Dyslipidemia, sense, antisense or nonsense?
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Chapter 1

Introduction and outline of this thesis
Introduction

**LDL-c lowering**
Cardiovascular disease (CVD) is still the cause of most morbidity and mortality worldwide. Low-density lipoprotein cholesterol (LDL-c) plays a pivotal role in atherogenesis. In line numerous epidemiologic studies have unambiguously demonstrated a positive linear relationship between LDL-c and the risk of CVD. As a consequence, LDL-c-lowering strategies have become the cornerstone of CVD prevention. At present, statins or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are the most effective LDL-c-lowering drugs. In a series of primary and secondary prevention trials, it was consistently shown that reducing LDL-c with statins resulted in profound decreases in cardiovascular event rates. After recent trials demonstrating a larger reduction in CVD risk by even more intensive lipid-lowering interventions\(^1\)\(^-\)\(^2\), the Third National Cholesterol Education Program advised more stringent LDL-c-target levels for the treatment of patients at risk of developing CVD\(^3\). However, the incremental LDL-c-lowering capacity when up titrating statins is limited and, moreover, increasing the dose leads to a clear increase in side effects\(^4\). In addition, more patients seem to experience side effects even at lower statin dose, precluding the long-term use of even moderately dosed statins. Therefore, alternative therapeutic approaches capable of effectively lowering LDL-c, as monotherapy or in combination with statins, are required. Apolipoprotein B (apoB) synthesis inhibition with mipomersen, is such a novel therapeutic approach\(^5\).

**Metabolic syndrome**
Although statins significantly reduce CVD risk, high risk patients who are treated with statins to desirable LDL-c goals continue to have residual CVD risk. This may especially be the case in patients with the metabolic syndrome\(^6\)\(^-\)\(^7\). The metabolic syndrome is a cluster of medical disorders including central obesity, metabolic dyslipidemia (elevated plasma triglycerides (TGs) and decreased plasma high-density lipoprotein cholesterol (HDL-c) levels), elevated blood pressure, and an increased fasting plasma glucose\(^3\). The metabolic syndrome is associated with increased risk for CVD and type 2 diabetes mellitus and is an increasing concern for public health worldwide. Treatment of the metabolic syndrome is directed towards its individual components whereas weight loss is currently the only overall effective treatment option. Development of novel therapeutic strategies targeting the metabolic syndrome is hampered by its complex pathophysiology, which is only partly elucidated.
Whereas visceral adiposity is an established risk factor for the metabolic syndrome, more recently non-alcoholic fatty liver disease (NAFLD) has emerged as an additional independent risk factor\textsuperscript{8,9}. NAFLD is the result of TG accumulation in the liver. Evidence from stable isotope studies indicates that serum free fatty acids (FFAs) resulting from lipolysis of visceral adipose tissue are the main source of hepatic TG in NAFLD. In addition, de novo hepatic lipogenesis is significantly increased whereas hepatic fatty acid oxidation is decreased in subjects with NAFLD, all contributing to the accumulation of intrahepatic fat\textsuperscript{10}.

All the above mentioned processes are direct consequences of central obesity and peripheral insulin resistance. In turn, fat accumulation in the liver has also been suggested to be causally involved in a variety of metabolic derangements. For example, fatty liver increases very-low-density lipoprotein (VLDL)-apolipoprotein B secretion resulting in hypertriglyceridemia (HTG), and decreases insulin clearance causing hyperinsulinemia\textsuperscript{11}. Furthermore NAFLD is believed to be causally related to hepatic insulin resistance through interference with the insulin signaling cascade in liver tissue\textsuperscript{12}. But if hepatic fat accumulation per se causes insulin resistance remains subject for debate.

Paralleling the increased prevalence of the metabolic syndrome, NAFLD has become the most common cause of chronic liver disease worldwide. Whereas steatosis observed in NAFLD usually remains stable, 30-40\% of patients with simple steatosis progress to non alcoholic steatohepatitis (NASH) whereas 74\% of NASH patients progress to more severe liver injury including fibrosis and cirrhosis\textsuperscript{13}. Increased adiposity, insulin resistance and oxidative stress are believed to contribute to the progression of NASH to fibrosis but its mechanisms are poorly understood\textsuperscript{14}.

**Hypertriglyceridemia**

TGs are lipid fractions used for energy storage. TGs are both synthesized by the liver as well as derived from external sources through uptake from the intestine. TGs are transported into VLDL and chylomicrons and are hydrolyzed in muscle, heart and adipose tissue by lipoprotein lipase (LPL) into free fatty acids for uptake. Since plasma TGs reflect the daily consumption of dietary fat they are highly variable. In addition life style factors such as alcohol intake but also the presence of insulin resistance are important determinants of plasma TG concentrations.

The metabolic syndrome is characterized by an atherogenic lipid profile of high serum TG levels together with low serum HDL-c and the presence of small, dense LDL-c particles. Studies have reported that the relationship between small, dense LDL particles and CVD risk largely depends on the presence of the metabolic dyslipidemia\textsuperscript{15;16}. In fact, high TGs and low HDL-c now constitute two of the five risk factors of the metabolic syndrome\textsuperscript{3}. Both elevated TGs as well as low
HDL-c levels have been associated with coronary heart disease (CHD)\(^{17,18}\). In line with the guideline committees, they have added non-HDL-c as a secondary goal for those with TG levels above 200 mg/dL (1.5 mmol/L)\(^3\). However, the evidence for TGs as an independent risk factor for CVD remains somewhat controversial since data obtained from large epidemiological studies, have not always been equivocal\(^{19,20}\). These inconclusive findings have been attributed to either the high variability in TG levels during the day, the increased presence of additional CVD risk factors, or the strong correlation between plasma TG and HDL-c levels. With respect to the latter, randomized placebo controlled intervention trials will not bring a solution since current available interventions such as fibrates, statins and nicotinic acid do not only affect TG but also LDL-c and HDL-c levels.

Whereas metabolic dyslipidemia is mainly associated with mildly elevated plasma TG levels, disorders such as LPL deficiency and familial partial lipodystrophy (FPLD) are characterized by extreme HTG (TG>10mmol/l) and associated pancreatitis. Whereas LPL is the key enzyme in TG catabolism, absence of LPL activity invariably leads to severe HTG. Most of these LPL deficient patients carry missense mutations in \(LPL\) which render the enzyme catalytically inactive\(^21\). But besides LPL deficiency several other rare loss of function mutations have been described in patients with severe HTG including mutations in the genes encoding apolipoprotein C2 (\(APOC2\)), Apolipoprotein AV (\(APOA5\)), glycosyl-phosphatidylinositol-anchored HDL binding protein 1 (\(GPIHBP1\)) and lipase maturation factor (\(LMF1\))\(^{22-25}\). Recent genome wide association studies (GWAS) replicated some of these known loci but also indentified novel promising loci associated with plasma TG levels for example common SNP’s in the genes encoding tribbles homolog 1 (\(TRIB1\)), glucokinase regulator (\(GCKR\)) and angiopoietin-like 4 (\(ANGPTL4\)). However, the majority of the phenotypic variation underlying HTG remains unidentified. Identification of novel genes attributing to HTG may improve our insight in the molecular basis of HTG and should enable diagnosis, prognosis, response to therapy, development of novel therapeutic strategies and might improve risk prediction. For example, genetic risk scores may provide an alternative approach to assess the relationship between circulating TG levels and risk for CVD.

Parts of this introduction have been published before in *Current Cardiology Reports* 2008;10:512–520.
Outline of this thesis

This thesis consists of three parts. Although the subjects of each part may seem divergent, they are in fact interrelated and all focus on different aspects of dyslipidemia.

**Part I** focuses on apoB synthesis inhibition, a novel treatment strategy for lowering LDL-c. Mipomersen is a second generation anti-sense targeting apoB. ApoB is an essential component of LDL-c and all other atherogenic lipoprotein particles and as a consequence apoB synthesis inhibition may be an attractive approach to lower LDL-c. Mipomersen is designed to lower LDL-c in patients at high risk for CVD who are either not on target despite the use of statins or who are intolerant to statins. The drug has shown to produce potent reductions in LDL-c and all other atherogenic lipids. Whereas mipomersen is generally well tolerated, safety concerns have focused on potential hepatic fat accumulation. Our research contains Phase II randomized controlled trials to study the safety and efficacy of mipomersen in different patient groups.

**Part II** of this thesis consists of 2 chapters describing research on rare dyslipidemic disorders and the metabolic syndrome. The first chapter contains the results of a hyperinsulinemic euglycemic clamp study in patients with familial hypobetalipoproteinemia (FHBL). Patients with FHBL have extremely low levels of apoB and LDL-c. They are in fact the “natural variant” of apoB synthesis inhibition. The majority of patients with FHBL are characterized by severe hepatic steatosis. But, unlike NAFLD, patients with FHBL develop hepatic steatosis mainly independent from metabolic derangements. FHBL patients therefore provide the opportunity to investigate the relation between intrahepatic triglyceride (IHTG)-content and insulin sensitivity in humans. In this chapter we compared insulin sensitivity in FHBL patients to healthy matched controls.

The second chapter of this part focuses on FPLD, a rare metabolic disorder associated with dyslipidemia. Patients with FPLD have an abnormal distribution of subcutaneous fat resulting in severe metabolic derangements. In line with the extreme phenotype approach, studying patients with FPLD may help to improve our understanding of the more common metabolic syndrome. FPLD, however, may not be readily recognized due to similarities with the metabolic syndrome. We show how to identify cases of FPLD amongst non-obese patients with type 2 diabetes mellitus and marked insulin resistance.

**Part III** focuses on TGs. The first chapter of part III deals with the association between metabolic dyslipidemia and CHD. We evaluate the risk of CHD associated
with metabolic dyslipidemia, defined as elevated plasma TG and low HDL-c levels, in men and women enrolled in the EPIC-Norfolk prospective study. The other chapters of part III contain research on the genetic background of HTG. In chapter 10 we looked for mutations in candidate genes in patients with severe HTG. We also performed a non-biased GWAS in patients with HTG to identify common variants associated with high TG levels. These results are described in chapter 11.
Reference List


