Inflammatory mediators in diet-induced cardiac dysfunction

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CHAPTER 8

General discussion

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Despite advances in diagnosis and therapy, cardiovascular disease (CVD) remains a major cause of morbidity and mortality in Western society and is an increasing problem especially in low-to-middle income countries. The most prevalent cardiovascular disorders are myocardial infarction (MI) and stroke, both are predominantly caused by atherosclerosis. Atherosclerosis is a complex disease associated with a variety of genetic and environmental factors. Main pathogenic phenomena underlying atherosclerosis are disturbed lipid metabolism and inflammation. The studies described in this thesis have explored the effects of specific aspects of these two components on atherosclerosis and MI. In this chapter, the main conclusions and implications of these findings are discussed.

Gender, nutrition and cardiac function

In the first part of this thesis, the effect of high-fat diet feeding (HFD) on cardiac function with and without an MI was studied. We first set out to investigate if feeding a diet rich in saturated fatty acids (SFA) resulted in cardiac dysfunction. **Chapter 2** describes that long term HFD feeding led to alterations in cardiac function solely in male mice, although the observed increase in body weight and plasma lipids due to HFD feeding was independent of gender. We therefore concluded that male mice are the preferred model to investigate effects of ischemia and drug treatments on cardiac function in a setting of HFD-induced obesity.

The observation that female mice seem protected from HFD-induced cardiac dysfunction is probably related to beneficial effects of sex hormones and in particular estrogen. In animal studies beneficial effects of estrogen treatment are reported in models of cardiac hypertrophy[1, 2], ischemia/reperfusion[3, 4] and atherosclerosis[5]. These effects are likely mediated by effects of estrogen on cardiomyocytes and endothelial cells, but effects on other cell types including inflammatory cells and stem cells may be involved as well. In addition, estrogen exerts a part of its protective effect via beneficial effects on serum lipoproteins, e.g. an increased high-density lipoprotein (HDL)-cholesterol and a decreased low-density lipoprotiein (LDL)-cholesterol, accounting for approximately one third of the observed clinical benefits.[6] Thus, in animal studies, treatment with estrogen results in predominantly favorable effects.

In premenopausal women, the incidence of atherosclerotic diseases is relatively low and a sharp increase is observed with the occurrence of the menopause.[7] Results from these observations and established results of positive effects of estrogen on the cardiovascular system, including increased vasodilatation and an inhibited response of blood vessels to injury[8], suggested that hormone replacement therapy could have beneficial effects on CVD in postmenopausal women. However, two large prospective studies, the Women's

Health Initiative and the Heart and Estrogen/Progestin Replacement study, found that hormone replacement therapy did not lead to an improvement in cardiovascular outcomes, even though lipid profiles were improved and incidence of type 2 diabetes was reduced.[9-11] A possible explanation for these outcomes could be age-related metabolic changes, partly independent of estrogen or the estrogen receptor, thereby reducing the protective effect or even leading to detrimental rather than cardioprotective effects.[12] In addition, the Women's Health Initiative study was prematurely aborted since an increased risk for invasive breast cancer was observed.[10] Therefore, hormone replacement therapies for prevention of CVD, and possibly other chronic diseases, are currently not recommended.

The results in **Chapter 2** underscore the detrimental effects of a SFA rich HFD on cardiac health. From meta-analysis of randomized controlled trials[13] it is recommended to replace dietary SFA by polyunsaturated fatty acids (PUFA) to reduce coronary heart disease (CHD). Consumption of PUFAs lowers the total cholesterol to HDL-cholesterol ratio and may improve insulin resistance and reduce systemic inflammation. The two best known PUFAs are omega-3 and omega-6 PUFAs which are present in mainly fish, shellfish and seeds, respectively. Although the beneficial effects of omega-6 regarding CHD have been under debate, the American Heart Association recommended recently that a minimum daily consumption of 5-10% of energy from omega-6 PUFAs is sufficient to reduce CHD risk.[14]

Regarding omega-3 PUFAs; national and international guidelines have converged on consistent recommendations for adults to consume at least 2 servings of fish (particularly fatty species) per week. However, clinical trials have not indisputably proven that omega-3 PUFAs have beneficial effects on CVD.[15, 16] Possibly omega-3 does not solely contribute to the observed effects and the interplay between omega-3 and other nutrients in fish is also important.[17] For the vulnerable CVD patient population, especially those on suboptimal pharmaceutical treatment or those who have additional risk factors as a result of comorbidities, intake of omega-3 fatty acids in form of oily fish is advised as they may benefit the CVD patient.[17]

Chapter 3 continues with examining the effect of HFD feeding in presence of an MI. Unexpectedly, no aggravated cardiac dysfunction was observed upon HFD feeding and subsequent induction of MI. However, there was a tendency towards an increase in the amount of macrophages in the infarcted area compared to the low-fat diet (LFD) fed group. After an MI an extensive inflammatory response takes place which is a critical factor in the balance between cardiac repair and adverse ventricular remodeling. Since macrophages have an important contribution in this inflammatory process occurring post-MI, it is possible that in the longer term this secondary effect could aggravate cardiac dysfunction after MI. However, since the follow-up period after MI was only 2 weeks no long-term conclusion could be drawn from this study.

Although it seems relevant to adapt diet and nutrition style after an MI and other symptoms of heart failure, scarce information is provided in the major heart failure management guidelines.[18, 19] The most recent guidelines from the Heart Failure Society of America present a comprehensive recommendation on diet and nutrition.[19] They

report that, besides a sodium restriction for all heart failure patients, dietary instructions are mainly recommended in patients with heart failure in combination with diabetes, dyslipidemia, or severe obesity. Often patients need personalized dietary advice and it has been shown that supervision by dieticians benefits their wellbeing with a decreased sodium intake, fluid retention and increased quality of life score.[20, 21] Unfortunately, it is difficult to provide general recommendations since diet is generally disregarded as factor in observational studies and dietary intake is highly heterogeneous.

Inflammation and cardiac function

The metabolic system and the inflammatory system are evolutionary conserved, as survival of organisms depends on the ability to fight infections and to withstand starvation.[22] Coupling of metabolism and immunity is important since mounting an immune response requires much energy and thus regulation of energy homeostasis is essential. As a result, there is a delicate balance between the two systems and dysfunction of one leads to dysfunction of the other, with malnutrition and immune suppression on one side and obesity and immune activation on the other side.[23] The current chronic caloric overload in the Western world results in complications at the intersection of metabolism and immunity, including obesity-linked inflammatory diseases like diabetes, fatty liver disease and CVD.

The liver, adipose tissue, immune cells and blood cells, which perform key metabolic and inflammatory functions in humans, have evolved from one functional unit, the 'fat body', which is still present in the fruit fly *Drosophila melanogaster*.[22] The fat body coordinates a variety of actions including nutrient sensing, pathogen and survival responses. Although in humans, the different organs acquired specialized functions, they maintained their developmental heritage. Partly overlapping pathways regulate both metabolic and immune functions through shared signaling systems.[22] Consequently, nutrients and especially fatty acids (FAs) act through pathogen-sensing systems like Toll-like receptors (TLRs), giving rise to metabolically or nutritionally induced inflammatory responses.[22, 24] It was discovered that in particular TLR2 and TLR4 dependent pathways are activated by FAs and contribute via this pathway to the low-grade inflammation in several metabolic diseases. However, in **Chapter 6** we demonstrate that HFD-induced cardiac dysfunction, as observed in **Chapter 2**, is not likely to be mediated by TLR2 and/or TLR4. Very mild beneficial changes in cardiac function are observed in TLR-deficient mice, suggesting that TLR2 and TLR4, if anything, play a moderately protective role in HFD-induced cardiac dysfunction. A possible explanation for the absense of cardiac dysfunction might be that in absence of one TLR, another TLR is upregulated as a compensatory effect, resulting in a comparable inflammatory status that does not lead to cardiac dysfunction. Such a compensatory mechanism is not inconceivable in an evolutionary conserved process.

Although we did not observe HFD-induced TLR2 and TLR4-mediated dysfunction in the heart, others reported that TLR4 exerts an important role in myocardial infarction

healing and left ventricular (LV) remodeling.[25] This difference is probably caused by the strong inflammatory activation which is exerted post-MI and the subsequently released 'danger' signals activate the TLR pathway. The importance of the TLR signaling pathway is also emphasized in **Chapter 5** where we show that RP105, a negative regulator of TLR4, is involved in post-infarction cardiac remodeling. Deficiency of the accessory molecule RP105 aggravates systolic and diastolic cardiac function indices after MI, indicating that reduced inhibition (=activation) of TLR4 signaling aggravates cardiac dysfunction.

The influence of the TLR pathway on infections in general and cardiac function specifically opens opportunities as a therapeutic target. Targeting of TLRs is explored for the treatment of e.g. cancer, rheumatoid arthritis, sepsis and cardiac malfunctioning.[26] Different strategies are being considered to pharmaceutically modify TLR2 and TLR4 including receptor agonists and antagonists and inhibition of the signal transduction pathway.[27] Despite the evidence shown by animal studies that TLRs are involved in several pathological cardiovascular processes, this has so far not been translated into clinical applications. The only drugs thus far that have been evaluated for treatment of ischemia/reperfusion injury are the TLR4 antagonist Eritoran and the TLR2 antibody OPN-305. Preclinical results showed that inhibition of TLR2 by OPN-305 preserved cardiac function.[28] TLR4 inhibition by Eritoran, in a similar murine model, led to a reduced infarct size and decreased cytokine production.[29] Although Eritoran was studied in a Phase III clinical trial for sepsis, cardiovascular events were not part of the study end points.[30] However, the results in **Chapter 5** provide new opportunities for therapeutic approaches to reduce cardiac remodeling through RP105 and thereby improve cardiac function after a myocardial event.

High-density lipoprotein, atherosclerosis and cardiac function

An inverse correlation of plasma HDL-cholesterol with CVD risk is found in numerous epidemiological studies [31, 32], resulting in the concept that by increasing HDL-cholesterol levels, the risk for CHD could be reduced. This risk reduction can be achieved via therapeutic elevation of HDL-cholesterol levels, including treatments with niacin and cholesteryl ester transfer protein (CETP) inhibitors. Niacin is one of the most potent HDL-cholesterol-raising compounds clinically available. Relatively small secondary prevention studies have shown positive effects of niacin in combination with statin therapy leading to a reduced progression or even regression of atherosclerosis compared to statin treatment alone.[33-35] These results were supported by animal studies demonstrating anti-atherogenic properties of niacin.[36-38] Despite these initially promising data, two large clinical trials investigating the effects of niacin on top of LDL-lowering treatment revealed no beneficial effect of niacin treatment (HPS2-THRIVE[39] and AIM-HIGH[40]). In **Chapter 7** we aimed to investigate the reason for the failure of these trials and showed that niacin decreases atherosclerosis development mainly by reducing non-HDL-

cholesterol. In addition, the additive effect of niacin on top of simvastatin treatment is mostly dependent on the non-HDL-cholesterol-lowering capacities, thereby suggesting that beneficial effects of niacin for patient treatment may only be observed when niacin lowers LDL-cholesterol on top of related lipid-lowering therapy.

Niacin is not the only HDL-raising drug that did not meet the expectations. Trials with the CETP inhibitors torcetrapib and dalcetrapib were also prematurely terminated due to off-target toxic effects and the absence of effect, respectively.[41, 42] These results indicate that the relation between increased plasma HDL-cholesterol levels and reduction of CVD risk is rather complex. Although increasing plasma HDL-cholesterol levels as such could be relevant, HDL function might be more important in preventing CVD. An increase in plasma HDL-cholesterol levels *per se* does not necessarily translate into a decreased CVD risk when the HDL particle is 'dysfunctional'. However, assessment of HDL quality in clinical situations is rather difficult, whereas quantification of HDL quantity is easily achieved by measuring plasma HDL-cholesterol levels.

HDL exerts many functions such as mediating cholesterol efflux from macrophages as a first step of reverse cholesterol transport (RCT). Although in **Chapter 7** we concluded that RCT is not the driving force behind the anti-atherogenic effects of niacin, in general RCT is thought to be an important contributor to cardioprotection. Other properties of HDL, including the anti-inflammatory, anti-thrombotic and anti-apoptotic effects, may contribute to the anti-atherogenic effects of HDL. It remains difficult to discriminate between these different functions especially since the effects are achieved via a variety of mechanisms.

Recently, it was proposed that the cholesterol efflux capacity by macrophages may be an appropriate tool to measure HDL functionality.[43] Consistent with studies in animals, an inverse association was observed in patients between early steps in the RCT pathway and carotid intima-media thickness and the likelihood of coronary artery disease assessed by angiography. However, these effects were independently of HDL-cholesterol levels, indicating that increasing HDL levels *per se* does not necessarily lead to a reduction in the atherosclerotic burden. Importantly, issues related to the reported clinical results[44, 45], including the measurement of cholesterol efflux transport[46], remain the subject of on-going discussions, therefore requiring further investigations before measuring cholesterol efflux as a marker of HDL functionality can be of clinical value.

Alternative strategies are actively being explored to develop HDL-targeted therapeutics and specifically drugs that increase cholesterol efflux from the arterial wall. One of the mechanisms by which cholesterol content in the atherosclerotic lesion, and indirectly CVD risk, is reduced is via RCT by removal of cholesterol from the arterial macrophage foam cells to the liver for biliary excretion. Active cholesterol removal is mediated by the ATP-binding cassette transporters ABCA1 and ABCG1. Deletion of both transporters in macrophages results in an impaired cholesterol efflux and a rapid development of atherosclerosis.[47, 48] Furthermore, prevention of ABCA1 mRNA degradation by administration of antagomirs of miR-33, resulted in an enhanced expression of ABCA1 in the macrophages present in the lesion and a subsequent improved cholesterol removal.[49] These results support the anti-atherogenic properties of increasing RCT

and suggest a promising tool for the treatment of CVD. Although numerous studies have been conducted on the effects of ABCA1 on atherosclerosis, no studies have been published on the role of ABCA1 after an MI. In **Chapter 4** we found much to our surprise, that despite its protective effects regarding the development of atherosclerosis, ABCA1 exerts detrimental effects on cardiac function after MI. This is possibly caused by a reduced activation status of immune cells resulting in less efficient repair after MI. Although ABCA1 is considered as a potential therapeutic target for the treatment of atherosclerosis, our data clearly indicate that it is of great importance to carefully consider potential adverse effects of ABCA1 on cardiac remodeling post-MI.

Concluding remarks

The ever increasing prevalence of CVD in the Western world urges industry and society to develop novel solutions to decrease this burden. Since lifestyle interventions to reduce CVD risk prove difficult to realize, novel solutions and therapies are needed. However, for the development of new drugs and therapies it is essential to evaluate their effects on both lipid metabolism and inflammation in all aspects of the development of cardiac pathophysiology. In this thesis we show that HFD feeding has mild but significant detrimental effects on cardiac function. In contrast to our expectations, this effect is not likely to be mediated by TLR2 or TLR4. Also, we demonstrated in mice that HFD feeding does not significantly aggravate cardiac dysfunction post-MI. Despite these findings, it would be of great scientific and societal interest to conduct a more in depth investigation on these effects in a prospective obese patient population.

Deficiency of RP105, a modulator of inflammation, improved cardiac function after induction of MI. In addition, ABCA1 had adverse effects on cardiac function post-MI, possibly via a reduced activation of immune cells. This confirms the important role of inflammation in recovery after an MI. Furthermore, we elucidated a possible reason for the failure of niacin in recent clinical trials and found that niacin's anti-atherogenic properties are most potent by lowering LDL-cholesterol on top of statin treatment. Altogether, these results provide novel insights and targets for the prevention or treatment of CVD.

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