Summary

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Cardiovascular diseases (CVD) are the leading cause of death in the Western world and despite greatly improved treatment options during the last decades, the number of patients suffering from CVD is still increasing. The risk for CVD is determined by a variety of factors, including genetic variation, gender, age, lifestyle, blood pressure and plasma lipid levels. Myocardial infarction (MI) and stroke caused by atherosclerosis are the most prevalent cardiovascular disorders. Atherosclerosis is a complex disease mainly caused by a disturbed lipid metabolism and inflammation. The studies in this thesis address specific aspects of inflammation and dietary lipids in the development of CVD. These two components are of particular interest as each, both individually and collectively, can lead to the onset or maintenance of pathological cardiac conditions.

An increased availability of high-fat diet (HFD) derived fatty acids (FA) may lead to excess FA uptake in non-adipose organs like the heart, as adipose tissue has a limited capacity to expand. The ectopic storage of FA in the form of TG is probably inert, but accumulation of toxic FA intermediates in the heart may affect cardiac function. In **Chapter 2** we investigated to what extent HFD feeding leads to alterations in left ventricular function and whether this depends on gender and (or) duration of HFD feeding. For this purpose we used wild-type (WT) male and female C57Bl/6J mice and subjected them to a low fat diet (LFD) or HFD for an intermediate (6 weeks) or long term (12 weeks) period. We demonstrated that HFD gradually increased body weight and plasma lipid levels in mice independent of gender. Furthermore, cardiac function assessed by combined echocardiography and invasive pressure-volume loop (PV-loop) measurements, was only affected in male mice, but not in female mice after long term HFD feeding. This was indicated by an increased end-systolic volume and end-systolic pressure and decreased ejection fraction, all symptoms of a reduced systolic function. We concluded from these data that male mice are the preferred model to investigate effects of ischemia and drug treatment on cardiac function in a setting of diet induced obesity.

Obesity is accompanied by low-grade systemic inflammation that is associated with increased risk of CVD including MI. Furthermore, inflammation in the heart, by for example local macrophage accumulation, might impair cardiac function. We therefore investigated in **Chapter 3** if HFD feeding results in aggravated cardiac dysfunction after an MI, due to an increased inflammatory response as compared to LFD feeding. We subjected WT male mice to a long term LFD and HFD and induced MI by permanent ligation of the left anterior descending coronary artery or performed sham surgery. Two weeks hereafter, cardiac function was determined by echocardiography and PV-loop measurements. Comparable with previous findings, HFD feeding increased body weight and induced dyslipidemia in mice. In addition, the sham surgery group showed that HFD feeding *per se* induced mild cardiac dysfunction. No differences in mortality rate nor in infarct sizes were observed between LFD and HFD mice with MI. Additionally, HFD did not further aggravate cardiac dysfunction after MI, despite a more prominent macrophage infiltration in the infarcted myocardial area in HFD MI mice compared to LFD MI mice. We concluded that long term HFD did not aggravate cardiac dysfunction two weeks after MI induction.

ATP-binding cassette transporter A1 (ABCA1), a transporter involved in cellular cholesterol transport, exerts anti-atherogenic functions in the pathogenesis of atherosclerosis. Since the actual role of ABCA1 during MI, is not established, we explored the role of ABCA1 after MI induction in **Chapter 4**. To this end, we induced an MI in ABCA1 knockout (KO) mice, WT controls and WT mice transplanted with ABCA1 KO or WT bone marrow. Surprisingly, compared to their respective WT controls, reduced infarct sizes were found in ABCA1 KO mice as well as WT mice transplanted with ABCA1 KO bone marrow. No effect of ABCA1 deficiency on infarct size was found in isolated hearts, as investigated in an *ex vivo* Langendorff perfusion system. The smaller infarct size *in vivo* in ABCA1 KO mice was thus likely due to a secondary effect of ABCA1 deficiency on myocyte function. Interestingly, after *in vivo* MI, ABCA1 KO mice compared to WT controls, showed higher levels of circulating leukocytes. After bone marrow transplantation no differences in leukocyte numbers were observed. However, white blood cell counts were increased compared to the first experiment. We concluded that despite the fact that ABCA1 has a protective role in atherosclerosis, it has adverse effects on cardiac function after MI. In addition, the observed detrimental effects are possibly caused by a reduced activation status of immune cells resulting in less efficient repair after MI. As a consequence, strategies aiming at up-regulation of ABCA1 function should be pursued with care in the light of potential adverse effects on cardiac remodeling post MI.

The innate immune system of the heart becomes activated after MI injury. Toll-like receptor 4 (TLR4), a receptor of the innate immune system, is suggested to negatively affect cardiac function after MI. RP105 is a TLR4 homolog which lacks the intracellular signalling domain that competitively inhibits TLR4-signalling. In **Chapter 5,** we hypothesized that deficiency of RP105, via an amplified signalling of TLR4, aggravates cardiac dysfunction after MI. First we proved that an increased inflammatory response was present in RP105 KO mice after TLR4 stimulation by LPS as compared to WT male C57Bl/6N mice. Subsequently, we found that baseline heart function, assessed by PV-loop measurements, revealed no differences between RP105 KO and WT mice. In addition, MI was induced and 15 days post MI, infarct size measurements revealed no differences between groups. However, PV-loop measurements showed that RP105 deficiency promoted cardiac dilatation after MI Furthermore, histological analysis of the myocardium and the infarcted area showed that in the area adjacent to the infarct zone, expression of RP105, TLR4 and the endogenous TLR4 ligand fibronectin-EDA and macrophage count were increased compared to healthy myocardium. However, no differences between RP105 and WT were found. Taken together, we showed that deficiency of the endogenous TLR4 inhibitor RP105 led to an enhanced inflammatory status and more pronounced cardiac dilatation after MI induction. Our findings underscored the role of the TLR4 pathway in post MI remodelling and modulating RP105 may be an interesting new therapeutic strategy.

TLR2 and TLR4 are hypothesized to be activated by ligands associated with HFD exposure such as (modified) FAs. Although TLR2 and TLR4 are involved in inflammation in cardiac disease, a direct relationship between HFD, TLR activation and cardiac dysfunction has not been reported. In **Chapter 6** we studied the role of TLR2 and 4 in the impairment of cardiac function after exposure to HFD. TLR2 KO, TLR4 KO mice and

their corresponding WT littermates received a long term HFD. No differences in body weight or plasma lipid levels were observed between the genotypes after HFD feeding. In TLR2 KO mice PV-loop measurements showed an increased end-diastolic volume, whereas other parameters of cardiac function were not different from WT littermates. In TLR4 KO mice trends were observed of a decreased pressure half time and an increased end-systolic elastance. These findings showed that deficiency for TLR2 or TLR4 only mildly impaired cardiac function in mice on HFD. This suggested that TLR2 and TLR4,

The primary goal of lipid-modifying therapy in patients with atherosclerotic disease is the lowering of LDL-cholesterol. Statins are currently the standard treatment for CVD, however a substantial residual risk for adverse cardiovascular outcomes remains. Treatment of low HDL-cholesterol is currently considered a secondary lipid target in the reduction of cardiovascular risk, since low HDL- cholesterol is an independent risk factor for CVD. Niacin is the most potent HDL-cholesterol raising drug clinically used and is considered as a novel therapy for reducing atherosclerosis. Despite evidence for an atheroprotective effect of niacin from previous small clinical studies, large outcome trials did not reveal additional beneficial effects of niacin on top of statin treatment. In **Chapter 7** we aimed to elucidate this apparent discrepancy by investigating the effects of niacin without and with simvastatin on atherosclerosis development and determine the underlying mechanisms in APOE*3Leiden.CETP mice. To this end, mice were fed a cholesterol-rich diet without or with niacin, simvastatin or their combination. Niacin treatment and the combination reduced total cholesterol and triglycerides, whereas simvastatin reduced total cholesterol. The combination treatment revealed a greater reduction of total cholesterol compared to simvastatin. Niacin decreased total cholesterol and triglycerides primarily by increasing VLDL clearance. Niacin increased HDL-cholesterol and mildly increased reverse cholesterol transport. All treatments reduced monocyte adhesion to the endothelium, atherosclerotic lesion area and severity. Compared to simvastatin, the combination increased the atherosclerotic plaque stability. Niacin and the combination reduced T-cells in the aortic root. Lesion area was strongly predicted by non-HDL-cholesterol and to a much lesser extent by HDL-cholesterol. In conclusion, niacin decreased atherosclerosis development mainly by reducing nonHDL-cholesterol with minor HDL-cholesterol-raising and additional anti-inflammatory effects. The additive effect of niacin on top of simvastatin was mostly dependent on its nonHDL-cholesterol-lowering capacities. These data suggest that clinical beneficial effects of niacin are largely dependent on its ability to lower LDL-cholesterol on top of concomitant lipid-lowering therapy.

Taken together, the studies in this thesis described the effects of both lipid metabolism and inflammation on several aspects of cardiac pathophysiology. We showed that HFD aggravated cardiac function, although this effect was likely not mediated by TLR2 or TLR4. Also, HFD has no detrimental effects on cardiac function post-MI. Deficiency of RP105 improved cardiac function after MI induction, whereas ABCA1 had adverse effects on cardiac function post MI. Last, we found that the anti-atherogenic properties of niacin were mostly dependent on its non-HDL-cholesterol-lowering capacities on top of statin treatment. Altogether, these studies offer novel strategies and insights for the prevention or treatment of CVD.