcCer, offered by the non-lysosomal glucocerebrosidase GBA2. This membrane-bound enzyme was firstly described by van Weely and colleagues (van Weely, 1993). Much later the corresponding gene was independently cloned by Yildiz and coworkers and Boot and colleagues (Yildiz, 2006; Boot, 2007). GBA2 and GBA1 show no homology and differ markedly in enzy-
matic features (van Weely, 1993). The GlcCer-laden macrophages are viable cells releasing specific proteins of which some can be detected in elevated levels in plasma. An example of a protein massively produced by Gaucher cells is chitotriosidase, a chitinase. The existence of the enzyme chitotriosidase was first discovered in plasma of GD patients where it is on average thousand-fold elevated (Hollak, 1994). Chitotriosidase is produced by Gaucher cells in various body locations and plasma levels reflect the total body burden of Gaucher cells (Bussink, 2006). Measurement of plasma chitotriosidase in GD patients is now used to monitor disease progression and efficacy of therapeutic intervention by chronic intravenous administration of recombinant GBA1 (Aerts, 2008). Chitotriosidase is also produced by lipid-laden macrophages in other conditions such as other lysosomal storage disorders, arthritis, sarcoidosis, and certain infectious diseases (Aerts, 2008). Of interest, chitotriosidase is also expressed in lipid-laden macrophage in atherosclerotic lesions (Boot, 1999). Clinical observations on GD patients offer hints for a connection between glycosphingolipid and cholesterol homeostasis. Patients with GD show relatively low plasma total cholesterol, LDL cholesterol, and HDL cholesterol (Ginsberg, 1984), and often develop gallstones in association with bile rich in cholesterol (Taddei, 2010). Despite their low plasma HDL levels, GD patients have not been found to be at high risk for atherosclerosis and CAD (de Fost, 2009)

Glycosphingolipids and atherosclerosis

The literature provides several indications for a relationship between atherosclerosis and glycosphingolipid abnormalities. For example, accumulation of glycosphingolipids in atherosclerotic lesions has been documented (Breckenridge, 1977). In addition, plasma GSL concentrations have been reported to be associated with risk for atherosclerosis (Mukhin, 1995; Garner, 2002). These associations may not be causative but rather by proxy since GSL are components of various lipoproteins and hyperlipidemia by itself causes increased plasma GSL levels (Clarke, 1981). Pro-atherogenic properties have been proposed for specific GSL, particularly for lactosylceramide (LacCer). Garner and colleagues proposed that this GSL actively promotes cholesterol accumulation in macrophage foam-cells (Garner, 2002). Furthermore, it has been reported that LacCer inhibits cellular cholesterol efflux via the ABCA1/apoA-I pathway (Glaros, 2005). Moreover it has been claimed that LacCer induces monocyte adhesion to endothelial cells (Gong, 2004) and stimulates vascular SMC proliferation (Bhunia, 1997). Other studies have reported that ganglioside GM3 accelerates low-density lipoprotein (LDL) uptake by macrophages and subsequent formation of lipid-laden
foam cells (Prokazova and Bergelson, 1994). Next, GM3 and GD3 have been described to promote the adhesion of platelets to atherosclerotic lesions. GD3 has been found to stimulate production of reactive oxygen species, to regulate SMC phenotype and inhibit metalloproteinase-9 expression, all events potentially contributing to plaque instability and atherosclerosis (Wen, 1999; Moon, 2006). Based on reports in the literature, GSLs could thus promote atherogenesis at various levels. Targeting the glycosphingolipid biosynthetic pathway may therefore offer an avenue to ameliorate atherosclerosis. Several studies indeed showed that myriocin, an inhibitor of SPT and thus of biosynthesis of any sphingolipid, inhibits atherogenesis in APOE(−/−) mice (Glaros, 2007; Glaros, 2008). A relatively poor in vivo inhibitor of glucosylceramide synthase, EtDO-P4, when tested in APOE(-/-) mice, had no effect on plasma cholesterol and atherosclerosis (Glaros, 2008). Macrophage specific deletion of sphingomyelin synthase 1 on the other hand was found to decrease atherosclerotic plaque size of LDLR(-/-) mice (Li, 2012). The present literature on the role of specific (glyco)sphingolipids in atherogenesis as well the effect of pharmacological modulation of their synthesis is puzzling and conflicting. More studies with specific inhibitors in various steps of the biosynthesis of sphingolipids are needed to better elucidate the role of this complex class of lipids in atherogenesis.

**AMP-DNM, a modulator of glucosylceramide metabolism**

In 1998, Overkleeft and co-workers reported the generation of a hydrophobic deoxynojirimycin (DNM) derivative linked via a pentyl-spacer to a heterocyclic adamantane, N-(5-adamantane-1-yl-methoxy)pentyl)-DNM (AMP-DNM) (Overkleeft, 1998). The compound was designed as inhibitor of the non-lysosomal glucosylceramidase (GBA2). Indeed, AMP-DNM is an extremely potent GBA2 inhibitor, the IC50 value being in the low nanomolar range. AMP-DNM was found to inhibit also some other glycosidases like lysosomal glucocerebrosidase (GBA1), however at much higher concentration (IC50 being around 200 nM). Following the report by Platt and coworkers that N-butyl-DNM is an inhibitor of glucosylceramide synthase (GCS) (Platt, 1994), the structurally related AMP-DNM was investigated also in this respect. It was demonstrated that AMP-DNM is actually a much more potent GCS inhibitor than N-butyl-DNM, the IC50 value being about 180 nM. AMP-DNM was next found to be an attractive pharmacological agent, being hardly metabolized and due its amphiphilic nature showing a nice biodistribution (Wennekes, 2010).

Given the ability of AMP-DNM to lower GSL levels, its effect on insulin sensitivity has been experimentally investigated in cell and mouse models (Aerts, 2007). The motivation for this stems from the fact that GSL are implicated to play a role in in-
sulin homeostasis. For example, insulin resistance in adipocytes is accompanied by elevated GM3 levels, and insulin receptor substrate 1 (IRS1) phosphorylation is reduced by addition of ganglioside GM3 to cells (Tagami, 2002). Mice deficient in GM3 synthase, unable to produce GM3, are found to be protected against diet–induced insulin resistance (Yamashita, 2003). Vice versa, hepatic expression of neuraminidase, an enzyme degrading ganglioside GM3, improved insulin sensitivity and glucose tolerance (Yoshizumi, 2007). AMP-DNM treatment of several rodent models of obesity and insulin resistance resulted in improved glycemic control with lowered blood glucose levels, reduced glycated hemoglobin (HBA1c) levels and ameliorated insulin sensitivity in muscle and liver measured by euglycemic clamps in ob/ob mice and ZDF rats (Aerts, 2007). Comparable effects were noted in Zucker rats, db/db mice and diet-induced obese (DIO) mice. A similar beneficial effect on glucose homeostasis and insulin sensitivity was reported for a ceramide-analogue inhibitor of GCS (Zhao, 2007). Improvements of the insulin signalling pathway have been demonstrated in tissues following AMP-DNM treatment, e.g. liver and adipose tissue (Bijl, 2009; Van Eijk, 2009). In adipose tissue, adipogenesis was restored as the number of larger adipocytes was reduced and the expression of peroxisome proliferator-activated receptor (PPAR)-gamma, GLUT-4 or adiponectin was increased. In addition, inflammation was reduced by AMP-DNM treatment. Less crown-like structures were observed and expression of inflammatory markers such as monocyte chemoattractant protein-1 (MCP1/CCL2) and osteopontin (OSN) were reduced (Van Eijk, 2009). AMP-DNM was also found to prevent hepatosteatosis in ob/ob mice (Bijl et al 2009), to improve cholesterol homeostasis and bile secretion (Bijl, 2009), to normalize body weight and food intake in leptin-deficient mice (Langeveld, 2012), and to exert an anti-inflammatory effect in colitis models (Shen, 2004)

Outline of the thesis

Excessive concentrations of GSLs were hypothesized by us to constitute a risk factor for the development of atherosclerosis. The iminosugar AMP-DNM is a known potent inhibitor of GCS, the enzyme catalyzing the initial step in biosynthesis of GSLs. Pharmacological lowering of GSLs by AMP-DNM in mouse models of atherosclerosis could therefore test whether GSLs indeed play a detrimental role in atherogenesis. Based on this concept we designed the research presented in this thesis.

In the investigations we used APOE3* Leiden transgenic (APOE3* Leiden) and LDL receptor null LDLR(-/-) mice. APOE3* Leiden mice are a model for familial dysbetalipoproteinemia characterized by defective clearance rate of remnant particles (van Vlijmen, 1994). On a high cholesterol diet, these mice develop
atherosclerotic plaques of which the severity positively correlates with the serum levels of cholesterol-rich VLDL/LDL lipoproteins (Leppänen, 1998). LDLR(-/-) mice are a model for familial hypercholesterolemia (Ishibashi, 1993). When fed a western type diet, LDLR(-/-) mice develop high plasma cholesterol levels and severe atherosclerotic lesions. Similarly, APOE deficient (APOE(-/-)) mice, develop spontaneously atherosclerotic lesions, a process stimulated by a high fat diet (Véniant, 2001).

Chapter II describes that AMP-DNM treatment can spectacularly prevent hypercholesterolemia and associated lesion formation in two different models, ApoE3* Leiden and LDLR(-/-) mice. The study revealed that iminosugar treatment markedly promotes biliary and fecal cholesterol excretion. Chapter III reports on a comparison of the outcome of treatment of LDLR(-/-) mice with AMP-DNM and its L-ido derivative, L-ido-AMP-DNM. The latter compound inhibits GCS and GBA2 on a par with AMP-DNM, but it does not significantly inhibit the lysosomal enzyme GBA1. The investigation showed that AMP-DNM promotes better biliary and fecal cholesterol secretion and consequently has a more potent anti-atherogenic effect. Chapter IV describes a study that addresses the question whether existing advanced lesions in LDLR(-/-) mice can also be reverted by AMP-DNM treatment. Chapter V reports on the beneficial effect of physical activity in slowing atherosclerotic lesion progression and modulating bile salt homeostasis. Chapter VI describes an investigation on the value of plasma chitotriosidase as marker for atherosclerotic lesion development in APOE3*Leiden, LDLR(-/-) and APOE(-/-) mice. Chapter VII provides additional data on the action of AMP-DNM on cholesterol homeostasis, focusing on its effects on cholesterol absorption and excretion. Chapter VIII deals with the marked reversal of liver steatosis in LDLR(-/-) mice treated with AMP-DNM. The positive effect of AMP-DNM treatment on insulin sensitivity in obese mice is reviewed in Chapter IX. Finally, in chapter X a general discussion is provided, concerning the outcomes of the studies performed in this thesis.
Reference List


Rothblat, G. H.; Phillips, M. C. High-density lipoprotein heterogeneity and function in reverse cholesterol transport. Curr. Opin. Lipidol. 2010, 21, 229-238.


CHAPTER I


CHAPTER I


