GENERAL INTRODUCTION AND OUTLINE OF THE THESIS
Chapter 1

Introduction

Cardiovascular disease and the cardiometabolic syndrome

Cardiovascular disease (CVD) remains a leading cause of death worldwide. With an estimated number of 17.5 million per year, it represents 30% of all global deaths. 1 Due to the emerging prevalence of obesity and type 2 diabetes mellitus (T2DM), in 2030 almost 25 million people will die from any form of CVD. The cardiometabolic syndrome is a cluster of medical disorders often seen in subjects with type 2 diabetes mellitus including obesity, impaired glucose tolerance, insulin resistance and metabolic dyslipidemia (elevated plasma triglycerides (TGs) and decreased plasma high-density lipoprotein cholesterol (HDL-C) levels). Treatment of the cardiometabolic syndrome is currently aimed at treating its individual components, but still a considerable residual risk remains in these subjects. Although hampered by its complex pathophysiology, development of new therapeutic strategies targeting the cardiometabolic syndrome is urgently needed. The focus of this thesis therefore lies on the origin and treatment modalities of triglycerides and glucose metabolism that accompany the cardiometabolic syndrome.

Hypertriglyceridemia

TGs are required for energy storage. They are derived from both exogenous (dietary) and endogenous (liver) sources. 2 TGs are transported into chylomicrons and very low-density lipoprotein (VLDL) particles and hydrolyzed in muscle, heart and adipose tissue by lipoprotein lipase to release free fatty acids for uptake. Triglyceride-depleted remnant particles are then transported to the liver where they are taken up and cleared. Because of the smaller particle size, these remnant particles are able to penetrate the vessel wall, where they accelerate atherogenesis most likely via an inflammatory reaction. 3 As plasma TGs reflects daily dietary fat consumption, they are highly variable. Hypertriglyceridemia, defined as plasma triglycerides levels ≥1.7 mmol/L (≥150 mg/dL), often arises from overconsumption of lipid-rich diets, obesity, physical inactivity, insulin resistance and related conditions resulting in increased production or decreased uptake of TGs. 4 Despite its coherence with insulin resistance induced dysglycemia, low HDL-C plasma levels and increased small-dense low-density lipoprotein (LDL) levels; elevated (both fasting and non-fasting) TG levels are an independent risk factor for CVD. 5, 6 However, nowadays therapeutic interventions to lower plasma TGs including ezetimibe and fibrates have failed to consistently reduce cardiovascular risk in prospective randomized studies. 7, 8 Thus, novel partakers in human lipid metabolism need to be identified in order to provide novel therapeutic targets.

Heparan sulfate proteoglycans

Diabetes-associated, metabolic dyslipidemia, characterized by increased triglyceride levels with concomitant small dense LDL-cholesterol, is a major contributor to diabetic macrovascular complications. 4 Current strategies comprising statin and fibrate therapy prevent only 25% of all cardiovascular events, highlighting the need for additional lipid-modulating strategies in T2DM. 4 Historically, hyperglycaemia-associated changes in heparan sulfate (HS)-synthesis have been put
forward as causal factor for cardiovascular complications in T2DM. Heparansulfate proteoglycans (HSPG) play a role in many biological processes including fine-tuning most of the (patho) physiological processes such as organ development, inflammatory pathways and lipid metabolism. HSPGs comprise a proteoglycan/glycoprotein backbone (syndecans and glypicans) on which sulfated polysaccharides (heparan sulfates) are attached as side-chains that are able to “catch” and then internalize plasma proteins like chylomicron/VLDL remnant particles. Synthesis of HSPGs results from the action of multiple enzymes, that built HS chains on proteoglycans, involved in chain initiation (xylosyl transferase or XT), elongation (exostosin or EXT) or sulfation (N-deacetylase/N-sulfotransferase or NDST and O-sulfotransferase or OST), see Figure 1, 12.

Figure 1 - Effects of different heparansulfate synthesis genes on chain architecture. (adapted from Forsberg E, J Clin Invest. 2001).
With respect to dyslipidemia, pioneering work showed that these negatively-charged HS facilitate binding and uptake of triglyceride-rich lipoprotein remnants (TRL) in hepatocytes. Following lipoprotein lipase-mediated hydrolysis of TRLs, the ensuing chylomicron- and VLDL-remnant particles are cleared by the liver via three receptors: the LDL receptor (LDLR), LDLR-related protein 1 (LRP1) and HSPG, see Figure 2 and references 16, 17. Intrigued by the observation that reduction in the degree of hepatic HS-sulfation was associated with increased TG-levels 18, Esko unambiguously demonstrated that the proteoglycan syndecan-1 (SDC1) is of pivotal importance for hepatic remnant clearance in conditional knockout mice on a normal C57BL6 background. 19, 20

Recent animal studies have implicated that HSPG degradation in T2DM might attribute to its characteristic metabolic dyslipidemia. 21 Hepatic glucosamine-6-O-endosulfatase-2 (Sulf2), a HSPG degrading enzyme that selectively removes 6-O-sulfates from HS chains, was strongly over-expressed in livers of diabetic mice which coincided with a diminished TRL binding to primary hepatocytes and concomitant elevations in plasma TG. In addition, genome wide association studies have linked
HSPG genes to the development of T2DM. Human studies exploring the role of HSPG in glucose and lipid homeostasis are lacking.

**OUTLINE OF THE THESIS**

This thesis explores the first steps in linking HSPG homeostasis to cardiometabolic diseases. Although it takes many steps for medical findings to develop from bench to bedside, we aim to address a number of steps to achieve translation insights: we studied in-vitro and animal models and translated these findings into specific patient groups as well as performing observational studies in large cohorts. Our results in cell, mice and men form part of the initial steps towards possible targets for therapy.

This thesis consists of two parts. The first part focuses on the causes of hypertriglyceridemia and novel targets in the cardiometabolic syndrome. In chapter 2 we review novel aspects in TG metabolism and the pathophysiology of hypertriglyceridemia. In chapter 3 we explore the value of a gene risk score for hypertriglyceridemia to improve cardiovascular risk prediction. To achieve this, we evaluated the ability of single nucleotide polymorphisms (SNPs) in TG modulating genes to predict plasma triglyceride levels as well as first cardiovascular event in a prospective case-control (EPIC) study. Chapter 4 describes the additional benefit of increasing statin dose in patients with elevated TGs on coronary artery disease. In this chapter we performed a post hoc analysis in a large randomized clinical statin trial in order to evaluate the additional benefit of atorvastatin 80 mg versus 10 mg in high risk patients stratified by HDL-C and TG levels. Another potential novel candidate, with promising results from animal studies 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 therapeutics to weight-lowering strategies. In chapter 5 we performed a randomized, placebo-controlled, double-blind trial to investigate the effect of TRC150094, a thyroid hormone mimetic, on insulin sensitivity, liver fat content and lipid profile, as well as on safety markers in obese male subjects with an increased cardiometabolic risk. Chapter 6 describes the future of gene sequencing in therapeutic strategies aimed at lowering plasma triglyceride levels.

The second part of this thesis explores the role of HSPG in the development of hypertriglyceridemia and hyperglycaemia. Chapter 7 describes a study investigating the postprandial lipid handling by heparan sulfates in subjects with hereditary multiple exostosis (HME) and familial hypercholesterolaemia (FH). Given that subjects with HME suffer from an inborn error in HSPG synthesis (loss-of-function mutations in EXT) they form a unique model to evaluate the role of HSPGs in human lipid metabolism. In chapter 8 we used a translational approach to study the effect of EXT mutations on pancreas volume, insulin secretion capacity (beta cell reserve) and subsequent glucose handling in both mice and humans (using heterozygous EXT mice and HME subjects with...
heterozygous EXT mutations). In chapter 9 we evaluate the effect of hepatic Sulf2 inhibition by antisense strategy on diabetic dyslipidemia in diabetic mice as previous data indicated that hepatic Sulf2 expression is strongly induced and linked to elevated plasma TG levels in these diabetic animals. In chapter 10 we aim to translate earlier in-vitro and animal findings linking acquired errors in Sulf2 regulation in diabetes and dyslipidemia to humans. In this chapter, we study the human hepatic SULF2 expression as well as the effect of genetic variation in SULF2 on fasting and postprandial lipid levels in subjects with T2DM and diabetic dyslipidemia. Furthermore, chapter 11 describes the effect of a predetermined common genetic variation in SULF2 on metabolic responses following oral glucose tolerance tests (OGTTs) and a meal tolerance test in a population-based non diabetic cohort. Finally, in chapter 12 we provide a summary and conclusion of this thesis.
REFERENCE LIST


