Studies on the role of glycosphingolipids in metabolism
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Chapter 11

SUMMARY AND GENERAL DISCUSSION
Summary

Type I Gaucher disease, a glycosphingolipid storage disorder, is associated with metabolic aberrations, such as an increased basal metabolic rate and altered glucose and cholesterol homeostasis. In Part I of this thesis it is described how these metabolic abnormalities are related to the glycosphingolipid excess present in Gaucher disease patients and whether the metabolic changes put patients at risk for developing complications such as overweight, type II diabetes and cardiovascular disease. In addition, the influence of treatment with enzyme replacement therapy (ERT) on metabolic parameters is studied.

In Chapter 3 plasma adiponectin levels in type I Gaucher disease patients are studied. Adiponectin, an adipocytokine secreted by adipocytes, has insulin-sensitizing properties. Low adiponectin concentrations, usually found in obese individuals, are associated with peripheral and hepatic insulin resistance. Serum adiponectin concentrations were shown to be significantly reduced in untreated type I Gaucher disease patients compared to BMI and age matched healthy control subjects. Treatment with ERT for several years resulted in an increase in adiponectin concentrations. The abnormally low adiponectin concentrations in type I Gaucher disease patients may contribute to reduced insulin responsiveness in this disorder.

The study described in Chapter 4 shows that symptomatic type I Gaucher disease is indeed associated with peripheral insulin resistance. Using the hyperinsulinemic euglycemic clamp technique, peripheral glucose uptake was shown to be lower in six type I Gaucher disease patients compared to matched healthy control subjects. Plasma concentrations of the ganglioside GM3, a lipid metabolite known to be involved in obesity induced insulin resistance, were elevated in the Gaucher patients. Excess gangliosides may therefore play a role in the observed insulin resistance in type I Gaucher disease patients. Non-insulin mediated glucose uptake was not significantly different between Gaucher patients and control subjects. This finding was unexpected since the presence of the large number of glucose-utilizing Gaucher cells in patients was presumed to be reflected in an increased non-insulin mediated glucose uptake.

Chapter 5 demonstrates that the concentration of the ganglioside GM3 was on average threefold elevated in plasma of 40 untreated type I Gaucher disease patients. The plasma GM3 concentrations correlated with disease severity as assessed by excess liver volume,
plasma chitotriosidase activity and the Severity Scoring Index. Increased GM3 levels were also found in spleen samples from four Gaucher patients.

In Chapter 6 the effect of Gaucher disease and treatment with ERT on the occurrence of overweight and type II diabetes is reported. In untreated type I Gaucher disease patients the prevalence of overweight is lower compared to the general population. Long term treatment with ERT induces weight gain, leading to the development of overweight in many patients. The resulting prevalence of overweight in Gaucher patients is similar to that in the general population. The prevalence of type II diabetes also increases significantly during treatment with ERT, resulting in a prevalence of type II diabetes in the ERT treated patients comparable to that in the general population.

In Chapter 7 the possibility that hypermetabolism in Gaucher disease is associated with altered thyroid hormone metabolism is explored. Baseline thyroid hormone levels were within the reference range in the majority of the 22 investigated patients. No cases of Non-Thyroidal Illness, a condition associated with diseases that cause an increase in the basal metabolic rate, were found. Also, there was no correlation between the increased Resting Energy Expenditure (REE) and the plasma T3, rT3 or fT4 concentration. REE decreased, but not normalized in most patients after several months of treatment with ERT. There was no correlation between the changes in REE and changes in fT4 and T3 concentrations. Concluding, in type I Gaucher disease there seems to be no influence of thyroid hormone metabolism on hypermetabolism, and vice versa.

Chapter 8 explores the consequences of the altered lipoprotein metabolism in type I Gaucher disease patients and carriers of a Gaucher disease mutation. Lipid profiles, apolipoproteins, and carotid artery intima-media thickness (cIMT) were analyzed in 40 type I Gaucher disease patients, 34 carriers and 41 control subjects. cIMT is a non-invasive validated biomarker for the status of atherosclerosis and for present and future cardiovascular disease risk. Compared to control subjects, patients showed decreased high-density lipoprotein cholesterol (HDL-c), as well as mildly decreased low-density lipoprotein cholesterol (LDL-c) levels, with an increased ApoB/ApoA1 ratio. In carriers HDL-c levels were normal, but LDL-c levels were decreased. Mean cIMT measurements were not different in the three study groups. It may be concluded that low HDL-c levels do not lead to premature atherosclerosis in type I Gaucher disease patients.

Sphingolipids and gangliosides have been described to be involved in obesity induced
insulin resistance. In rodent models for insulin resistance increased concentrations of sphingolipids and gangliosides were found in muscle, liver and adipose tissue and inhibition of (glyco)sphingolipid synthesis ameliorates insulin resistance. In Part II of this thesis we further explore the effects of reducing glycosphingolipid concentrations on obesity induced metabolic pathology. This was done by treating genetically obese leptin deficient ob/ob mice with the glycosphingolipid synthesis inhibitor N-(5-adamantane-1-yl-methoxy)-penty1-1-deoxynojirimycin (AMP-DNM).

In Chapter 9 ob/ob mice were treated with 100mg/kg body weight/day AMP-DNM, which reversed hepatic steatosis, restored insulin signaling in the liver and subsequently corrected blood glucose and insulin concentrations. In addition, AMP-DNM treatment almost completely corrected the gene expression profile in liver towards the profile of lean mice. The correction of gene expression included SREBP1-c target genes involved in fatty acid synthesis, as well as inflammatory and fibrotic markers.

In Chapter 10 ob/ob mice were treated with a similar dose of AMP-DNM for five weeks. Treatment initiation resulted in a rapid increase in the fat oxidation rate, a decrease of carbohydrate oxidation and a reduction in food intake and energy expenditure. During the third and fifth week of treatment fat oxidation levels remained high and food intake and energy expenditure reduced. After five weeks of treatment, a strong decrease in liver triglyceride concentrations of AMP-DNM treated animals was observed. In line with the high fat oxidation rates, liver CPT-1a expression was increased. In a second experiment, four hours of AMP-DNM exposure induced activation of regions of the hypothalamus known to be involved in the regulation of food intake. This short treatment span had no detectable peripheral metabolic effects in ob/ob mice, suggesting that the brain activation precedes the peripheral effects.

**General discussion**

*Energy and glucose homeostasis in type I Gaucher disease*

Gaucher disease is characterised by the presence of large numbers of lipid-laden macrophages in liver, spleen and bone-marrow which leads to the primary disease symptoms; hepatosplenomegaly, bone disease and cytopenia. In addition, several other phenomena
have been recognized in the past years as part of the clinical expression of the disorder. In untreated Gaucher disease patients resting energy expenditure is significantly increased\textsuperscript{1,2}. We hypothesized that the massive amount of storage cells is responsible for the excess energy usage. However, we were unable to show an increase in non-insulin mediated glucose uptake, which would have been indicative of increased glucose utilization by the storage cells. The study was performed in a relatively small number of patients, which may have hampered the detection of a (small) difference in non-insulin mediated glucose uptake between patients and control subjects. A separate study invalidated the hypothesis that altered thyroid hormone metabolism could be responsible for the hypermetabolism observed in Gaucher disease. A more definite conclusion regarding the cause of the hypermetabolism in Gaucher disease may come from studies in recently developed inducible knockout mouse models for type I Gaucher disease\textsuperscript{3,4}, which will allow precise assessment of metabolic rate and of labelled glucose uptake in different organs.

As shown in this thesis, and recently confirmed by Ucar and co-workers\textsuperscript{5}, symptomatic type I Gaucher disease is associated with insulin resistance. It is hypothesized that the reduced responsiveness to insulin in Gaucher disease is due to the formation of excess gangliosides, for which the accumulating glucosylceramide serves as substrate. Plasma levels of the ganglioside GM3 are increased in type I Gaucher disease patients. In insulin resistant rodents, elevated concentrations of gangliosides were found in liver, muscle and adipose tissue\textsuperscript{6-8}. Surplus gangliosides, especially GM3, are thought to induce insulin resistance by hampering insulin receptor phosphorylation\textsuperscript{9-11}. Whether increased plasma GM3 concentrations in Gaucher patients reflect increased levels of gangliosides in their insulin responsive tissues, thus explaining their blunted response to insulin, remains to be established. Muscle biopsies for ganglioside measurements were not performed because of the increased bleeding tendency in symptomatic type I Gaucher patients. Future research, for example in the mouse models for type I Gaucher disease, is needed to establish whether insulin resistance in Gaucher disease is indeed caused by excess gangliosides.
Long-term treatment of Gaucher disease patients affects the pattern of complications and associated conditions

Treatment of type I Gaucher disease with ERT reverses most of its primary disease manifestations. In the majority of the patients organomegaly and cytopenia respond very well\textsuperscript{12} and bone marrow infiltration by Gaucher cells also reduces in response to treatment with ERT\textsuperscript{13}. This means that, especially in those patients that did not suffer from bone complications prior to treatment initiation, near normal health status can be achieved\textsuperscript{14}. However, new complications and conditions associated with Gaucher disease, that were previously less well recognized, now become apparent in long term treated patients.

As was described in the early work by Ginsberg and co-workers\textsuperscript{15}, Gaucher disease is associated with reduced plasma levels of low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c). A low plasma HDL-c level is an independent risk factor for the development of atherosclerotic cardiovascular disease in the general population\textsuperscript{16,17}. During treatment with ERT cholesterol levels increase\textsuperscript{18}, but in this thesis we established that HDL-c concentrations remain below the normal range in many long term treated Gaucher disease patients. We further show that this does not lead to an increased risk for atherosclerotic cardiovascular disease in Gaucher patients. This was assessed by measuring the Intima Media Thickness (IMT) of the carotid arteries. An increase in IMT is considered a surrogate marker for atherosclerosis. In Gaucher disease patients, IMT is comparable to control subjects. Interestingly, all patients in whom the HDL-c concentration remained significantly reduced during treatment with ERT had high chitotriosidase activity levels in plasma. Chitotriosidase is secreted by Gaucher cells and its plasma activity is thought to reflect total body Gaucher cell burden\textsuperscript{19,20}. This indicates that the low HDL-c level may be a sign of ongoing Gaucher disease activity, which seems to alter lipoprotein metabolism. The low HDL-c concentration in type I Gaucher patients is not indicative of a higher risk for atherosclerosis related events. Therefore, in the assessment of cardiovascular disease risk in an individual Gaucher disease patient, low HDL-c concentrations should not be weighed as a contributing factor. We show that carriers of a glucocerebrosidase mutation have decreased plasma LDL-c concentrations, whereas their HDL-c concentrations were within the normal range. In an earlier study by Pocovi and co-workers, the opposite was found in Gaucher disease carriers: a lower HDL-c concentration and a normal LDL-c plasma concentration\textsuperscript{21}. The outcome of the study reported in this thesis provides a theoretical survival advantage for
Gaucher disease carriers, since lower LDL-c concentrations could potentially reduce the risk of cardiovascular disease and thus benefit survival. This adds another option to the list of possible advantages of being a carrier of glucocerebrosidase mutation that may explain the high carrier frequency for Gaucher disease\(^22\). On the other hand, the reproductive potential may not be influenced by the risk for cardiovascular complications, because these complications usually occur later in life. In addition, this potential beneficial effect was not reflected in a reduced IMT in the Gaucher disease carriers compared to the control subjects. Larger studies are needed to confirm the abnormalities in cholesterol profile in Gaucher disease carriers as well as their influence on cardiovascular risk and survival in these individuals. One of the features of Gaucher disease patients that is most dramatically changed by treatment with ERT is the metabolic phenotype. Untreated patients, with considerable disease activity, are generally slim, sometimes even underweight and hypermetabolic\(^1\). In this thesis, we show that long term treatment with ERT is associated with weight gain, resulting in overweight in many patients. The resulting incidence of overweight in ERT treated Gaucher disease patients is similar to that in the general population in western countries. The actual increase in fat mass in Gaucher patients may be even larger than the body weight gain in kilograms since organomegaly is reduced by ERT. Treatment of Gaucher disease with ERT reduces plasma concentrations of glucosylceramide, the main storage product in this disorder\(^23\). If this reflects identical alterations in tissue glycosphingolipid levels, an improvement in insulin sensitivity as a result of treatment can be envisioned. On the other hand, development of overweight is generally associated with a decrease in insulin sensitivity\(^24\). Obesity associated insulin resistance may, at least partially, be mediated by increased (glyco)sphingolipid formation in adipose tissue and muscle\(^25,26\). In Gaucher disease patients, development of significant overweight or even obesity may thus once more lead to an increase in glycosphingolipid levels in insulin responsive tissues. The net result, improvement or worsening of insulin sensitivity during ERT is difficult to predict for the individual Gaucher disease patient. If insulin sensitivity declines, the risk for the development of type II diabetes increases\(^27,28\). During treatment, four out of the 49 patients receiving ERT in our cohort developed type II diabetes. Although this was not significantly different from the incidence of type II diabetes in studies in the general population, definite conclusions about the risk for development of type II diabetes during ERT requires a study in a larger patient cohort, including a well defined control group.
Treatment considerations regarding the metabolic complications of Gaucher disease

The metabolic abnormalities associated with symptomatic type I Gaucher disease and changes in these parameters during treatment should be taken into consideration when monitoring patients. Treatment with ERT reduces energy expenditure. Weight gain during treatment with ERT may thus be due to the fact that patients do not adjust their caloric intake to the reduced energy expenditure. It is therefore advisable to discuss the risk of weight gain with patients that start with ERT. If weight gain does occur, advice from a dietician may be helpful. Regular monitoring of the fasted plasma glucose concentrations in overweight and obese type I Gaucher disease patients is justified since it is unknown whether their risk for the development of type II diabetes, that may be already increased due their altered glycosphingolipid metabolism is further augmented by the development of overweight Gaucher disease can also be treated by inhibiting glucosylceramide synthesis, a principle referred to as substrate reduction therapy (SRT). The only currently registered drug for SRT in Gaucher disease is the iminosugar N-butyldeoxyxojirimycin (miglustat, Zavesca, Actelion Pharmaceuticals, Switzerland). In contrast to ERT, treatment with miglustat is not associated with weight gain but, in the initial phase of treatment, with weight loss. This is most likely due to the limited specificity of miglustat. Miglustat inhibits the activity of disaccharidases in the intestine resulting in diminished absorption of complex carbohydrates. This is most likely the cause of the osmotic diarrhoea and perhaps also the cause of the observed weight loss in many patients after start of treatment with miglustat. These side effects are transient and in a study on the longer-term efficacy of miglustat most patients had regained weight and returned to their initial body weight by 24 months of treatment. Whether weight gain continues during subsequent years of treatment with SRT is unknown. More potent and specific iminosugars, that inhibit glucosylceramide synthase without affecting intestinal disaccharidases, have recently been generated. Another class of small molecules, ceramide analogues (for example Genz-112638, Genzyme) have also been shown effective in reducing glucosylceramide synthesis, with limited aspecific activity. A phase 2 study, evaluating efficacy and safety of Genz-112638 in type I Gaucher patients, is currently conducted. A potential advantage of SRT is that, if reduction in energy expenditure results in overweight in treated Gaucher patients, the resulting increase in ganglioside synthesis is counteracted and the negative metabolic consequences of increased adiposity may thus be reduced.
A role for iminosugars in treatment of obesity associated pathology?

In addition to their role in treatment of lysosomal storage diseases, glycosphingolipid synthesis inhibitors are promising candidates for the treatment of obesity associated metabolic complications. Obesity has far reaching metabolic consequences in the majority of individuals. Surplus nutrient supply to adipose tissue, due to overfeeding, eventually exceeds the storage capacity of this organ. This has several negative consequences. First, pathological adaptations to lipid oversupply result in inflamed and dysfunctional adipose tissue. Second, due to the exhausted buffering capacity of the adipose tissue, spill of lipids to other organs occurs. This leads to reduced insulin sensitivity of muscle and liver due to the formation of lipid metabolites that negatively influence insulin signalling, a phenomenon referred to as lipotoxicity. Interestingly, increased fat content of the liver is associated with a mixed resistance for the different metabolic actions of insulin. The glucose pathway becomes insensitive to insulin signaling, resulting in impaired suppression of hepatic glucose production by insulin. The fatty acid synthesis pathway on the other hand, remains insulin sensitive and increased insulin concentrations thus enhance fat synthesis. The pathological changes in liver resulting from fat accumulation are united under the name Non Alcoholic Fatty Liver Disease (NAFLD) and consist of steatosis, inflammation, hepatocyte injury and fibrosis. In addition to insulin resistance and NAFLD, obesity is associated with hypertension and dyslipidemia, resulting in a strongly increased risk for the development of cardiovascular disease.

In this thesis we show that treatment with the iminosugar N-(5-adamantane-1-yl-methoxy)-pentyl-1-deoxynojirimycin) AMP-DNM corrects several of these complications of obesity. Previous studies already showed that the iminosugar AMP-DNM improves hepatic and peripheral insulin sensitivity in rodent models of obesity induced insulin resistance. AMP-DNM partially inhibits the enzyme glucosylceramide synthase, resulting in reduced glucosylceramide and ganglioside synthesis. This may improve insulin sensitivity by abolishing the inhibition of insulin receptor phosphorylation by surplus gangliosides. Treatment with AMP-DNM results in lower plasma glucose and insulin levels and lower glycated hemoglobin concentrations, all signs of improved glucose homeostasis. Moreover, AMP-DNM has been shown to correct pathological changes in adipose tissue. Inflammation was reduced and adipocyte function improved in genetically obese ob/ob mice treated with AMP-DNM.
In the first study on the effect of AMP-DNM treatment in *ob/ob* mice described in this thesis, the positive effect on NAFLD is described. Central in this effect was a spectacular reduction in hepatic fat content in the treated animals and this was shown to be, at least partially, the result of lower fat synthesis. Concomitantly, insulin sensitivity improved, resulting in lower circulating insulin levels. Since insulin is one of the driving forces behind fat synthesis in the liver via activation of SREBP1-c, the lower insulin concentrations may have contributed to the reduction in fat synthesis.

In the second study in *ob/ob* mice described in this thesis, a surprisingly rapid effect of AMP-DNM treatment on substrate oxidation patterns, food intake and energy expenditure is described. AMP-DNM increases fat oxidation and reduces carbohydrate oxidation in *ob/ob* mice. Moreover, food intake almost immediately starts to decline after treatment initiation. The AMP-DNM effect on food intake may be mediated by a change in appetite, since brain areas involved in the regulation of food intake show an altered activation pattern after short-term exposure to AMP-DNM. Determination of the mechanisms behind the effects of AMP-DNM on food intake and substrate oxidation, and to which extent these effects are independent of each other, requires further investigation.

The positive effects of AMP-DNM on insulin sensitivity, fatty liver, substrate oxidation patterns and inflammation of adipose tissue and liver make this drug an attractive option for the treatment of obesity associated pathology. Part of the effects of AMP-DNM may be mediated by a reduction in food intake. In this thesis we show that treatment with a relatively high dose (100 mg/kg/day) of AMP-DNM is associated with a reduction in food intake, resulting in less weight gain than normally observed in *ob/ob* mice. The insulin sensitizing effects of AMP-DNM seem to be directly related to the glycosphingolipid lowering capacity of the drug, since this effect is also seen in mice treated with a low dose (25 mg/kg/day) at which food intake and body weight gain are not affected. Moreover, the non-iminosugar glucosylceramide inhibitor Genz-123346 also improved insulin sensitivity in insulin resistant rodent models, without a change in body weight.

**Suggestions for future studies**

Glycosphingolipids may negatively influence insulin sensitivity. Future studies are needed to validate this hypothesis in humans. Measurement of sphingolipid concentrations in insulin responsive tissues are needed to confirm their supposed excess in the insulin resistant state.
Since ceramide and GM3 are the sphingolipid species most frequently associated with obesity induced insulin resistance\textsuperscript{25,26}, determination of their concentrations in liver, muscle and adipose tissue from insulin resistant individuals would contribute to the understanding of their contribution to pathology in human obesity. Moreover, if animal studies show acceptable safety profiles, a clinical trial using glycosphingolipid synthesis inhibitors should be carried out to establish whether glycosphingolipid lowering also improves insulin resistance in humans. If so, this would be of interest for the treatment of both obese insulin resistant individuals as well as Gaucher disease patients. AMP-DNM would be an especially good candidate for treatment of obese individuals since, apart from improving insulin sensitivity through glycosphingolipid lowering, it seems to have an effect on food intake, reducing caloric intake and subsequently lowering body weight gain. This reduction in body weight will further improve insulin sensitivity and it would therefore be interesting to what extent the metabolic effect of high dose AMP-DNM treatment is mediated by either glycosphingolipid lowering or by weight reduction. This could be done by performing a pair fed study, limiting the caloric intake of 'control' animals to that of AMP-DNM treated animals. The rapid effect of AMP-DNM on food intake is an intriguing finding and the mechanism behind this phenomenon will be an interesting topic for future research.

References

38. Unger RH. Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic


