CHAPTER 1

General introduction

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Cardiovascular diseases

Cardiovascular diseases (CVD) are the leading cause of death in the Westernized world. In the Netherlands, 30% of the reported deaths were a result of CVD.[1] Similar percentages are listed globally, according to the World Health Organization.[2] Despite substantially improved treatment options during the last decades, the number of patients suffering from CVD is still increasing.[2]

Risk factors for CVD can be subdivided into non-modifiable and modifiable risk factors. Non-modifiable factors include genetic variation, gender and age, whereas modifiable factors include lifestyle (smoking, physical inactivity, diet, overweight), hypertension, hyperlipidemia and hyperglycemia.[3] Adequate changes in lifestyle such as a more healthy diet, smoking prevention, increased physical activity and weight control can avoid over 75% of deaths attributed to CVD.[4] However, harsh reality shows that this is rarely feasible for individuals. As a result, in the last decade research on the treatment options for CVD is increasingly focused on the role of non-modifiable factors. Primary prevention strategies focus on the identified risk factors, such as lowering of plasma lipid levels using statins. Despite all efforts CVD are projected to remain the leading cause of death in the future.[2] Therefore it is urgent to expand the knowledge of underlying mechanisms in order to develop new therapies based on targeting different pathways.

The main CVD events are myocardial infarction (MI) and stroke, both caused by atherosclerosis. An MI occurs when, due to a vicious cycle of lipid accumulation and inflammation, the assembled atherosclerotic lesion in the coronary artery ruptures thereby releasing thrombotic factors, causing formation of a thrombus that can occlude the blood flow. This results in ischemia in distal areas, which can cause damage and death of the myocardial cells, eventually leading to cardiac remodeling and dysfunction.

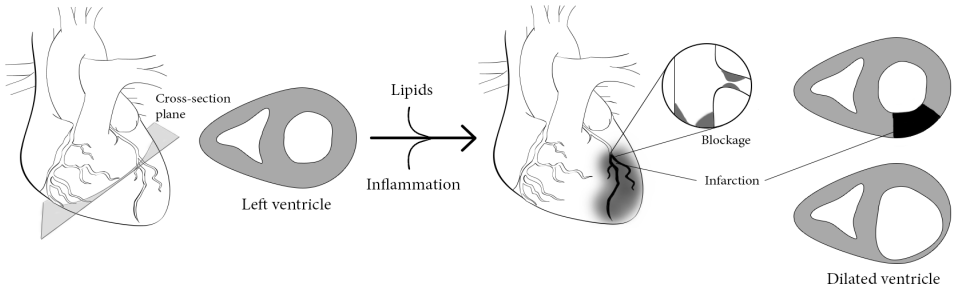

Figure 1 **Schematic representation of the role of inflammation and lipids on cardiac pathophysiology.** In a healthy heart with a normal functioning left ventricle a disturbed lipid metabolism as well as inflammation can contribute to cardiac dysfunction and atherosclerosis. The latter may lead to coronary occlusion and subsequent ischemia or even infarction, finally resulting in a dilated and less functional left ventricle.

The research reported in this thesis investigates the role of inflammation and lipid metabolism in the development of CVD (see figure 1). These two components are of particular interest as each, both individually and collectively, can lead to the onset or maintenance of pathological cardiac conditions. To study the influence of lipids and inflammation on cardiac function, specialized hemodynamic measurements were performed in mice. This chapter offers a general introduction to heart physiology and pathophysiology as well as to lipids and inflammation, two effectors of CVD that are both related to metabolic dysfunction.

Physiology

Heart function

Basal heart function

A well-functioning heart is essential for life as it pumps oxygen-rich blood with life-maintaining nutrients via the circulation to every part of the body. The blood flow originates by the coordinated contraction of the four chambers of the heart; two superior atria and two inferior ventricles. Via the right-sided cardiac chambers deoxygenated blood is pumped into the pulmonary circulation where it is enriched with oxygen. The left chambers of the heart pump blood into the systemic circuit. When left ventricular pressure is plotted as a function of its instantaneous volume, a pressure-volume loop (PV-loop) is generated, describing the function of the left ventricle (LV) during the different phases of one contractile cycle. These include a: filling phase, isovolumic contraction, ejection phase and isovolumic relaxation (see figure 2; upper panel).

The first phase in the cardiac cycle is passive filling of the LV (A), when blood flows from the atrium to the ventricle as a result of the atrial-ventricular pressure gradient. A second active ventricular filling is established by atrial contraction thereby increasing the atrial pressure and thus re-establishing a pressure gradient between the atrium and ventricle. The end-diastole (B), is the point where the ventricular volume is maximal, just prior to activation of the myocardium. Then isovolumic contraction takes place (C). After mitral valve closing, the ventricular chamber gradually develops sufficient pressure that exceeds the pressure in the aorta, thereby opening the aortic valve (D), followed by active ejection of the blood from the ventricular chamber into the aorta (E). Once the LV pressure drops below the aortic pressure the aortic valve closes as a result (F). This is described as the end-systolic point, with the lowest ventricular volume and a high ventricular pressure. During the subsequent isovolumic relaxation period (G) the ventricle muscle relaxes. When the LV pressure falls below atrial pressure the mitral valve opens (H), thereby starting the filling phase of the LV. From this loop a range of indices can be derived characterizing systolic and diastolic function of the LV and general hemodynamic indices (figure 2; middle and lower panel).

The following parameters can be obtained from PV-loops (figure 2):

Systolic function:

- end-systolic pressure (ESP): pressure in the ventricle at the end of systole, approximates mean arterial blood pressure
- end-systolic volume (ESV): blood volume remaining in the ventricle at end of systole (normally approximately the end of ejection)
- ejection fraction (EF; calculated as [EDV-ESV]/EDV): fraction of blood ejected from a ventricle per heartbeat, under normal circumstances ejection fraction is >60%
- dP/dt_{MAX}: maximum rate of pressure change in the LV, characterizing cardiac contractility
- end-systolic elastance (E_{ES}): the slope of the end-systolic pressure-volume relation (ESPVR). An increased slope as well as a leftward shift of the ESPVR indicates an increase in contractility

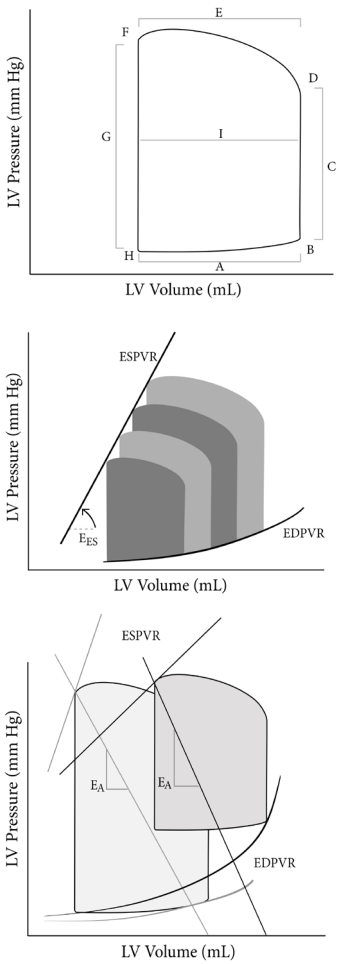

Figure 2 **Illustrations of left ventricular PV-loops.**

Upper panel: PV-loop of a regular cardiac cycle; see text for comprehensive explanation.

Middle panel: Calculations of end-systolic pressure–volume relation (ESPVR) and end-diastolic pressure–volume relation (EDPVR). A cluster of PV-loops under different loading conditions reveals the ESPVR and the EDPVR. The slope of the ESPVR indicates the end-systolic elastance (E_{ES}).

Lower panel: Illustration of a PV-loop from a dilated and failing left ventricle. A decrease in contractility is indicated by a decreased slope and a rightward shift of the ESPVR. The end-diastolic stiffness has increased indicated by the slope of the EDPVR. The left ventricular distensibility has decreased indicated by the upward shift of the EDPVR. Furthermore, LV performance has decreased indicated by decrease in the ventricular-arterial coupling ratio (E_{ES}/E_A). ESPVR, end-systolic pressure-volume relation; EDPVR, end-diastolic pressure-volume relation; E_A, effective arterial elastance; LV, left ventricle.

Diastolic function:

- end-diastolic pressure (EDP): pressure in a ventricle at the end of diastole, a measure of cardiac preload
- end-diastolic volume (EDV): blood volume at end of diastole at maximal stretch of cardiomyocytes prior to contraction (measure of preload)
- Tau: time constant of isovolumic exponential pressure decay during relaxation, measurement of LV relaxation
- $-dP/dt_{MIN}$: minimal rate of pressure change in the LV
- end-diastolic elastance (E_{ED}): the slope of the end-diastolic pressure-volume relation (EDPVR), representing the diastolic stiffness of the ventricle

General function:

- heart rate (HR): number of heart beats per minute
- stroke volume (SV; calculated as EDV-ESV): volume of blood ejected from a ventricle at each heartbeat, during heart failure stroke volume usually decreased or maintained only at the expenses of substantially increased filling pressure
- cardiac output (CO; calculated as HRxSV): total blood volume expelled by a ventricle per minute
- stroke work (SW): external work performed by the ventricle by ejecting stroke volume, represented by the area within the PV-loop
- effective arterial elastance (E_A; calculated as ESP/SV): index for afterload, load that the heart must eject blood against
- E_{ES}/E_A: ventricular-arterial coupling ratio, describes the coupling between LV performance and the systemic arterial system. Normal coupling ratio is approximately 1, the values decreases with heart failure

Assessment of left ventricular function in mice

Humans and mice display a 99% gene similarity[5], making mice suitable models for studying the function of human genes in health and disease. In addition, mice and rats are the most commonly used species for basic animal studies, as the understanding of their biology and genetics is well developed, breeding and housing are relatively inexpensive and a large number of specifically designed tools and techniques exist for this very purpose.

To reach the objectives of the current research program, functional assessment of the heart is essential. Considering the small size of the mouse heart and the high heart rate (approximately 500 beats per minute), highly specialized techniques are required to assess hemodynamic and ventricular function in the intact mouse. Magnetic resonance imaging (MRI), echocardiography and invasive PV-loop measurements have become available for cardiac phenotyping of small animals.

In this thesis, PV-loops by conductance catheter and transthoracic echocardiography were used to acquire cardiac function data. PV-loops are considered the gold standard in hemodynamic measurement, as they provide a direct volume signal and by combining these with LV pressure, pressure volume-relations can be obtained.[6] In addition, the pressure volume-relations yield very sensitive and load-independent indices. A disadvantage is that PV-loop measurements are highly invasive as the catheter is inserted in the right carotid artery and positioned into the LV. As a result, these measurements are routinely performed in mice as study ending. In contrast, echocardiography is a safe and non-invasive measurement and is one of the most widely used imaging tools in cardiology.[7, 8] It can be used relatively easy to obtain two dimensional movies and provides valuable information about cardiac chamber shape and size and allows tissue damage visualization.

Metabolism of the heart

Energy supply to the heart

Continuous contraction and relaxation of the heart results in a high energy demand by this organ. However, the heart has almost no internal energy reserves and thus has to produce energy (as adenosine triphosphate (ATP)) to maintain contractile function. Under normal conditions the heart preferably oxidizes fatty acids (FA), generating 70% of the cardiac energy demand. The remainder is covered by utilization of glucose (20-30%), lactate and ketones.[9] If necessary, the heart can shift its substrate utilization preferences during changed substrate availability and certain pathological conditions.[10] As *de novo* synthesis of FAs is low in heart tissue, the majority of FAs is derived from the circulation. FA uptake occurs via passive diffusion or via FA transporters (CD36/FATP)[11] by the cardiomyocytes which represent 75% of the cardiac cells and are the main site for fat oxidation.[12] After several modification steps FAs undergo β-oxidation yielding acetyl CoA, which subsequently enters the Krebs cycle and result in nicotinamide adenine dinucleotide hydrogen (NADH) and flavin adenine dinucleotide hydrogen ($FADH_2$) production, which are both used by the electron transport chain to generate ATP. Glucose is taken up via the glucose transporter (GLUT) and converted via the glycolysis pathway to acetyl CoA. Similar to FA derived Acetyl CoA, glucose derived Acetyl CoA is further processed in the Krebs cycle and electron transport chain. Since glucose utilization results in less production of ATP per carbon molecule, FAs are the preferred substrate in the aerobic heart.

Oxygen supply to the heart

To maintain adequate ATP concentrations in the heart, aerobic production of ATP is necessary since insufficient energy is produced in anaerobic conditions. Consequently, a constant supply of oxygen is essential to sustain cardiac function and viability.[13] The heart consumes large amounts of oxygen, especially compared to other organs. Under basal conditions the heart uses ~5–10 mL O_2/min/100g tissue, which can increase to more than ~70 mL O_2/min/100g myocardial tissue during heavy exercise. This is significantly

more than that consumed by the brain (~4 mL O_2/min/100g tissue) or skeletal muscle (~0.2 mL O_2/min/100 g) tissue under basal conditions.[14]

Adequate cardiac oxygen supply is achieved via the coronary circulation, as the myocardium itself is too thick to allow direct oxygen delivery from the chambers. Blockage of a coronary artery, for example by thrombus formation, therefore has major implications for the oxygen supply to the surrounding myocardium.

Pathophysiology

Atherosclerosis

Atherosclerosis is a complex, chronic and progressive inflammatory disorder which is characterized by thickening and hardening of medium-sized and larger arteries. Lipids, smooth muscle cells (SMC) and immune cells inside the vessel wall accumulate, resulting in narrowing of the blood vessel. Rupture of an advanced atherosclerotic lesion can lead to an MI, one of the most common causes of death in the Western world.[15]

The first step in the development of atherosclerosis is endothelial dysfunction, which has an impact on the cellular structure and function of the endothelium. Dysfunction can, among others, be caused by hypercholesterolemia, cigarette smoking, hypertension, diabetes mellitus or infections.[15, 16] As a consequence of the endothelial dysfunction, the vessel wall expresses adhesion molecules, such as vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1 and E-selectin. Leukocytes, like monocytes and T-cells are attracted from the circulation by adhesion molecules and migrate through the endothelial layer into the intima via monocyte chemoattractant protein-1 (MCP-1).[17, 18] Monocytes differentiate into macrophages upon migration into the sub-endothelial space, where the macrophages start to ingest modified lipids, resulting in lipid-rich macrophages or foam cells. The foam cells excrete cytokines and chemokines that attract and activate additional immune cells, thereby amplifying the intralesional inflammatory response.[19] Lesions consisting of macrophage foam cells and T-cells are the earliest type of lesions, the so-called fatty streak. However, these lesions do not result in clinical symptoms.[20] At this stage the development of the lesion is still reversible, since cholesterol can be exported to cholesterol acceptors as high density lipoprotein (HDL) and lipid-poor apoA1 via cholesterol transporters like ATP-binding cassette transporter A1 (ABCA1).[15] Inflammatory stimuli activate SMC to migrate into the intima leading to the formation of an advanced lesion.[16, 20] In the lesion SMC are able to take up cholesterol and thereby contributing to the foam cell formation. Furthermore, they secrete extracellular matrix components like elastin and collagen which form an elastic fibrous cap covering the lesion. This cap forms a barrier between the circulating blood on one side and the content of the lesion on the other side, consisting of a core of extracellular lipid, necrotic material derived from death foam cells, macrophages, SMC, T-cells and other inflammatory cells.[16]

The lesion stability depends on the thickness of the fibrous cap and on the lesion composition. Matrix metalloproteinases, produced by foam cells, degrade the extracellular matrix, thereby reducing the stability of the atherosclerotic lesion.[20] In addition, a large number of macrophages and a relative high lipid content of the lesion also diminish the stability. Lesion instability can lead to rupture, thereby releasing pro-thrombotic material into the bloodstream which triggers thrombus formation. This can result in an occlusion of a blood vessel which may lead to an MI or stroke.

It is important to emphasize that atherosclerotic lesions in mice are not prone to rupture, although mouse lesions resemble human lesions in several aspects. As a consequence, atherosclerotic mouse models do not develop MIs and in order to study the effects of an MI an alternative intervention is required. The model of permanent ligation of the left anterior descending (LAD) artery is often used to induce MI in mice as well as the ischemia/reperfusion model.[21]

Myocardial infarctions

Occlusion of a coronary artery interrupts the blood flow towards the downstream myocardial tissue, causing ischemia and initiating tissue damage and starvation. Damage can be reduced by timely reperfusion of the tissue, which is the preferred clinical therapy after an MI. Prolonged occlusion of the coronary arteries initiates a slow cascade of events which transforms the endangered zone in order to maintain cardiac output.[22] This process is usually completed in 6 to 8 weeks in humans and is referred to as infarct healing or LV remodeling.

Infarct healing can be divided into three, somewhat overlapping, stages: inflammation, proliferation and maturation.[23] In the first stage apoptosis occurs, which is a major contributor to cardiomyocyte loss.[24] Subsequently, danger signals are released by the necrotic cardiomyocyte, thereby activating the innate immune mechanism, e.g. complement system and Toll-like receptors (TLR), causing an intense inflammatory response.[25, 26] Chemokines and cytokines are released, resulting in leukocyte infiltration and clearance of necrotic cells and debris in the infarcted zone. After approximately 3 days the acute inflammatory response diminishes, marking the onset of the next stage. Concomitantly, activated macrophages produce growth factors and cytokines leading to the formation of granulation tissue which replaces the dead cardiomyocytes.[25] Proliferation of fibroblasts deposits a network of extracellular matrix and collagen, thereby strengthening the wound.[27] Furthermore, neovascularization realizes renewed blood supply to the infarcted area, via new vessels derived from pre-existing blood vessels or from migrating endothelial cells.[28] As repair proceeds, in the maturation stage, fibroblasts undergo apoptosis and a collagen-rich scar is formed.

The ischemic inflammatory response is a critical factor in the balance between cardiac repair and adverse LV remodeling. Experimental models suggest that prolonged or expanded inflammation or a non-controlled infarct healing result in adverse remodeling of the infarcted heart.[29, 30] For instance, both TLR2 and TLR4 have shown to play an unfavorable role in LV remodeling, since disrupted TLR4 signaling prevents adverse remodeling and TLR2 inhibition reduces ischemia/reperfusion injury and improves

cardiac function.[31-33] Although morbidity after an MI is decreasing due to improved reperfusion strategies and medical therapy, 5-year morbidity remains high. Therefore, strategies to interfere with inflammatory pathways after MI are intensively studied.

Metabolic dysfunction

Lipid metabolism

FAs are preferentially used as energy source by the heart and derive from our diet or are produced *de novo* in the liver. Since FAs themselves are cytotoxic, they are transported through the circulation either bound to serum albumin or incorporated as triglycerides (TG) in lipoproteins. TG-derived FAs are also a main source of energy for activity of skeletal muscle, for heat production by brown adipose tissue or for storage in white adipose tissue (WAT) for usage at a later point in time. In addition to TGs, cholesterol is also an essential lipid for many of our body's functions. Cholesterol is of critical importance for maintenance of cellular membranes and serves as a precursor for steroid hormone and bile acid synthesis.[34, 35]

Lipids are insoluble in an aqueous environment like blood, therefore they are transported in the circulation in water-soluble spherical particles called lipoproteins. Lipoproteins consist of a lipid-rich core of neutral lipids containing TGs and cholesteryl esters (CE), surrounded by a single layer of phospholipids (PL), free cholesterol and proteins called apolipoproteins. The latter are necessary for lipoprotein formation, modulation of enzymes and proteins, and act as ligands for receptor-mediated binding.

Based on their composition and size, lipoproteins can be divided in several subclasses: chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL).[36] The two particles with the lowest density, chylomicrons and VLDL, contain mainly TGs, whereas LDL and HDL particles mainly transport cholesterol in the form of CE. The metabolism of these lipoproteins and the subsequent distribution and cellular processing of TG-derived FA is described in the following sections and a schematic overview is depicted in figure 3.

After a meal, in the postprandial state, dietary TGs are lipolyzed by pancreatic lipases into 2-monoacylglycerol and FA. Together with dietary cholesterol these components are taken up by the intestinal cells and assembled into chylomicrons. These particles are transported from the lymph to the blood circulation, where TG in the core of the particle is hydrolyzed into glycerol and FA by endothelium-bound lipoprotein lipase (LPL).[37] FAs are taken up by the underlying peripheral tissue as an energy source for activity, thermogenesis or for storage.[38] The chylomicron-remnant particles, which are relatively enriched in CE and apolipoprotein E (apoE), are removed from the circulation by the liver e.g. via recognition of apoE by the LDL receptor (LDLr) and the LDLr-related (LRP).[39] Since remnant clearance results in the uptake of dietary cholesterol by the liver, it is clear that the liver plays a central role in cholesterol metabolism.

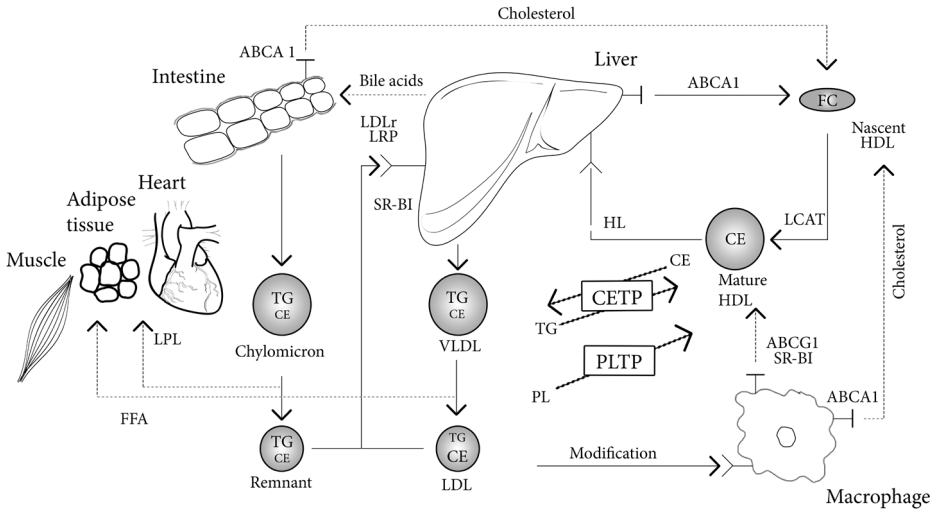

Figure 3 **Schematic representation of lipoprotein metabolism.** See text for explanation. ABCA1, ATP-binding casette transporter A1; ABCG1, ATP-binding casette transporter G1; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FFA, free fatty acids; HDL, high density lipoprotein; HL, hepatic lipase; LCAT, lechitin-cholesterol acyltransferase; LDL, low density lipoprotein; LDLr, LDL receptor; LPL, lipoprotein lipase; LRP, LDLr related protein; PL, phospholipid; PLTP, phospholipid transfer protein; SR-B1, scavenger receptor B1; TG, triglycerides; VLDL, very low density lipoprotein

In the fasted state no food is taken up in the intestines and the body's energy supply rely on the production of TG-rich VLDL particles by the liver. These TG-rich VLDL particles serve as main donor of TG-derived FA for extrahepatic tissues. Cholesterol and FA, derived from WAT or *de novo* synthesis, are packed as TG into VLDL particles in the hepatocytes of the liver.[40] They are secreted into the circulation and, similarly to chylomicrons, the core TGs are hydrolyzed by LPL. The depletion of TG from the VLDL particle results in the formation of VLDL remnants. These remnant particles are partly cleared by the liver, while the remaining particles undergo further lipolysis that results in the formation of LDL particles. LDL particles are relatively enriched in CE as compared to TG and apolipoprotein B (apoB) is its primary apolipoprotein. The majority of LDL particles are taken up from the plasma by the LDLr, while the remainder is taken up by extrahepatic tissues where the CE are used for e.g. maintenance of cellular membranes. High levels of apoB-containing lipoproteins (VLDL, VLDL remnants and LDL) can lead to deposition of these lipoproteins in the vessel wall, where they are modified and taken up by macrophages. This is the initial step of atherosclerosis development.

In contrast to apoB-containing lipoproteins, HDL is generally believed to have anti-atherogenic properties. This is attributed to its involvement in reverse cholesterol transport (RCT). In RCT, excessive cholesterol from the periphery is transported via the plasma to the liver. In the liver, HDL-derived cholesterol can be recycled or converted to degradation products like bile acids which are excreted via the bile into the intestine and feces. ApoA1, the major HDL apolipoprotein, is synthesized in the

liver and the intestine and is secreted into the plasma in complex with PL.[41] The apoA1-rich nascent HDL particle is subsequently further lipidated with PL and cholesterol from peripheral cells, through the involvement of the cholesterol transporter protein ABCA1. The initial lipidation of HDL via ABCA1 is a crucial step in HDL formation since patients with ABCA1 gene mutations as well as mice lacking ABCA1 have very low HDL levels.[42,43] The acquired cholesterol is esterified by lecithin-cholesterol acyl transferase (LCAT) generating spherical mature HDL. This mature particle can take up PL from chylomicrons and VLDL particles, via interaction with the phospholipid transfer protein (PLTP). In addition, loading with cholesterol from the periphery can occur via Scavenger Receptor B1 (SR-B1) and/or another ABC-transporter, ABCG1. The accumulated CE are stored in the core of the HDL particle and these CE can be taken up directly by the liver via SR-B1.[41] Indirect uptake of CE can take place after exchange of CE from HDL with TGs from apoB-containing lipoproteins and this transport is facilitated by the cholesteryl ester transfer protein (CETP).[44] Importantly, CETP is not expressed in rodents, except for hamsters, and therefore in mice there is no bidirectional exchange of CE and TG between HDL and (V)LDL.

Cholesterol and triglycerides as risk factors for CVD

Numerous clinical studies have shown that both elevated LDL-cholesterol levels and low HDL-cholesterol levels correlate with an increased CVD risk.[45-48] Several anti-atherogenic drugs have been developed aimed at reduction of LDL-cholesterol levels in plasma. Statins (or HMG-CoA reductase inhibitors) are the most widely used drugs that efficiently lower plasma (V)LDL-cholesterol levels, leading to a decreased cardiovascular morbidity and mortality.[49] However, a substantial residual cardiovascular risk remains, thereby indicating that additional treatment is required.[47, 50] One of the experimental strategies aims at increasing the anti-atherogenic HDL-cholesterol concentration. It is thought that the removal of excess cholesterol from macrophages and foam cells by HDL is one of the important mechanisms underlying the atheroprotective property of HDL. Increase in HDL levels can be achieved through inhibition of CETP. As described above, CETP facilitates the transfer of CE from HDL to (V)LDL in exchange for TGs. Via this mechanism, cholesterol in the anti-atherogenic HDL particles is lowered whereas it is increased in the atherogenic (V)LDL particles. Lowering CETP activity can be achieved by using drugs such as anacetrapip and niacin.[51, 52]

In addition to cholesterol, elevated levels of plasma TGs, known as hypertriglyceridemia, are also associated with an increased risk for CVD. It has been debated to which extent TG independently promote CVD risk[53]. Previous population studies showed that high TG levels are inversely related with HDL-cholesterol levels. Thus, an adjustment of HDL-cholesterol attenuates the relationship between TG and CVD. Nevertheless many clinical studies provided evidence that increased TG levels act as an independent risk factor.[54-57]

Ectopic fat deposition

In a healthy person, FA that are not directly used as energy source, are stored in WAT for usage at a later point in time. Through a tightly regulated process only a small portion

of FAs are directed for storage to non-adipose tissue such as liver, pancreas, skeletal muscle, kidney and heart.[58] Despite the ability of WAT to increase adipocyte cell size (hypertrophy) as well as cellular number (hyperplasia), the expandability of WAT is limited, particularly when the FA flux is increased over a long period of time.[59] As a result, transportation of FAs towards the non-adipose tissue will be increased, leading to ectopic fat accumulation. While limited ectopic FA accumulation may be physiological, in pathological conditions, excess ectopic fat may disrupt cellular function probably via toxic intermediary metabolites like ceramides and diacylglycerol. This is believed to contribute to insulin resistance, non-alcoholic hepatosteatitis (NASH) or heart dysfunction.[60, 61]

Metabolic inflammation and the innate immune system

Although the exact molecular mechanisms responsible for the activation of inflammatory pathways in obesity are still poorly understood, both adipose tissue and the liver are thought to be involved in the onset and development of metabolic inflammation. In obese subjects, expanded adipocytes secrete chemokines and cytokines, including tumor necrosis factor alpha (TNFα) and monocyte chemotactic protein-1 (MCP-1), resulting in the recruitment and activation of macrophages.[62] These pro-inflammatory macrophages in turn produce large amounts of TNFα and interleukine-1ß thereby providing a negative feedback loop and further amplification of the inflammation.[63] Similar to adipose tissue, the liver also becomes inflamed during obesity, which is reflected by an increase in hepatic inflammation markers such as serum amyloid A and C-reactive protein and nuclear factor κB (NF-κB) activation in high-fat diet fed mice.[64, 65] In general, the consequent chronic low-grade systemic inflammation increases the risk for associated inflammatory diseases like atherosclerosis and type 2 diabetes mellitus.

TLRs, which are part of the innate immune system, are suggested to play an important role in the development of obesity, insulin resistance and atherosclerosis.[66-68] Several studies demonstrated that saturated FAs exert pro-inflammatory effects through these receptors, particularly via the subtypes TLR2 and TLR4. TLRs are pattern-recognition receptors (PRRs) and are predominantly expressed on immune cells, like macrophages and dendritic cells, but have also been identified on non-immune cells such as adipocytes and cardiomyocytes. TLRs, of which 12 different types have been identified thus far, are expressed on the cell surface or in intracellular compartments.[69] These PRRs detect microbial components and 'danger' signals released by injured host cells, thereby activating the immune system as a first line of defense.[70] In mammals, ligand recognition by TLRs leads to recruitment of cytoplasmic myeloid differentiation factor (MyD)88, a universal TLR adaptor protein. MyD88 recruits proteins of the IRAK family and TRAF6, resulting in the phosphorylation and activation of the IKK complex. Finally, this process causes translocation of NFκB to the nucleus and the transcription of pro-inflammatory cytokines (figure 4).[71]

It is interesting to note that medium-chain FAs might serve as exogenous ligands for TLR2 and TLR4, provoking a nutritionally or metabolically induced inflammatory response.[72, 73] The lipoid structures in bacterial membranes recognized by TLR2 (lipoteichoic acid,

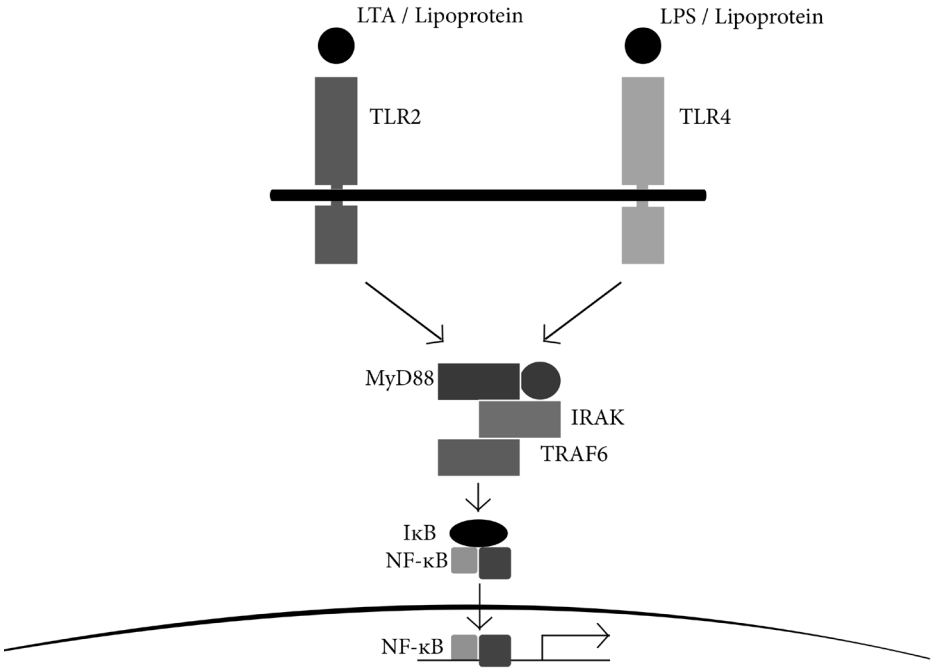

Figure 4 **TLR2 and TLR4 mediated signaling pathway.** Ligand recognition by TLR2 or TLR4 leads to the activation of MyD88 and the IRAK family, which in turn activates TRAF6. This results subsequently in the phosphorylation and activation of the IKK complex allowing NFκB to translocate into the nucleus. LTA, lipoteichoic acid; LPS, lipopolysaccharide,; IRAK, Interleukin-1 receptor-associated kinase; TRAF6, TNF receptor associated factor 6; IKK, IκB kinase.

(LTA)) and TLR4 (lipopolysacharide, (LPS)) share homology with endogenous lipids. Consequently, those TLRs can be activated by endogenous lipids and lipoproteins[74], including oxidized LDL[75], medium-chain FA component of LPS, as well as saturated FA.[76] In vivo animal experiments demonstrated that inhibition of TLR4 in mice resulted in protection from acute infused lipid induced insulin resistance[73], and also protection against diet-induced obesity has been reported.[68, 77] Similar findings, including improved insulin sensitivity[78] protection against died-induced adiposity and hepatic steatosis[79], are reported for TLR2 knock-out models. Furthermore, deletion of TLR2 or TLR4 reduces atherosclerotic lesion development in murine models.[80, 81]

Outline of thesis

The studies in this thesis are performed to provide additional and improved insight into the effects and interplay of dietary lipids and metabolic inflammation on cardiac performance in the presence or absence of atherosclerosis and myocardial infarctions. All studies are performed in mice and during the beginning of this PhD project little was known about the effect of gender on the relationship between diet-induced obesity, lipid metabolism and cardiac function in mice. Therefore, the effects of LV function after intermediate and long-term high-fat diet feeding in male and female mice are studied in **Chapter 2**. Subsequently, in **Chapter 3** the question is addressed if high-fat diet feeding results in more severe cardiac dysfunction after an MI, due to an increased inflammatory response induced by the dietary lipids, as compared to low-fat diet feeding. **Chapter 4** also examines effects after an MI, as the role of ABCA1 after an infarction is studied. **Chapter 5** focuses on the role of RP105, the physiological inhibitor of TLR4-signalling, in the process of LV remodeling after an MI. These effects are studied to investigate the role of TLR4 in cardiac function after an infarction. In addition, **Chapter 6** reports on the direct effects of TLR2 and TLR4 deficiency on cardiac function after high-fat diet feeding. In **Chapter 7**, it is investigated whether treatment with the LDL lowering drug simvastatin, in combination with the HDL raising drug niacin, leads to beneficial effects regarding plasma lipids and atherosclerotic development. **Chapter 8** provides a summary of the results obtained from the studies and discusses their implications.

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