Glycobiology in cardiometabolic homeostasis
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SUMMARY

The research presented in this thesis addresses several pathophysiological pathways in glucose and lipid metabolism, two major parameters of the cardiometabolic syndrome. The main findings reported in this thesis are presented below. Whereas the first part of the thesis deals with the cause of hypertriglyceridemia and novel treatment candidates targeting the cardiometabolic syndrome, the second part of this thesis explores the role of heparan sulfates in hyperglycaemia and hypertriglyceridemia. Moreover, using a translational approach we show that defects in heparan sulfate proteoglycan (HSPG) synthesis affects lipid and glucose metabolism.

Part I: Causes of hypertriglyceridemia and novel treatment targets

Fasting and non-fasting triglyceride (TG) levels are recognized as independent risk factors for cardiovascular disease (CVD) \(^1\), a finding which bears direct relevance in view of the pandemic of obesity and type 2 diabetes mellitus (T2DM). However, current risk prediction models do not include TG levels and therapeutic strategies to lower the CVD risk in cardiometabolic subjects are limited. In chapter 2 we review novel aspects in TG metabolism including the role of heparan sulfate proteoglycans (HSPGs) in hepatic lipid remnant clearance. Atherogenic TG remnant particles bind to hepatic HSPGs after which they are internalized and cleared by the liver. \(^2\) In murine models, abnormalities in HSPG result in a mild hypertriglyceridemic (HTG) phenotype due to delayed clearance of these TG-rich lipoprotein remnant particles. \(^3\) Therefore, HSPG modulation might be an interesting target for future therapeutic targets in humans. \(^4\)

Early identification of subjects at risk for HTG could reduce their risk of coronary artery disease by inducing early interventions. A DNA sequence variant could be such an early indicator for future risk of HTG and coronary artery disease. In chapter 3, we hypothesized that the combination of several polymorphisms associated with plasma TG levels improves CVD risk prediction. Accordingly we evaluated if a panel of validated single nucleotide polymorphisms (SNPs) in TG-modulating genes predicts plasma triglyceride levels as well as first cardiovascular event in a prospective case-control study in the EPIC-Norfolk cohort. This study shows that a gene score composed of three TG modulating SNPs in APOA5 and LPL was linearly associated with plasma TG concentrations (+0.32 mmol/l [95% CI 0.257-0.379] per allele change, \(p<0.0001\)) and other lipid parameters representing an atherogenic lipid profile including decreased low-density lipoprotein (LDL) size, increased LDL number, increased very low-density lipoprotein (VLDL) particle number and decreased high-density lipoprotein (HDL) particle size. In line, the risk of future coronary artery disease was elevated in individuals with the highest gene score compared to those with lowest gene scores (odds ratio 1.88 [95% CI 1.11-3.18]; \(p=0.02\)).

Nowadays, lifestyle interventions and statin and fibrate based therapy are the primary treatment steps in hypertriglyceremic patients. However, a residual cardiovascular risk remains in these subjects, even when LDL-cholesterol target levels are achieved. \(^5\) It is unclear what the optimal
treatment strategy is for patients who are treated with statin therapy and still display a metabolic lipid profile. In chapter 4 we investigated whether high-risk patients with metabolic dyslipidemia gain additional benefit from switching to high dose statin therapy. To address this question we performed a post-hoc analysis in the Treating to New Targets (TNT) trial including 9,994 patients with coronary artery disease who were randomly assigned to receive either atorvastatin 10 mg or 80 mg per day following a run-in phase on atorvastatin 10 mg. We found that increasing atorvastatin dose from 10 mg to 80 mg results in significant incremental cardiovascular benefit in patients with high TG levels and low HDL-cholesterol levels.

Another potential novel candidate, targeting cardiometabolic sequelae are the thyroid hormone mimetics. Since thyroid hormone mimetics are capable of uncoupling the beneficial metabolic effects of thyroid hormones from their deleterious effects on heart, bone and muscle, this class of drug is considered as adjacent therapeutic to weight-lowering strategies. In chapter 5 we performed a randomized, placebo-controlled, double-blind trial to investigate the safety and efficacy of TRC150094, a thyroid hormone mimetic, in male cardiometabolic subjects. In this study, TRC150094 dosed 50 mg once daily was safe and well tolerated, however, insulin sensitivity, hepatic fat content and lipid profiles did not improve following 4 weeks of administration. However, subgroup analysis indicated that TRC150094 might have beneficial effects in patients with more severe metabolic derangements (including as overt diabetes mellitus and hypertriglyceridemia), suggesting beneficial effects in specific patients groups.

Finally, the ever-expanding genomic information has led to an explosion of novel approaches to unravel the pathophysiology of dyslipidemia, varying from the use of classical approaches for monogenetic disorders, to the growing amount of genome wide association studies that have increased our understanding of the complex processes involved in (postprandial) lipid metabolism. In chapter 6 we review the therapeutic use of genomic sequencing in monogenetic and polygenetic hypertriglyceridemia.

Part II: Acquired and inborn errors of heparansulfates in hyperglycaemia and hypertriglyceridemia

As mentioned previously, experimental data indicates that hepatic HSPGs are involved in the clearance of atherogenic remnant lipoproteins by the liver. In chapter 7 we investigate the postprandial lipid handling by heparan sulfates following both a monogenetic (subjects with hereditary multiple exostosis or HME) and SNP based approach in patients with familial hypercholesterolemia (FH). HME subjects are characterized by loss-of-function mutations in exostin (EXT), involved in HS chain elongation, and therefore display inborn defects in HSPGs. FH subjects are already characterized by a defect in another hepatic remnant receptor namely LDL receptor, and therefore their lipid clearance might be more HSPG-dependent. We observed only a trend towards small changes in postprandial lipid handling in carriers of loss-of-function EXT mutations (HME subjects), indicating a negligible contribution of HS chain length to lipid metabolism. Using a HSPG SNP based approach in heterozygous FH subjects we did find small differences in postprandial retinyl palmitate. These
findings suggest that, although modest compared to previous murine findings, heparan sulfates may indeed contribute in human lipid metabolism.

Several genome wide association studies have identified exostosin 2 (EXT2) as a novel risk factor for the development of type 2 diabetes mellitus. As EXT genes (involved in the chain elongation step of heparan sulfate biosynthesis) are intricately involved in organ development, we hypothesized that mutations in these genes might affect pancreatic volume and subsequent insulin secretion/beta cell reserve capacity. In chapter 8 we used a translational approach showing an effect of EXT mutations on pancreatic volume, glucose stimulated insulin secretion and beta-cell function (by hyperglycaemic clamp) in mice and humans with heterozygous EXT mutations underlying HME syndrome. Although no differences in oral glucose tolerance and insulin sensitivity were found in both mice and men with EXT mutations when compared to controls, we did find a significantly reduced glucose stimulated first phase insulin secretion as well as a decreased absolute insulin secretion capacity (upon arginine stimulation) in HME subjects. In line with these findings, abdominal Magnetic Resonance Imaging revealed a significantly smaller pancreas volume in HME subjects compared to controls providing the first evidence that heparan sulfates are indeed important for normal pancreas development and subsequent beta-cell function in humans.

Postprandial hepatic clearance of triglyceride-rich lipoprotein (TRL) remnants is known to be impaired in T2DM and thereby attributing to its metabolic dyslipidemic phenotype. It was previously shown that glucosamine-6-O-endosulfatase-2 (Sulf2), a HSPG desulfase enzyme, is highly expressed in livers of diabetic mice. Increased levels of Sulf2 result in decreased sulfation of HSPG, a diminished negative charge and subsequently a decreased binding of TRLs in cultured hepatocytes. In chapter 9 we show that inhibition of hepatic Sulf2, by a targeted allele-specific antisense approach, restores the TRL-binding capacity of primary hepatocytes, normalizes non-fasting plasma TG levels and reduced postprandial hypertriglyceridemia in db/db mice. These findings provide an in vivo proof-of-concept for Sulf2 inhibition as potential target to improve metabolic dyslipidemia.

In chapter 10 we aimed to translate previous findings implicating a role of SULF2 in diabetic dyslipidemia to humans. We investigated whether genetic variation in SULF2 associates with plasma triglyceride levels in T2DM subjects. This study shows that carriership of the SULF2 rs2281279 minor G allele predisposes to lower postprandial TRL levels in dyslipidemic T2DM patients, underscoring the relevance of our Sulf2 antisense findings in mice. Chapter 11 further illuminates the impact of the SULF2 rs2281279 on metabolic responses in non-diabetic individuals. Although we were not able to find any effect on postprandial plasma TG levels in these non-diabetic subjects, we did find that the SULF2 rs2281279 genotype was associated with a significant decrease in glycaemic excursions following an OGGT in a stepwise manner, which was accompanied by improved insulin sensitivity in these genotypes. This intriguing finding suggests a potential role of SULF2 in peripheral or hepatic insulin sensitivity in humans and further studies are warranted to study this potential mechanism.
SUMMARY

PERSPECTIVES

As stated previously, the prevalence of the cardiometabolic syndrome and its sequelae is expanding worldwide. Hence, there is an immense, unmet medical need for approaches to unravel its complex pathophysiology and the identification of novel therapeutic targets to reduce morbidity and mortality rates.

In this thesis, we suggested a gene score based on the presence of TG-raising polymorphisms which predicts plasma TG levels as well as future coronary artery diseased risk. The use of a mini-TG gene chip might thus bolster future individualized patient therapeutic approaches aimed at improved cardiovascular risk prediction and/or therapeutic efficacy in guiding choice of TG lowering therapy and therapeutic rigor of treating hypertriglyceridemia. In the past few years, the efficacy of various drugs for treatment of hypertriglyceridemia including fibrates combination with statins was evaluated in large prospective randomized clinical trials showing negative outcomes. Unfortunately, also novel therapeutic interventions including nicotinic acid, ezetimibe and CETP inhibition have failed in consistently reducing cardiovascular end-points on top of statin therapy. Thus, despite extensive clinical research efforts, intensive statin therapy remains the only evidence based pharmacotherapeutic strategy to reduce cardiovascular risk in patients with metabolic dyslipidemia in clinical practice. Fortunately, novel approaches keep entering the cardiometabolic arena including antisense therapy (i.e. antisense APOC3) and monoclonal antibodies (i.e. PCSK9 antibody). Large, long-term randomised trials with predefined clinical cardiovascular endpoints are now eagerly awaited to substantiate the promise of these new drugs to establish safety, tolerability, acceptability, and cost-effectiveness in dyslipidemic patients. One of these promising novel potential candidates in our battle against the cardiometabolic syndrome is the thyroid hormone mimetics. Thyroid analogues increase basal energy expenditure and oxygen consumption leading to a reduction in body weight with concomitant favourable improvements in lipid and carbohydrate metabolism. Indeed, 3 months treatment with the thyroid hormone analogue Eprotirome was associated with decreases in levels of atherogenic lipoproteins in patients receiving treatment with statins. However, reports on toxicity warrant caution and call for further research to identify compounds that are able to increase basal metabolism without toxicity.

This thesis opens a new research area focussing on the role of HSPGs in human glucose and lipid metabolism. For example, soon after the report on a role of hepatic over-expression of Sulf2 in diabetic dyslipidemia we were able to report that restoration of HSPG derangements in diabetic mice corrected their (postprandial) dyslipidemic phenotype. Although the effect of HSPG genes, including Sulf2, on postprandial TG levels seems rather modest in human lipid metabolism, it is still tempting to speculate about targeted (antisense based) therapeutic intervention aimed at normalizing hepatic Sulf2 in human diabetic subjects. This based on the selectivity of antisense aimed at hepatic targets on the one hand, whereas redundancy of HSPG genes on the other hand
CHAPTER 12

will prevent a complete loss of HSPG function. As minor genetic disturbances in HSPGs, results in human metabolic derangements varying from glucose-insulin metabolism, beta-cell mass and hepatic TG clearance, it is tempting to speculate on the lipid- and glucose lowering effects of HS mimetics or modulation of HSPG core proteins in type 2 diabetes mellitus subjects with hypertriglyceridemia. With so many unknown, unidentified and mystifying HSPG targets in human biology, the research field linking (hepatic) glycobiology and cardiometabolic homeostasis could be a fruitful area for future attempts targeting HSPG biosynthesis in a safe and effective manner in order to reduce cardiometabolic sequelae.
REFERENCE LIST


