The role of glycosphingolipids in insulin signaling and lipid metabolism
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Chapter

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1.1 General Introduction

The benefit of a rich food supply in our modern society seems to have a major drawback. Our average bodyweight is steadily increasing and the World Health Organization (WHO) formally recognized obesity as a global epidemic in 1997 (i). Indeed an epidemic, as obesity prevalence in the United States doubled among adults between 1980 and 2004 and more than 30% of Americans are now considered overweight ([1]). The same trend in body weight increase can be found throughout Europe, albeit a few years delayed compared to the United States (ii). Alarmingly, the demographics of the obesity epidemic mirror those of the insulin resistance epidemic (the diabesity epidemic [2]) and other metabolic abnormalities such as dyslipidemia. The causal relation between insulin resistance, dyslipidemia and obesity is currently unknown although some hypotheses exist. This thesis deals with a novel hypothesis that focuses on a specific class of lipids, the glycosphingolipids, as causal mediators of obesity induced insulin resistance.

1.2 Metabolic actions of insulin

Insulin flows through the blood after release by the pancreas until it encounters and activates the insulin receptor on the plasma membrane of different target tissues. The insulin receptor is a heterodimer consisting of two extracellular α-units, capable of insulin binding, and two intracellular β-units with intrinsic tyrosine kinase activity. Binding of insulin to the extracellular units induces a conformational change resulting in autophosphorylation of the intracellular units, further increasing their kinase activity ([3, 4]). This step is followed by activation of a signal transduction cascade, started by the phosphorylation of insulin receptor substrate (IRS) proteins, eventually leading to phosphatidylinositol 3-kinase (PI3-kinase) activation, and production of PIP2 and PIP3. The importance of PI3-kinase in insulin signaling is highlighted by studies showing that virtually all of insulin’s effects on glucose transport, lipogenesis, and glycogenesis are abolished by either inhibitors or dominant-negative mutants of PI3-kinase ([5, 6]). The different effects of insulin on glucose homeostasis and lipid and protein synthesis are regulated downstream of PI3-kinase. Insulin signaling can also activate the MAP kinase pathway. This pathway is mainly involved in cell growth and differentiation (figure 1).
Insulin and glucose homeostasis
PI3-kinase activates the serine/threonine kinase Akt (also known as protein kinase B, or PKB). Akt has numerous and diverse intracellular targets. Importantly, it stimulates glycogen synthesis by activation of the enzyme glycogen synthase. The activity of this enzyme (GS) is repressed in the absence of insulin signaling thereby keeping the glucose available for use in other tissues such as the brain. Activation of Akt leads to the phosphorylation and deactivation of glycogen synthase kinase 3 (GSK3)[7]. Deactivation of GSK3 in its turn leads to dephosphorylation and activation of glycogen synthase. At the same time insulin inhibits the breakdown of glycogen. Activated AKT promotes insulin-stimulated glucose uptake by promoting the translocation of the glucose transporter vesicle GLUT4 to the plasma membrane in fat and muscle cells ([8]). Akt also exerts effects at the level of gene transcription. Various genes involved in gluconeogenesis, such as phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, are actively transcribed in the absence of insulin. Their transcription factor FOXO-1 is phosphorylated downstream of PI3-kinase, at least partially by Akt, leading to its inactivation and repression of gene transcription ([9, 10]).
Insulin and lipid homeostasis

The synthesis of fatty acids is regulated at the level of gene expression by the transcription factor sterol regulatory-element binding factor 1c (SREBP-1c). This transcription factor activates the genes coding for the enzymes needed to synthesize fatty acids from acetyl-CoA up to the final steps of elongation and desaturation (such as acetyl-CoA carboxylase, fatty-acid synthase, and stearoyl-CoA desaturase) ([11]). The activation of SREBP-1c is complex but well described. The inactive form of SREBP-1c is present as a precursor bound to the endoplasmic reticulum (ER) by Insig proteins. The maturation is initiated when the COOH-terminal domain of SREBP-1c binds to SREBP cleavage-activating protein (SCAP). SCAP is a sterol-sensing escort protein. When the ER membrane becomes depleted in cholesterol, SCAP escorts SREBP-1c from the ER to the Golgi. Here two proteases cleave SREBP-1c liberating the active transcription factor domain. The much smaller mature form can now enter the nucleus to activate transcription ([12, 13]).

Insulin stimulates fatty acid synthesis and several lines of evidence point to direct effects of insulin on SREBP-1c transcription, maturation and activation. The main regulation of SREBP-1c is at the transcriptional level, but insulin stimulation leads to a parallel increase in both the membrane-bound precursor and the mature nuclear form. The promoter region of SREBP-1c contains amongst others SREBP-1c binding sites ([14]) and transcription is regulated in part by SREBP1c in an auto-regulatory loop. The transcription of SREBP-1c can also be induced by the activation of liver X receptor (LXR) ([15]). LXR is a nuclear hormone receptor with high expression in liver, and it is activated by oxysterols (intermediates of cholesterol metabolism). The significance and respective roles of these two factors in the regulation of SREBP-1c, however, are largely unknown and might differ from mice to human ([16-18]). The induction of SREBP-1c transcript by insulin appears to depend on PI3-kinase activity, through activation of PKCλ ([19]).

The maturation or post-translational processing is also influenced by insulin. Insulin induces phosphorylation of the precursor SREBP-1c in a PI3-kinase and Akt dependent fashion and this leads to activation of the transport process from the ER to the Golgi followed by proteolytic cleavage ([20]). Finally, after SREBP-1c is bound to its target genes, it can be inactivated via ubiquitination as is the case for many transcription factors. This process is induced by phosphorylation of SREBP-1c by the enzyme GSK3, reducing degradation of SREBP-1c, and thus enhancing transcriptional activity ([21]).
Whereas insulin activates synthesis of fatty acids in the liver and in adipose tissue, insulin decreases lipolysis at the same time. It does so by activating phosphodiesterase-3B resulting in a decrease in cAMP levels ([22, 23]). By this action, hormone sensitive lipase is deactivated, resulting in inhibition of lipolysis.

These metabolic properties of insulin on glucose and fat homeostasis are clearly shown in mice that lack the insulin receptor in specific tissues only. For example, mice with a disruption of the insulin receptor in their fat tissue, (the FIRKO mice: fat insulin receptor knockout mouse), are sensitive to insulin except in their fat tissue. The adipocytes (fat cells) from these mice show the expected defects in glucose and lipid metabolism, with a 90% decrease in insulin-stimulated glucose uptake and insulin-stimulated incorporation of glucose into triglycerides. Also the ability of insulin to prevent lipolysis is strongly reduced in these mice ([24]).

Mice that lack the insulin receptor in their liver only (the LIRKO mice: liver insulin receptor knock-out) are characterised by hyperglycemia ([25, 26]). This is most likely due to ongoing gluconeogenesis in the liver. In agreement with this, these livers have increased expression of gluconeogenesis enzymes. The circulating levels of free fatty acids and triglycerides are decreased in LIRKO mice. This was found to be due in part by the high insulin levels in these mice causing suppression of extrahepatic processes. In addition, the complete lack of insulin receptors in the liver prevents activation of SREBP-1c and the synthesis of fatty acids ([27]).

**Insulin and cholesterol homeostasis**

The liver is the main organ for the de novo synthesis of cholesterol. The synthesis starts with one molecule of acetyl-CoA and one molecule of acetoacetyl-CoA, which are dehydrated to form 3-hydroxy-3-methylglutaryl CoA (HMG-CoA). This molecule is then reduced to mevalonate by the enzyme HMG-CoA reductase. This step is an irreversible step in cholesterol synthesis and is the site of action for the statins (HMG-CoA Reductase Inhibitors). The synthesis of cholesterol is directly regulated by the cholesterol levels present: a higher intake from food leads to a net decrease in endogenous production, while lower intake from food has the opposite effect. The regulation is dependent on the transcription factor SREBP-2. As described above for SREBP-1c the activation of SREBP-2 is directly regulated by endogenous levels of sterols. Insulin also seems to be involved in SREBP-2 activation given the finding that LIRKO mice show a 40-80% decrease in levels of SREBP-2 mRNA (Srebf2). Transcripts of many of the targets of SREBP-2, including the enzymes HMG-
CoA reductase, squalene synthase, and farnesyl diphosphate synthetase, as well as the low-density lipoprotein receptor, are also decreased by 60%–90% in LIRKO mice livers ([27]). Interestingly, these mice develop atherosclerosis when they are challenged with a high cholesterol diet. Plasma cholesterol levels are dramatically increased compared to controls on the same diet. This hypercholesterolemia is thought to be due to a decreased uptake of cholesterol by the liver under control of SREBP-2.

Cholesterol can be metabolised in the liver by the conversion into bile salts. These bile salts are secreted from the liver together with cholesterol, phospholipids and waste products, stored in the gallbladder as bile and subsequently used for food digestion when needed. During storage in the gallbladder, cholesterol can crystallize from supersaturated bile and gallstones may develop. The fact that this process is associated with obesity is already known for a century but we only now start to understand how insulin resistance plays a role in this process. Investigations with LIRKO mice (see above) have helped to identify the pathophysiological mechanism. Feeding LIRKO mice a high cholesterol diet results in the formation of gallstones due to the fact that ABCG5/ABCG8, the transporters that efflux cholesterol into bile, are highly expressed in LIRKO mice. The bile of the LIRKO mice is therefore supersaturated with cholesterol molecules leading to gallstone formation. It was found that insulin directly regulates the expression of ABCG5/ABCG8 via a pathway involving Akt and Foxo1 ([28]).

1.3 Insulin Resistance Syndrome

In a healthy person, insulin is produced by the pancreas in response to increasing blood glucose levels after a meal. The subsequent rise in plasma insulin concentrations triggers insulin-sensitive tissues like muscle and adipose tissue to absorb glucose from the blood and to stop the production of glucose in the liver. These processes lower blood glucose levels and the pancreas stops the production and release of insulin (figure 2A). In an insulin-resistant person, normal levels of insulin do not have the same effect on muscle and fat cells, with the result that glucose levels stay higher than normal. To compensate for this, the pancreas in an insulin-resistant individual is stimulated to release more insulin. This leads to high levels of insulin in the blood, a condition called compensatory hyperinsulinemia. It is important to keep in mind that insulin is not only closely related to glucose homeostasis, but that
insulin also regulates the synthesis and storage of fat, protein synthesis, and non-metabolic processes such as cell growth and differentiation. In insulin resistance not all tissues and insulin-regulated processes become equally resistant to the actions of insulin. The elevated insulin levels found in insulin-resistant states lead to increased actions throughout the body, for example the stimulation of fatty acid synthesis in the liver and the inhibition of breakdown of fat from adipose tissues (figure 2B).

**Figure 2A** Normal response to a glucose load. Insulin leads to decreased gluconeogenesis and increased synthesis of fatty acids and triglycerides (TG) in the liver. Insulin leads to uptake of glucose by fat and adipose tissue.

**Figure 2B** Type 2 diabetes. Insulin fails to decrease gluconeogenesis, but it continues to stimulate synthesis of fatty acids and TG in the liver. Insulin fails to stimulate uptake of glucose by fat and adipose tissue.
There are many metabolic abnormalities that can originate from insulin resistance ([29, 30]). Amongst these are central obesity, dyslipidemia (raised triglycerides, raised small dense LDL and reduced HDL cholesterol), glucose intolerance, and hypertension. The co-occurrence of these metabolic abnormalities is clustered under the definition of Insulin Resistance Syndrome or Metabolic Syndrome. The above mentioned metabolic abnormalities are risk factors for the development of type 2 diabetes, cardiovascular disease, cancer, polycystic ovary syndrome, and non-alcoholic fatty liver disease (figure 3) ([31]). Identification of individuals with Metabolic Syndrome allows physicians to institute preventive measures to reduce these health risks. In 2006 the International Diabetes Foundation (IDF) standardized the criteria for the definition of Metabolic Syndrome (Table 1).

![Figure 3. Metabolic Syndrome. Metabolic abnormalities that originate from insulin resistance (light grey), increase the risk to develop a number of diseases (dark grey).](image)

**Table 1.** Metabolic Syndrome criteria (as defined by the IDF in 2006):

<table>
<thead>
<tr>
<th>Metabolic Syndrome criteria</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Central obesity plus any two of the following four factors:</td>
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| Raised triglycerides | $\geq 150 \text{ mg/dL (1.7 mmol/L)}$  
or specific treatment for this lipid abnormality |
| Reduced HDL cholesterol | $< 40 \text{ mg/dL (1.03 mmol/L) in males}$  
$< 50 \text{ mg/dL (1.29 mmol/L) in females}$  
or specific treatment for this lipid abnormality |
| Raised blood pressure | systolic BP $\geq 130$  
or treatment of previously diagnosed hypertension  
diastolic BP $\geq 85 \text{ mm Hg}$  
| Raised fasting plasma glucose | (FPG) $\geq 100 \text{ mg/dL (5.6 mmol/L)}$,  
or previously diagnosed type 2 diabetes |
1.4 Causes of obesity-induced insulin resistance

Adipose tissue dysfunction
If insulin resistance is the underlying cause of a variety of metabolic abnormalities, then what causes this insulin resistance? Is obesity an important factor in this connection? Humans are able to survive periods of scarcity by using up the reserve that was stored in fat tissue in times of surplus. Fat, as long as it can be safely stored in adipose tissue, seems to be quit harmless. Indeed not all people that are largely overweight develop obesity-associated problems such as type 2 diabetes or high blood pressure. On the other hand, mildly obese individuals that show severe insulin resistance and type 2 diabetes, also exist. These phenotypes suggest that not the absolute amount but rather adipose tissue function determines the development of insulin resistance. Adipocytes are not only storage cells for fat, but they are highly secretory cells producing a wide range of hormones and cytokines involved in glucose metabolism (e.g. adiponectin, resistin), lipid metabolism (e.g. cholesteryl ester transfer protein, CETP), inflammation (e.g. TNF-α, IL-6), coagulation (PAI-1), blood pressure (e.g. angiotensinogen, angiotensin II), and feeding behaviour (leptin). Changes in adipocyte function will thus affect metabolism and the function of many organs and tissues including muscle, liver, vasculature, and brain. Several research groups focus on the function rather than the amount of adipose tissue as a predisposing factor to develop insulin resistance ([32]).

Inflammation
It has now been firmly established that obesity is associated with the appearance of a chronic, low inflammatory state. This low-grade inflammation is mediated by the inflammatory (classical) activation of recruited and resident macrophages that populate metabolic tissues, including adipose tissue and liver. The cytokines secreted by these tissue macrophages can directly promote insulin resistance ([33, 34]). For example, several studies show that the cytokine TNF-α represents a key mediator of obesity-linked insulin resistance. Treatment of adipocytes in vitro with TNF-α inhibits glucose uptake and the downstream insulin signaling cascade ([35]). TNF-α contributes to insulin resistance by inhibiting the expression of genes which are essential for insulin signaling (IRS-1) and glucose uptake (GLUT4) ([36, 37]).
Lipotoxicity
Another hypothesis linking obesity with insulin resistance is that the delivery of fatty acids to peripheral tissues in excess of their oxidative or storage capacities impairs the function of these tissues. This process has been referred to as lipotoxicity ([38]). Extensive research has been conducted to explain how free fatty acids can cause insulin resistance. First of all, free fatty acids such as palmitate are implicated in causing beta-cell dysfunction and lipotoxicity in insulin sensitive tissues ([39]). Other studies have shown that the fatty acid palmitate inhibits the activation of insulin receptor substrate 1, PI3-kinase, or Akt, causing insulin resistance ([40]). The complex effect of fatty acids on insulin resistance was recently reviewed ([41]). Besides being directly involved in causing insulin resistance, free fatty acids can also be used as building blocks of various classes of lipids such as sphingolipids, e.g. ceramide. Sphingolipid levels are selectively up-regulated by circulating factors associated with obesity and metabolic disease, and ceramides and related sphingolipids have been shown to accumulate in obese humans and rodents ([42]). Interestingly these high levels of ceramide were shown to inhibit downstream insulin signaling, at least partially at the level of Akt ([42]). More recently, a more complex class of sphingolipids, the so-called glycosphingolipids, has been implicated in insulin resistance ([43]). An introduction to glycosphingolipids and their role in obesity-induced insulin resistance will be given in the next paragraphs.

1.5 Glycosphingolipids in insulin resistance
Glycosphingolipids were initially discovered by J.W.L. Thudichum in 1882, studying the chemical composition of the brain. In brain lipid extracts he discovered a compound with an ‘alkaloidal nature’ that was named sphingosine, after the mythological Sphinx’s riddle. Thudichum demonstrated next the existence of (glyco)sphingolipids consisting of a so-called ceramide lipid moiety with covalently linked oligosaccharides or phosphorylcholine (sphingomyelin). His land-slide discovery did not receive recognition during his lifetime. Only by the 1930s, his claims were fully vindicated and Herbert E. Carter coined the term sphingolipids to honor Thudichum. Since then numerous complex glycosylated sphingolipid derivatives have been discovered ([44]).
Synthesis of glycosphingolipids
The fatty acid palmitate, which is abundantly available in states of nutrient excess, is the precursor for synthesis of sphingolipids and its availability determines the synthesis rate. Thus, excess intake or impaired oxidation of saturated fat due to insulin resistance likely contributes to the accumulation of sphingolipids in tissues ([45]). The enzyme serine-palmitoyl transferase (SPT) catalyzes the first reaction, in which L-serine condenses with palmitoyl-CoA. In subsequent steps at the cytosolic leaflet of the ER ceramide is formed. Extracellular stimuli such as cytokines that are elevated in obesity can increase the synthesis rate by increasing the expression of SPT. Then, ceramide is delivered to the Golgi apparatus by ceramide transfer proteins, and converted to sphingomyelin (SM) by SM synthase catalyzing the transfer of phosphorylcholine from phosphatidylcholine (PC) to ceramide. At the cytosolic side of the Golgi, ceramide is also converted to glucosylceramide (GlcCer) by GlcCer synthase that catalyzes the transfer of glucose from UDP-glucose to ceramide. The next glycosylation step in the formation of glycolipids is the transfer of galactose from UDP-galactose to glucosylceramide. This reaction occurs in the lumen of the Golgi and is facilitated by lactosylceramide synthase. Thereafter, the pathway for the biosynthesis of gangliosides involves sequential activities of sialyltransferases and glycosyltransferases that add one or more N-acetylneuraminic acid (sialic acid) and sugar residues. From lactosylceramide the most simple ganglioside, GM3, can be formed by the addition of one sialic acid residue ([46, 47]). High levels of glycosphingolipids are found in rodent models for obesity ([42]) and increased GM3 levels are found in adipose tissue of mice made insulin resistant by low levels of TNF-α ([48]). Besides increased availability of substrates and increased expression of the rate limiting enzyme SPT, other factors in obesity could contribute to increased glycosphingolipid levels. For example, increased GM3 synthase mRNA concentrations are found in adipose tissues of ob/ob mice and ZDF rats ([48]).

Glycosphingolipids interact with the insulin receptor
Glycosphingolipids are predominantly present at the plasma membrane of mammalian cells. The ceramide backbone is located inside the lipid bilayer of the plasma membrane and the carbohydrate moiety at the outside where it interacts with glycoproteins and other glycosphingolipids. Due to the ability to form hydrogen bonding, glycosphingolipids can form microdomains with cholesterol that are more rigid and ordered than other parts of the membrane. Caveolae are membrane invaginations characterized by the presence of caveolin-1 and enriched in cholesterol, sphingomyelin and glycosphingolipids. They are thought to serve as signaling
platforms for receptors such as the insulin receptor. Glycosphingolipids can also form specific membrane micro-domains with cholesterol in the absence of caveolin (detergent resistant domains). Some proteins preferentially insert into these highly organised membrane structures and form specialised platforms for cell signaling ([49]). The amount of GM3 seems to directly affect the ability of insulin to activate the receptor. It was shown that the addition of GM3 to cultured adipocytes reduces the phosphorylation of the insulin receptor and its downstream substrate IRS-1 by insulin. Reducing GM3 levels by inhibition of GM3 synthesis, using an inhibitor, results in activation of insulin signaling. The relation between glycosphingolipid levels and the ability of insulin to activate its receptor is further substantiated by observations in mutant mice. Mice that lack the enzyme for GM3 (GM3 synthase knockout mice) show enhanced activation of the skeletal muscle insulin receptor after stimulation with insulin ([50]). When these mice are challenged with a high fat diet to induce insulin resistance, these mutant mice remain sensitive to the actions of insulin ([50]). We can conclude from the above observations that increased levels of glycosphingolipids disturb insulin signaling and that the absence of GM3 protects against the development of insulin resistance.

Very recently Kabayama et al provided evidence that the interaction of GM3 with the insulin receptor is mediated by a specific lysine residue located just above the transmembrane domain of the receptor ([51]). Based on these observations and their earlier results, a model has been proposed how glycosphingolipids impair insulin signaling: excess levels of GM3 promote dissociation of the insulin receptor from its membrane location and hamper the downstream signaling events (figure 4) ([52]).

![Diagram](image)

**Figure 4.** An excess level of GM3 promotes the dissociation of the insulin receptor from its membrane location and its association with caveolin-1, thereby hampering the downstream signaling events.
Inhibitors of glucosylceramide synthase

The negative association of glycosphingolipids with insulin signaling has prompted researchers to test the effect of inhibitors of GSL synthesis on insulin signaling. Inokuchi and co-workers were the pioneers in this field and they used the ceramide analogue 1-phenyl-2-decaoylamino-3-morpholinopropanol (PDMP) to inhibit glucocylceramide synthase. In their studies they treated cultured adipocytes with TNF-α to increase glucosylceramide levels and showed that this indeed caused insulin resistance. Subsequently they added PDMP to these cells to prevent glycosphingolipid synthesis and noted that PDMP prevented TNF-α induced insulin resistance ([53]). However, the compound they used also inhibits transacylation of 1-O-acylceramide, and thereby increases cellular ceramide concentrations which makes interpretation of the results difficult ([54]). This compound formed the basis for the synthesis of the glucosylceramide synthase inhibitor (1R,2R)-Nonanoic acid[2-(2’,3’-dihydro-benzo [1,4] dioxin-6’-yl)-2-hydroxy-1-pyrrolidin-1-ylmethyl-ethyl]-amide-L-tartaric acid salt (Genz-123346) ([55, 56]). Unlike the original parent compound PDMP, Genz-123346 has little effect on 1-O-acylceramide synthase activity at concentrations that effectively inhibit glycosphingolipid synthase activity and therefore does not significantly elevate cell ceramide levels in vitro.

Besides ceramide based structures, N-alkylated iminosugars are also potent inhibitors of the enzyme glucosylceramide synthase. Already in 1994 N-butyl-deoxynojirimycin (NB-DNJ) was reported to be an inhibitor of glycosylceramide synthesis ([57]). NB-DNJ is used as an inhibitor of GCS in the treatment of patients with Gaucher’s disease. This compound is a very potent inhibitor of intestinal α-glucosidases which is the cause of adverse effects observed in the gastrointestinal tract in patients treated with this drug. On the other hand, the potential of N-alkylated iminosugars to inhibit intestinal α-glucosidases forms the basis of the pharmacological action of the registered anti-diabetic drug Miglitol (N-(1-hydroxyethyl)-1-deoxynojirimycin), by buffering the carbohydrate uptake from food. In 1998 a more specific inhibitor of GCS was developed by Overkleeft and co-workers ([58]). This compound is based on the structure of NB-DNJ, but the butyl chain is replaced by an adamantane structure. This compound, N-(5-adamantane-1-yl-methoxy)-pentyl-1-deoxynojirimycin (AMP-DNM) is a much more potent inhibitor for GCS (100nM) compared to the original compound NB-DNJ (IC50 25000nM). However, AMP-DNM still inhibits the intestinal glucosidases in the same order of magnitude as Miglitol. Structural analogues of AMP-DNM have since been developed. Of these, N-(5-adamantane-1-yl-methoxy)-pentyl-L-ido-1-deoxynojirimycin (L-ido-AMP-DNM) is the most specific
and potent iminosugar-based GCS inhibitor reported to date ([59]).

The impact of glycosphingolipid lowering by oral administration of the iminosugar AMP-DNM to various rodent models of insulin resistance has been studied in our laboratory. It was found that treatment with AMP-DNM markedly improves insulin resistance in these models in a dose dependent manner ([60]). Similar findings were independently made with Genz-123346 ([61]), further substantiating that inhibition of glycosphingolipid biosynthesis ameliorates insulin resistance. Consistently, it was very recently reported that inhibition of ceramide synthesis with myriocin, and thus synthesis of anabolite sphingolipids, improved insulin sensitivity in rodent models of obesity-induced insulin resistance ([62]).

1.6 Aim and outline of the thesis

The research described in this thesis is based on the hypothesis that glycosphingolipids are involved in the signaling actions of insulin and that by pharmacological reduction of glycosphingolipid levels the insulin signaling capacity is restored. These studies were aimed to gain further insight into effects of AMP-DNM on glucose homeostasis as well as on lipid homeostasis, to study the organs involved in the effects of AMP-DNM and look for possible side-effects. Finally, these studies should improve insight in the role of glycosphingolipids in insulin resistance.

The first chapters (chapters 2-5) of this thesis describe the effects of AMP-DNM on glucose and lipid homeostasis in insulin resistance. Leptin-deficient ob/ob mice, a well-established model of insulin resistance, were used in the investigations. The absence of leptin causes these mice to be hyperphagic. They also have a low energy expenditure resulting in obesity, hyperglycemia, hyperinsulinemia and massive hepatic steatosis. Chapter 2 describes the effects of AMP-DNM on glycemic control in these mice. AMP-DNM is shown to improve insulin signaling as well as to buffer carbohydrate assimilation. These two factors contribute to improved glucose homeostasis. Adipose tissue function is an important factor in the progression of insulin resistance to lipotoxicity and type 2 diabetes. Chapter 3 describes investigations demonstrating that AMP-DNM improves adipose tissue of ob/ob mice and reduces the inflammatory state. Massive hepatic steatosis is a hallmark of insulin resistance. Treatment with AMP-DNM reduces the steatosis in ob/ob mice, as is described in chapter 4. In an attempt to gain further insight in the cause of improvements in metabolism, energy expenditure of ob/ob mice receiving AMP-DNM was investigated using metabolic cages. The outcome of this study is reported in chapter
5. Chapter 6 and 7 describe studies that were performed to establish effects of AMP-DNM in non-diseased states. A liver derived cell line was used as a model to investigate the impact of glycosphingolipid on gene transcription. The outcome of this study is presented in chapter 6. The insulin receptor is not the only receptor found in close proximity to glycosphingolipids. In chapter 7 the effects of glycosphingolipid lowering on the functionality of ABC-transporters are described. AMP-DNM was found to show, besides effects on glucose and lipid homeostasis, a major effect on cholesterol and bile homeostasis (6 and 7). How bile and cholesterol metabolism are interrelated is described in chapter 8. Finally, in chapter 9, a general discussion of the study outcomes described in this thesis is provided.

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