Cardiac lipid storage and metabolism following myocardial ischemia

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ABSTRACT

Myocardial ischemia is associated with cellular- and metabolic adjustments within the heart, including accumulation of lipids. Myocardial lipids are stored in cytosolic droplets, consisting of a core of neutral lipids surrounded by a complex surface containing proteins, such as perilipins. Little is known about how myocardial lipid content and dynamics affect the function of the ischemic heart.

In this study, we investigated cardiac lipid accumulation and the consequences of altered lipid storage and metabolism following myocardial ischemia.

In **Paper I**, we investigated lipid accumulation of in a porcine model of ischemia/reperfusion and we found that cholesteryl esters accumulate in the myocardium following ischemia. The expression of the low density lipoprotein receptor (LDLr) and the low density lipoprotein receptor-related protein 1 (LRP1) was up-regulated, suggesting that cholesteryl ester uptake was mediated by these receptors.

In **Paper II**, we investigated the role of the lipid droplet protein Perilipin 5 (Plin5) in the pathophysiology of myocardial ischemia. In humans, we showed that a polymorphism in the PLIN5 gene is associated with reduced heart function following myocardial ischemia. In mice, Plin5 deficiency dramatically reduced the triglyceride content in the heart. Under normal conditions, *Plin5*$^{-/-}$ mice maintained a close to normal heart function by decreasing fatty acid uptake and increasing substrate utilization from glucose, thus preserving the energy balance. However, during stress or myocardial ischemia, Plin5 deficiency resulted in reduced myocardial substrate availability, severely reduced heart function and increased mortality.

In **Paper III**, we investigated the role of Plin2 in lipid storage and cardiac function following ischemia. We found that deficiency of Plin2 in mice surprisingly resulted in significantly increased levels of triglycerides. The heart function was not compromised in *Plin2*$^{-/-}$ mice in baseline and stress conditions. However, heart function was markedly reduced in *Plin2*$^{-/-}$ mice after induced myocardial infarction.

In conclusion, our findings indicate that dysregulation of myocardial lipid metabolism and storage influences heart function and survival following myocardial ischemia. Furthermore, our findings highlight a role for lipid droplet proteins perilipins in cardioprotection following myocardial ischemia.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Hjärtinfarkt är en av de vanligaste dödsorsakerna i västvärlden. Vanligen beror en hjärtinfarkt på att en blodpropp har bildats och täppt till ett av kärlen som försörjer hjärtat. Detta leder till syrebrist i hjärtmuskeln och om syrebristen pågår tillräckligt länge dör delen av hjärtat som normalt försörjs av det tilltäppta kärlet. När hjärtat drabbas av syrebrist så ställer hjärtat om sin metabolism från att främst använda fett till att använda socker, vilket inte generar lika mycket energi till hjärtats pumpfunktion som fett. Den förändrade metabolismen i hjärtat leder bland annat till att fett börjar lagras in i hjärtat i så kallade fettdroppar. Kunskapen om hur hjärtats förändrade lagring och hantering av fett påverkar hjärtfunktionen efter en infarkt är fortfarande begränsad.

I avhandlingen har vi studerat hur hjärtat påverkas av syrebristen som följer en hjärtinfarkt. Vi har studerat vilka typer av fett som lagras in i hjärtat efter en infarkt. Vidare har vi studerat hur proteiner som sitter runt fettdropparna i hjärtat (perilipiner) påverkar hjärtats funktion efter en infarkt.

Delarbete I. Här har vi undersökt fettinlagring efter en inducerad infarkt i grishjärta. Vi upptäckte att hjärtat lagrade in fettet kolesterol i de delar av hjärtat som drabbats av syrebrist. Kolesterol är en viktig komponent i hjärtcellernas membran, men de ökade nivåer att kolesterol har sannolikt ingen funktion för hjärtat och kan vara skadligt.

Delarbete II. Vi har studerat hur Perlipin5 (Plin5) påverkar hjärtfunktion och överlevnad efter infarkt. Efter att ha studerat Plin5 i patienter kunde vi visa att hur mycket proteins som tillverkades hade betydelse för hjärtfunktionen efter att hjärtat drabbats av syrebrist. Vi upptäckte även att avsaknad av Plin5 hos möss resulterade i kraftigt minskade nivåer att fett i hjärtat. När hjärtat var tvunget att arbeta hårdare ledde de minskade fettnivåerna och en reducerad användning av fett som energikälla till en sämre hjärtfunktion. Dessa möss hade även en sämre överlevnad efter hjärtinfarkt.

Delarbete III. Här undersökte vi hur Perilipin2 (Plin2) var involverad i fettinlagring och hjärtfunktion efter hjärtinfarkt. Avsaknad av Plin2 i möss ledde paradoxalt nog till ökade nivåer av fett i hjärtat, trots att Plin2 har som funktion att skydda fettdroppar. Efter en hjärtinfarkt var hjärtfunktionen

försämrad hos mössen som saknar Plin2, vilket visar att funktionen av Plin2 är viktig efter en hjärtinfarkt.

Från våra studier kan vi dra slutsatsen att reglering av fettinlagring i hjärtat påverkar hjärtfunktionen och utfallet efter en hjärtinfarkt. Förändrade fettnivåer i hjärtat efter en infarkt kan leda till sämre förmåga för hjärtat att återhämta sig. Forskning inom detta område kan därför leda till ny kunskap och på sikt resultera i utveckling av nya läkemedel mot hjärtkärlsjukdom.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Cholesteryl esters accumulate in the heart in a porcine model of ischemia and reperfusion

Drevinge C, Karlsson LO, Ståhlman M, Larsson T, Perman Sundelin J, Grip L, Andersson L, Boren J, Levin MC.

Plos One 2013; 8: e61942.

II. Perilipin 5 is protective in the ischemic heart

Drevinge C, Dalen KT, Nastase Mannila M, Scharin-Täng M, Ståhlman M, Klevstig M, Lundqvist A, Wramstedt A, Haugen F, Fogelstrand P, Adiels M, Asin-Cayuela J, Ekestam C, Gådin J, Lee YK, Nebb H, Romeo S, Redfors B, Omerovic E, Levin M, Valen G, Gan LM, Eriksson P, Andersson L, Ehrenborg E, Kimmel A, Borén J and Levin MC.

Manuscript

III. Increased myocardial lipid storage and reduced heart function after a myocardial infarction in *Plin2*$^{-/-}$ mice

Mardani I*, Drevinge C*, Dalen KT, Ståhlman M, Scharin-Täng M, Lundqvist A, Fogelstrand P, Redfors B, Andersson L, Omerovic E, Borén J and Levin MC.

*Equal contribution

Manuscript

CONTENT

ABBREVIATIONS

AAR	Area at risk
AMPK	AMP-activated protein kinase
ATGL	Adipose triglyceride lipase
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
CAT	Carnitine-acylcarnitine translocase
CCT	CTP:phosphocholine cytidylyltransferase
CGI-58	Comparative gene identification-58
CHOP	C/EBP homologous protein
CPT	Carnitine palmitoyltransferase
DGAT	Diglyceride acyltransferase
ER	Endoplasmic reticulum
FABPpm	Plasma membrane fatty acid–binding protein
FACS	Fatty acyl-CoA synthetase
FAT/CD36	Fatty acid translocase
FATP1	Fatty acid transport protein 1
G6P	Glucose-6-phosphate
GLUT	Glucose transporter
HIF	Hypoxia-inducible factor
HSL	Hormone-sensitive lipase

IA	Infarct area
IDL	Intermediate density lipoprotein
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IRE1	Inositol-requiring kinase 1
LAD	Left anterior descending artery
LCAD	Long-chain acyl-CoA dehydrogenase
LDL(r)	Low density lipoprotein (receptor)
LPL	Lipoprotein lipase
LRPI	Low density lipoprotein receptor-related protein 1
PDH	Pyruvate dehydrogenase
PDI	Protein disulfide isomerase
PERK	dsRNA-activated protein kinase-like ER kinase
PKA	Protein kinase A
Plin	Perilipin
PPAR	Peroxisome proliferator-activated receptor
TCA cycle	Tricarboxylic acid cycle
UPR	Unfolded protein response
WAT	White adipose tissue
VLDL(r)	Very low density lipoprotein (receptor)
WT	Wild type

1 INTRODUCTION

1.1 Cardiac anatomy and physiology

The heart consists of four chambers that function as two separate pumps: (1) the right heart that pumps blood to the lungs and (2) the left heart that pumps blood to the systemic circulation. The cardiac cycle consists of diastole, the period of relaxation in which the heart is filled with blood, and systole, the contraction of the heart. During diastole, the deoxygenated blood from the systemic circulation flows through the inferior and superior vena cava to the right ventricle through the right atrium. Simultaneously, the oxygenated blood from the pulmonary circuit flows through the pulmonary veins into the left ventricle through the left atria. About 80 percent of the blood flows directly through the atria to the ventricles, and only the remaining 20 percent is delivered by contraction of the atria. The ventricles contracts shortly after the atrial contraction. The right ventricle ejects blood through the pulmonary arteries to the lungs and the left ventricle delivers blood to the peripheral organs through the aorta. The blood is prevented from flowing backwards by the atrioventricular valves (the tricuspid and mitral valves) and the semilunar valves (the aortic and pulmonary artery valves).[1]

In the human heart, the left ventricle is filled to approximately 110-120 ml during diastole. This is called the end-diastolic volume. During contraction, the volume (about 70 ml) that is pumped out of the heart is called stroke volume. Thus, a volume of about 40 to 50 ml is remained in the ventricle and is referred to as the end-systolic volume. The ejection fraction is the fraction of the end-diastolic volume that is ejected from the heart and is around 60 % in a healthy heart. However, during physiological stress, such as exercise, the heart can the dramatically increase the cardiac output (the amount of blood ejected into the circulatory system in a minute). The major elements of this regulation are (1) the Frank-Starling mechanism and (2) the autonomic nervous system.[1] The Frank-Starling mechanism is the intrinsic ability of the heart to adapt to increased venous return. The greater the heart muscle is stretched during filling, the stronger is the force of contraction. Thus, the heart ejects venous blood that is returned to the heart within physiological limits.[1, 2] The autonomic nervous system of the heart consists of two

branches, the sympathetic and the parasympathetic systems. These systems work in a finely tuned but opposite fashion.[3] Sympathetic stimulation can increase cardiac output by 100 percent, whereas strong parasympathetic stimulation can lower the heartbeat to 20–40 beats per minute.[1]

1.1.1 Coronary circulation

The human heart is supplied with blood by coronary arteries; the blood within the ventricles only supplies the 100 μm of the inner endocardial surface. Two large epicardial arteries originate from the root of the aorta: the right coronary artery and the left coronary artery. The left coronary artery is branched into the left anterior descending artery (LAD) and circumflex artery, and they supply mainly the left atrium and ventricle, and the interventricular septum. The right coronary artery supplies most of the right side of the heart and parts of the left ventricle and septum. The coronary venous blood returns to the right atrium through the coronary sinus and anterior cardiac veins.[1, 4]

1.2 Myocardial ischemia

Myocardial ischemia and ischemic heart disease are leading causes of death in the industrialized world. Life style factors in the Western societies such as high calorie food intake and minimal physical activity aggravates the risk of developing atherosclerosis and subsequent ischemic heart disease.[5] Myocardial infarction occurs when the blood flow supplying the heart muscle is blocked, resulting in necrosis of parts of the myocardium. The most common cause is the rupture of an atherosclerotic plaque in one of the coronary arteries. Early mortal complications following a myocardial infarction are cardiogenic shock, cardiac rupture and arrhythmia. Further, the loss of contractile myocardium and the subsequent reduced pump function promotes development of chronic heart failure.[1, 6, 7]

1.2.1 Reperfusion

An early reperfusion of blood flow to the injured myocardium reduces the infarct size and thereby improves the clinical outcome after a myocardial infarction. Thrombolytic therapy and percutaneous coronary intervention (widening of the stenotic coronary artery by balloon inflation) are the most used methods in clinic to restore blood flow following a myocardial

infarction.[8] However, the reperfusion of the affected myocardium paradoxically also attenuates injury. Several mechanisms have been proposed to mediate the damage induced by the reperfusion, such as inflammation, increased radical oxygen species (ROS), increased levels of intracellular Ca^{2+} and the reduction of oxidative phosphorylation.[8, 9]

1.2.2 The healing myocardium

Profound morphologic and histological changes of infarcted myocardial tissue occur during and after a myocardial infarction. The lack of oxygen in the affected area leads to a rapid loss of cardiomyocytes. The cell death triggers inflammatory signals that recruits neutrophils and macrophages to the infarction. These inflammatory cells degrade the collagen framework surrounding the cardiomyocytes and aid the clearance of necrotic cells and their debris. The collagen structure is virtually vanished within the first week after the infarction. At this early time point, the ventricle wall is weakened and therefore susceptible to rupture. Approximately five days after the infarction, macrophages and endothelial cells promote angiogenesis and supply the new forming tissue with blood. Furthermore, myofibroblasts start to synthesize collagen which strengthens the ventricle wall. After several weeks, a solid scar has been formed with a stable cross-linked collagen structure. During this healing process, the left ventricle undergoes profound remodeling, involving myocyte hypertrophy and changes of the ventricular architecture to distribute the wall stress more evenly. A suboptimal remodeling of the heart can result in contractile dysfunction and development of heart failure. [10, 11 12]

1.3 Cardiac metabolism

The heart must contract continuously and have therefore a high energy demand. The mitochondria contribute to >95% of the ATP generated and occupies one third of the cardiomyocyte volume.[13] The heart has a relatively small content of ATP and a fast hydrolysis resulting in a complete turnover of the pool every 10 s. In order to generate the high rate of ATP and sustain the contractility, the heart is flexible and can use all energy substrates including lipids, carbohydrates, amino acids and ketone bodies. However, lipids are the predominantly used energy substrate and 50-70% of the ATP is generated from fatty acid β-oxidation.[14, 15]

1.3.1 Lipid metabolism

Sources of lipids

Lipids have a low solubility in plasma and are therefore supplied to the heart either as free fatty acids bound to albumin or as triglycerides and cholesteryl ester transported in lipoproteins.[16]

To provide energy to the heart and other peripheral tissues, triglycerides stored in the adipose tissue are hydrolyzed into fatty acids and released into the circulation. Once leaving the adipocytes, the fatty acids are ionized and bind to the plasma protein albumin.[1, 17] Normal concentrations of free fatty acids in the plasma vary between 0.2 and 0.6 mM. However, conditions such as fasting, poorly controlled diabetes and severe stress results in highly elevated levels of circulating free fatty acids.[18]

Lipoproteins consist of a core of triglycerides and cholesteryl esters surrounded by a monolayer of amphipathic phospholipids with embedded apolipoproteins. There are several classes of apolipoproteins that, among many functions, can act as ligands for cell-surface receptors. Circulating chylomicrons transport dietary lipids absorbed by the intestine. The triglycerides in this lipoprotein are hydrolyzed in peripheral tissues, and the resulting remnant chylomicron is then removed from the circulation by the liver. Very low density lipoprotein (VLDL) is the principal transporter of endogenous triglycerides. VLDL is secreted by the liver and has a high proportion of triglycerides but also contains cholesteryl esters. The triglycerides in the VLDL are hydrolyzed by the heart, adipose tissue and other peripheral tissues. As the triglycerides are removed, VLDL is subsequently transformed into intermediate density lipoprotein (IDL) and after that, low density lipoprotein (LDL). Hence, these lipoproteins contain larger proportions of cholesteryl esters and proteins and smaller proportion of triglycerides and phospholipids.[1]

Fatty acid uptake

Triglyceride rich lipoproteins such as chylomicrons and VLDL provide the heart with triglycerides. To enable the uptake of triglycerides into the cardiomyocyte, the triglycerides are hydrolyzed into fatty acids by lipoprotein lipase (LPL) located at the endothelial wall.[14, 19]

After being dissociated from albumin or hydrolyzed from triglycerides in lipoproteins, the fatty acids are transferred from the microvascular compartment through the capillary endothelium and the interstitial compartment to the sarcolemmal membrane of the cardiomycocyte.[14] Fatty acid uptake into the cardiomyocyte is facilitated either by passive diffusion or by protein-mediated uptake. The former alternative represents a non-saturable low affinity process where the fatty acids flip-flop through the membrane.[20] However, the predominant uptake of fatty acids are mediated via a family of transporters: fatty acid translocase (FAT/CD36) (hereafter referred to as CD36), plasma membrane fatty acid–binding protein (FABPpm), and fatty acid transport protein 1 (FATP1).[21, 22] CD36 is the predominately studied fatty acid carrier and it has been shown that 50-60 % of the fatty acid uptake is mediated via CD36.[23] Further, CD36 is able to relocate from the endosomes to the sarcolemmal membrane to increase fatty acid uptake in response to insulin, contraction, and AMP-activated protein kinase (AMPK) activation. Thus, CD36 has a key regulatory function of fatty acid uptake.[14]

The VLDL receptor (VLDLr) has also been implicated to play a role in triglyceride uptake.[24] The VLDLr is most abundant in the heart, but is also expressed in other tissues including skeletal muscle, adipose tissue and brain.[25] VLDLr mediates lipid uptake either by endocytosis of lipoproteins or by cooperation with LPL.[26, 27]

β-oxidation

Fatty acid β-oxidation is the catalytic process by which fatty acids are broken down in the mitochondria to produce ATP. After uptake of fatty acids into the cytoplasm of the cardiomyocyte, fatty acyl-CoA synthetase (FACS) activates the fatty acids by adding a CoA moiety (figure 1). The two main pathways of these fatty acid-CoAs are (1) delivery to the mitochondria for oxidation or (2) esterification to triglycerides for temporary storage in the triglyceride pool in cytosolic lipid droplets.[28] Storage of lipids in cardiac lipid droplets will be described in more detail in section “Lipid storage”.

In contrast to short- and medium-chain fatty acids, long-chain fatty acids, which constitute the major fraction of the fatty acids, cannot simply diffuse through the mitochondrial membranes. Therefore, long-chain fatty acids are converted to long chain acylcarnitine by carnitine palmitoyltransferase 1 (CPT1) located at the outer mitochondrial membrane. The fatty acids are

shuttled through the inner mitochondrial membrane by carnitine-acylcarnitine translocase (CAT), following by conversion back to acyl-CoA by CPT2.[29] The entrance of fatty acids into the mitochondria can be regulated by allosteric inhibition of CPT1 by malonyl-CoA.[14]

After the entrance into the mitochondrial matrix, β-oxidation of fatty acids occurs by cleaving two carbons of the fatty acid each cycle, forming acetyl-CoA as well as NADH and $FADH_2$. Each cycle produces theoretically 5 ATP from the generation of NADH and $FADH_2$. However, the entrance of acetyl-CoA into the TCA cycle yields additionally 12 ATP.[30]

TCA cycle

The TCA cycle (also referred as citric acid cycle or Krebs cycle) is the final common pathway for the oxidation of fuel molecules such as fatty acids, glucose and amino acids. The cycle is also an important source of precursors, *e.g.* for amino acids and nucleotide bases. In the first step, acetyl-CoA, derived from glucose or fatty acids, combines with oxaloacetate to form citrate. After a sequence of chemical reactions oxaloacetate is regenerated, allowing the cycle to continue. The tricarboxylic acid (TCA) cycle generates the reducing equivalents NADH and $FADH_2$ and also CO_2 as a byproduct. NADH and $FADH_2$ deliver electrons to the electron transport chain, resulting in ATP formation by oxidative phosphorylation.[1]

1.3.2 Glucose metabolism

Glucose uptake into cardiomyocytes is mediated by glucose transporters. GLUT4 and GLUT1 is the predominantly glucose transporters expressed in the heart. In the adult heart, GLUT4 is the isoform responsible for the majority of the myocardial glucose uptake. However, there are a variety of pathophysiological circumstances, for instance ischemia, in which GLUT1 expression is induced in the heart.[31] Further, GLUT3 and GLUT5 may also be upregulated during ischemia.[32] In order to increase the glucose uptake, GLUT4 is recruited to the sarcolemmal membrane from intracellular vesicles in response to insulin signaling, high work load or ischemia.[33]

After uptake, glucose is rapidly converted to glucose-6-phosphate (G6P). The negative charge prevents G6P from diffusing out of the cell, which lacks transporters for this substrate. G6P can enter several metabolic pathways of which glycolysis is the predominant one. The glycolytic pathway converts

G6P into pyruvate and generates two ATP for each molecule of glucose. Pyruvate can either (1) be converted to lactate in the cytosol in an anaerobic process or (2) be shuttled into the mitochondrial matrix to be transformed to acetyl-CoA by the pyruvate dehydrogenase (PDH) complex for subsequent oxidation in the TCA cycle (figure 1).[34]

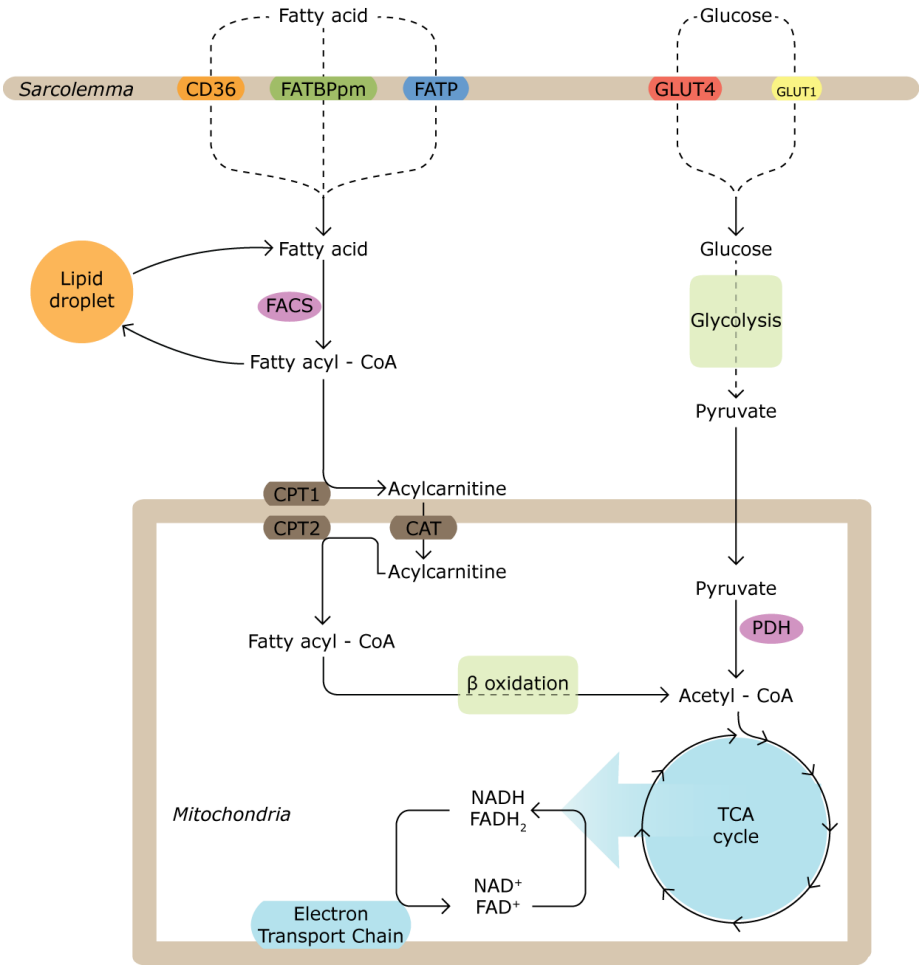

Figure 1. Schematic overview of fatty acid β-oxidation and glucose oxidation in the cardiomyocyte. CD36, cluster of differentiation 36; FATBPpm, plasma membrane fatty acid-binding protein; FATP, fatty acid transport protein; FACS, fatty acyl coA synthase; CPT, carnitine palmitoyltransferase; CAT, carnitine-acylcarnitine translocase; GLUT, glucose transporter; PDH, pyruvate dehydrogenase.

1.4 Cholesteryl esters

Cholesterol is important for the function and fluidity of the plasma membrane, and is also a precursor molecule in several biochemical pathways in liver and adrenal gland.[35] The heart is among the tissues with the lowest *de novo* cholesterol biosynthesis.[36] Thus, circulating lipoproteins is important for supplying heart with cholesteryl esters.

Cholesteryl ester delivery occurs via endocytosis of LDL via LDL receptor (LDLr) mediated uptake. LDLr is located on the cell surface and the internalized lipoprotein is delivered to the lysosome where the cholesteryl esters are hydrolyzed. The liberated cholesterol is either used by the cell or stored as cholesteryl esters in lipid droplets. The LDLr are recycled back to the plasma membrane.[35] The LDLr belongs to a family of lipoproteins receptors: LDLr gene family. Two other members of the family are VLDLr and low density lipoprotein receptor-related protein 1 (LRP1).[37]

In addition, selective uptake of cholesteryl esters in the core of lipoproteins has been reported in the heart and arterial wall.[38] [39] LPL increased selective uptake of LDL cholesterol in LDLr negative human fibroblasts and CHO cells,[40] suggesting a cholesteryl ester uptake independent of LDLr.

1.5 Metabolic alterations in the ischemic heart

In the ischemic heart, the coronary blood flow is inadequate to supply the myocardium resulting in a mismatch between the oxygen demand and the oxygen supply. The well-perfused heart has high oxygen consumption due to its high energy demand. Thus, low oxygen availability drives the heart into major alterations of the energy metabolism. The myocardial ischemia results in a decreased oxidative metabolism and a subsequent decline in ATP production (figure 2).

The extent of the reduction in glucose and fatty acid oxidation is dependent of the severity of the ischemia. During severe ischemia caused by a myocardial infarction, the oxygen demanding oxidative metabolism is dramatically reduced and the major source of ATP is anaerobic glycolysis:

the conversion of pyruvate to lactate via lactate dehydrogenase (LDH). Hence, anaerobic glycolysis is uncoupled from the glucose oxidation and thereby provides a limited amount of ATP. Further, the accumulation of deleterious byproducts of glycolysis, lactate and H^+, result in an increased expenditure of ATP to reestablishing of ionic homeostasis. Altogether, the reduction in ATP production results in a reduction in cardiac function and efficiency.[14, 41]

Hypoxia-inducible factor (HIF) and 5′-AMP-activated protein kinase (AMPK) are two important components in the cellular response to myocardial ischemia. The transcription factor HIF1α is degraded during normal oxygen concentration. However, during low oxygen availability HIF1α escapes degradation and promotes transcription of numerous of genes including GLUT1 and LDH. Thus, HIF1α activates genes involved in oxygen-independent ATP synthesis.[42] AMPK is highly activated during ischemia by increased levels of AMP, an indicator of compromised cellular energy availability. AMPK can be considered to be a fuel gauge, responsible for the activation a number of energy-producing metabolic pathways as well as inhibiting energy-consuming pathways. Thus, AMPK activation results in an increase in glucose utilization, *e.g.* by promoting the translocation of GLUT4 to the sarcolemma and by stimulation of glycolysis via phosphorylation of phosphofructokinase 2. Further, AMPK activation also stimulates β-oxidation *e.g.* by reducing the malonyl-CoA levels. Hence, during mild ischemia β-oxidation remains the major source of the residual oxidative metabolism.[41, 43]

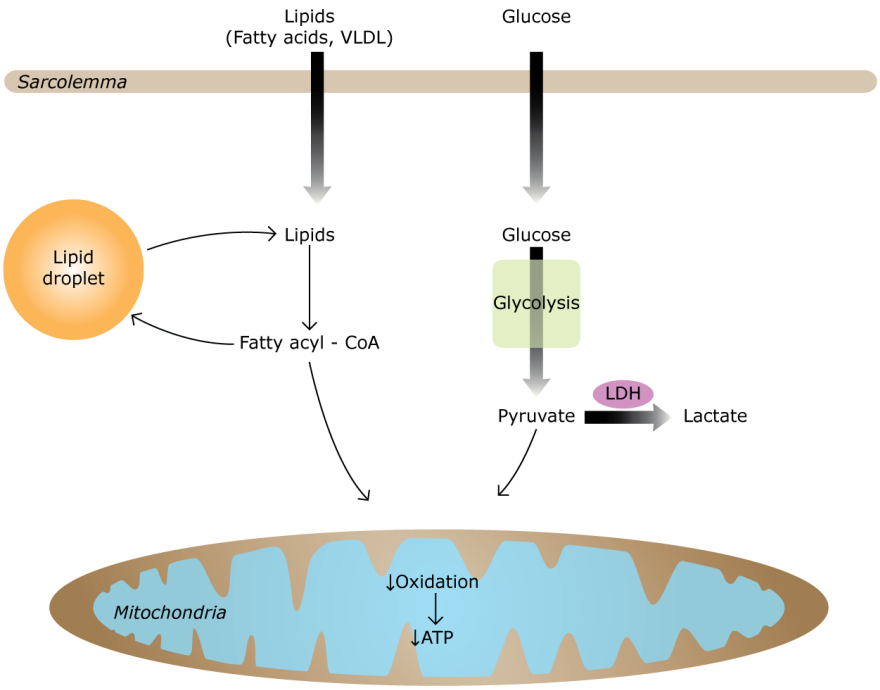

Figure 2. Schematic figure of metabolic alterations in the ischemic cardiomyocyte. LDH, lactate dehydrogenase.

1.5.1 Lipid accumulation in the ischemic heart

Reduced oxygen availability promotes myocardial accumulation of neutral lipids, such as triglycerides and cholesterol esters.[24, 44, 45] Due to the reduced fatty acid oxidation during ischemia, fatty acids are not used for energy production but are instead converted to triglycerides that accumulate in the cell. However, lipid uptake has also been reported to be increased in the ischemic heart contributing to the lipid accumulation.[24] During ischemic insult, plasma level of norepinephrine is elevated which promotes adipose tissue lipolysis. This results in increased concentration of circulating free fatty acids, thus, increases the delivery of fatty acids to the myocardium.[14, 46] In addition to increased availability of circulating fatty acids, cellular

response to hypoxia promoting lipid accumulation has been reported. Chabowski *et al* have demonstrated a hypoxia-induced translocation of fatty acid transporters CD36 and FABPpm to the sarcolemma resulting in an increased lipid accumulation in hypoxic isolated hearts.[47] Further, increased cardiac lipid uptake mediated via HIF1α induced up-regulation of VLDL receptor[24] and LRP1[45] has been described.

The issue whether lipid accumulation is detrimental for the ischemic heart or not is complex. Lipid accumulation in the heart has been associated with cardiac dysfunction, suggesting a detrimental role of excessive lipids in the heart.[48, 49] On the other hand, storage of lipids in the form of inert triglycerides has been postulated to be protective to heart function.[50, 51] Thus, sequestration of fatty acids in the triglyceride pool potentially protects the cell from toxic fatty acid metabolites, such as fatty acids, and ceramides. These lipids are regarded as bioactive lipids with cell signaling functions. An excess of free fatty acids have been reported to promote increased oxidative stress and apoptosis.[52] Further, increased availability of saturated fatty acids appears to be the primary trigger of synthesis of ceramides. Ceramides are synthesized either *de novo* from serine and palmitate or by breakdown from sphingomyelin.[53] Increased levels of ceramides are associated with cellular apoptosis and ROS production.[54, 55, 56] Further, ceramide reduction in LPL^{GPI} mice, a transgenic mouse model displaying increased levels of ceramides, was associated with improved cardiac function.[57]

1.6 Endoplasmic reticulum (ER) stress

Interference with protein folding or any of the major functions of ER results in ER stress. This leads to activation of the unfolded protein response (UPR), a signaling cascade from ER to the nucleus that induces a comprehensive gene expression program. The UPR triggers an adaptive response in order to reestablish ER homeostasis by reducing the protein synthesis, upregulating expression of ER resident chaperones as well as degrading misfolded proteins.[58] During ischemia, there are significant nutrient and oxygen starvation which lead to the induction of ER stress. For example, the activity of protein disulfide isomerase (PDI) is dependent on molecular oxygen. During hypoxia or ischemia, disulfide bonds are not formed resulting in accumulation of misfolded proteins and subsequent ER stress.[59]

The ER molecular chaperone BiP/GRP78 protein is involved in sensing misfolded protein accumulation in the ER and are responsible for the initiating the response to ER stress in combination with three ER integral membrane protein: dsRNA-activated protein kinase–like ER kinase (PERK), inositol-requiring kinase 1 (IRE1) and activating transcription factor 6 (ATF6). However, if the ER stress is severe or prolonged, the UPR may lead to apoptosis. This is promoted by transcriptional induction of C/EBP homologous protein (CHOP), the caspase 12-dependent pathway and activation of the c-Jun NH2-terminal kinase (JNK)-dependent pathway.[59, 60]

1.7 Lipid storage

Triglycerides and cholesteryl esters are hydrophobic lipids and thus insoluble in the cytosol. Hence, these lipids are stored in the core of so called lipid droplets. The lipid core is surrounded by a monolayer of amphipathic lipids, such as phospholipids and unesterified cholesterol (figure 3). The lipid droplet was long considered to be a passive storage pool of lipids, but is now recognized as a dynamic organelle that is involved in numerous of cellular processes. Thus, the lipid droplets are coated with a large number of proteins that are critical for the formation, trafficking and stability of the lipid droplet.[61, 62]

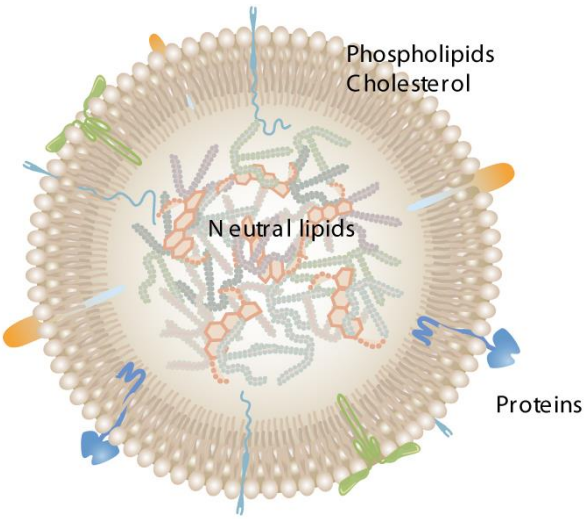

Figure 3. Schematic view of the lipid droplet.

The loading of hydrophobic lipids in a water free phase provides the most efficient form of energy storage. The lipid droplets are intracellular lipid reservoirs providing lipids for energy metabolism, membrane synthesis and steroid synthesis.[63] Nearly all cell types have the ability to generate lipid droplets when there is a surplus of fatty acids to be subsequently used as source of energy when conditions are sparse. The number and size of lipid droplets differ between different cell types, with sizes ranging from 100 nm to 100 µm. The lipid droplets in white adipocytes are large, with diameters up to 100 µm, and fill almost the entire cytoplasm.[64]

Lipid droplets are formed *de novo* from the ER. The exact mechanism of the lipid droplet formation is poorly understood. One model suggests that neutral lipids are synthesized between the two leaflets of the ER membrane. The formed lipids are highly hydrophobic and have a limited solubility in the membrane and therefore form a lens structure that is the core of the lipid droplets. The mature lipid droplet is then thought to bud from the ER membrane to form an independent organelle with a monolayer of phospholipids.[65, 66]

The storage capability of lipids is increased by formation of new lipid droplets and by growth of existing droplets.[64] Lipid droplet has been proposed to grow by *de novo* triglyceride synthesis directly in the droplet. DGAT2 catalyzes the final step of triglyceride synthesis and have been associated to the lipid droplet in conditions of oleate loading.[67] In addition, the increase in surface area of the lipid droplet during expansion requires large increases of the phospholipid content. CTP:phosphocholine cytidylyltransferase (CCT), catalyze the rate-limiting step in PC biosynthesis and have been shown to be activated by binding to lipid droplets.[68, 69]

When energy is required, the triglycerides in the lipid droplet are hydrolyzed to generate fatty acids.[70] Triglyceride lipolysis is a three-step process where adipose triglyceride lipase (ATGL) selectively performs the first and rate-limiting step generating diglycerides and non-esterified fatty acids.[71] Hormone-sensitive lipase (HSL) is rate-limiting for diglyceride hydrolysis.[72] Finally, monoglyceride lipase efficiently cleaves monoglyceride into glycerol and non-esterified fatty acids.[73]

Analyses of the lipid droplet proteome have revealed a large number of lipid droplet–associated proteins. However, many of those are most possibly not genuine associated proteins but are artifacts originating from the purification process.[74] One group of proteins associated with the lipid droplets are the Rab proteins, which are GTPases involved in trafficking event.[66] Rab18 have been suggested to localize to lipolytically active lipid droplets and mediate an increased association with the ER.[75] However, the best characterized and most abundant proteins on the lipid droplets are the perilipins, a family of five related proteins.[74]

1.8 Perilipins

The family of perilipins has undergone a change in nomenclature and was formerly known as the PAT family, named after the three first discovered perilipins: perilipin, ADRP, TIP47. The mammalian genome encodes five so far discovered perilipin (Plin) genes: Plin1-5. Plin1, 2, 3, and 5 share a highly conserved N-terminal domain and an 11-mer repeating helical organization. Plin4 is the most divergent member of the perilipin family with a highly expanded 11-mer repeat region.[76, 77]

The perilipins appear to play an important role in regulating storage of lipids and to protect lipid droplets from unregulated hydrolysis. The perilipins share the ability of binding to lipid droplets, but display different tissue and subcellular localization. This suggests that each perilipin has a unique role in the lipid dynamics. Plin1 and 2 are located exclusively on lipid droplets, and Plin2 is rapidly degraded in absence of lipid droplets. In contrast, Plin3, 4 and 5 are stable in absence of LDs and have been shown to translocate from a cytosolic pool to nascent lipid droplet in response to fatty acid supplementation or other conditions promoting lipid droplet formation.[78, 79, 80]

1.8.1 Plin1

Plin1 is primarily expressed in white adipose tissue (WAT), brown adipose tissue (BAT), and steroidogenic tissue.[81] Early studies have identified Plin1 as a major regulator of adipocyte lipolysis.[82] Under basal lipolytic conditions, the lipases ATGL and HSL are cytosolic whereas Plin1 is located on the lipid droplet in association with comparative gene identification-58 (CGI-58), the co-activator of ATGL. This prevents CGI-58 from binding to ATGL and to

facilitate ATGL-mediated triglyceride hydrolase activity. Thus, the lipolysis is maintained at low rates. However, upon β-adrenergic stimulation, Plin1 and HSL are phosphorylated by protein kinase A (PKA). This allows (1) CGI-58 to dissociate from Plin1 and recruit ATGL and (2) HSL to translocate to the lipid droplet. Thus, during basal conditions Plin1 functions as a barrier to lipases, whereas Plin1 participates in their recruitment after β-adrenergic stimulation.[83, 84]

1.8.2 Plin2

Plin2 is ubiquitously expressed in the body. Overexpression of Plin2 results in increased formation of lipid droplets.[85, 86] Phospholipids as well as triglycerides and cholesteryl esters are increased upon Plin2 overexpression, suggesting that Plin2 may play a role in increasing lipid droplet membrane size to support lipid droplet expansion.[87] The stability of is Plin2 is mediated by lipid droplets and the protein is degraded by the proteasome in the absence of neutral lipids.[88, 89]

In WAT deficient in Plin1, Plin2 becomes the major protein coating lipid droplets. In the *Plin1*$^{-/-}$ adipocytes, the basal lipolysis is elevated compared to WT, but the stimulated lipolysis is decreased.[90, 91] This suggests that unphosphorylated Plin1 is more protective to lipases than Plin2, which in turn is more protective than phosphorylated Plin1.

Plin2 is the major protein coating lipid droplets in the liver. Deletion of *Plin2* protects against lipid droplet accumulation and chronic inflammation in liver in mice on a high-fat diet.[92, 93] Further, *Plin2*$^{-/-}$ lacking leptin have improved systemic glucose and lipid homeostasis compared to leptin deficient controls. As expected, muscle-specific PLIN2 overexpression resulted in increased lipid droplet accumulation. Interestingly, PLIN2 overexpression improved skeletal muscle insulin sensitivity.[94]

In humans, a missense polymorphism of Plin2, Ser251Pro, resulting in structural changes in the protein has been described. The polymorphism was associated with increased lipid accumulation and decreased lipolysis in cells, and carriers of the minor Pro251 allele had decreased circulating triglycerides and VLDL concentrations.[95]

1.8.3 Plin3

Similar to Plin2, Plin3 is widely expressed in the body and also shares the highest sequence homology with Plin2 of all perilipins. In addition to its role in lipid droplet biogenesis, Plin3 was reported to be involved in the intracellular transport of mannose 6-phosphate receptors between the trans-Golgi and endosomes.[96] Structural analysis of the protein have indicated the existence of two distinct 'functional modules', which may explain the dual function of Plin3.[97, 87] Although Plin2 is the dominating perilipin in the hepatocytes, depletion of Plin3 has been reported to suppress hepatic steatosis.[98]

1.8.4 Plin4

Plin4 have a divergent amino acid structure compared to Plin2, 3 and 5 and also show a limited tissue distribution. Plin4 is mainly found in white adipose tissue and are expressed in low levels in heart and skeletal muscle. Although Plin4 and Plin1 both are expressed in adipocytes, they are located in different lipid droplet pools. Plin4 have been shown to translocate to nascent LDs upon lipid loading in adipocyte, thus participating in the early formation of lipid droplets.[99] However, Plin4 deficiency in mice resulted in an unperturbed adipocyte differentiation and development. Interestingly, the *Plin4*$^{-/-}$ had a dramatically reduced triglyceride content in the heart. This was associated with a reduction in Plin5, whose gene is located immediately upstream of Plin4. It remains to be investigated whether the targeting of *Plin4* had an impact on the transcriptional action at the *Plin5* locus.[91,100]

1.8.5 Plin5

Plin5 is predominantly expressed in tissues with high mitochondrial β-oxidation such as the heart, skeletal muscle, liver and BAT. Plin5 expression is increased under conditions that promote fatty acid elevation, including fasting and exercise.[101] The Plin5 gene has been reported to be transcriptionally regulated by members of the peroxisomal proliferator-activated receptors (PPARs). PPARα is expressed in fatty acid metabolizing tissues and is activated under conditions of energy deprivation. Cardiac expression of Plin5 can be induced by PPARα agonists.[80, 102, 103] However, although PPARα$^{-/-}$ mice displays reduced levels of Plin5 in the basal state, the expression of Plin5 was induced by fasting, suggesting additional

regulatory control of Plin5.[104] One additional regulator may be PPARβ/δ, which has been shown to induce Plin5 in skeletal muscle.[105]

Similar to other perilipins, Plin5 prevents uncontrolled lipolysis of lipid droplets. ATGL mediated lipolysis was shown to be inhibited in lipid droplets in cells either derived from cardiac tissue of mice overexpressing cardiac Plin5 or from COS-7 cells overexpressing recombinant Plin5.[106] Plin5 has been shown to interact with ATGL, HSL as well as CGI-58. [107, 108, 109] In contrast to Plin1-regulated lipolysis, the exact mechanism is not fully understood. However, the binding of Plin5 to ATGL or CGI-58 has been proposed to prevent their interaction and thereby reduce lipolysis.[101] Further, Plin5 is a substrate for PKA phosphorylation,[107] and PKA treatment have been reported to stimulate fatty acid release *in vitro* from lipid droplets enriched with Plin5.[110] Altogether, this suggests that Plin5 functions as a barrier towards lipolysis of the lipid droplet in the basal state. Possibly, in response to β-adrenergic stimulation, PKA unlocks the Plin5 barrier function and thereby promotes hydrolysis of the triglycerides.

Global *Plin5*$^{-/-}$ mice have normal growth rates, organ weights, and lean and fat masses compared with their wild type littermates.[111, 112] Deletion of Plin5 resulted in reduced levels of triglycerides in the heart and red oxidative muscle.[111] Overexpressing Plin5 in skeletal muscle by gene electrophoresis led to an increased triglyceride pool and lipid droplet size, supporting the role of Plin5 in protection of lipid droplets.[113]

Close physical and functional interaction between lipid droplets and mitochondria is crucial for the function of highly oxidative tissues with a fluctuating energy demand. A mitochondrial recruitment domain positioned in the C terminal of Plin5 has been identified and overexpression of Plin5 in fibroblasts mediates a close association of lipid droplets and mitochondria.[114] Also, mitochondrial and Plin5 association are increased after electrically induced contraction in rat skeletal muscle together with a reduction in the triglyceride pool.[115] Thus, this suggests that Plin5 facilitates the transfer of fatty acids from lipid droplets to mitochondria.

2 AIM

The overall aim of this study was to investigate cardiac lipid accumulation and the consequences of altered lipid storage and metabolism following myocardial ischemia.

Specific aims

1) To investigate the derangements of cardiac lipid metabolism in a porcine model of ischemia/reperfusion.

2) To investigate the role of the lipid droplet protein Plin5 in myocardial lipid dynamics, cardiac function, and outcome after myocardial ischemia.

3) To investigate the role of the lipid droplet protein Plin2 in myocardial lipid metabolism, heart function and outcome after an induced myocardial infarction.

3 METHODOLOGICAL CONSIDERATIONS

In this section, considerations regarding selected methods in human subjects, animal models and cell culture are discussed. A more detailed descriptions of all methods and material and are included in the enclosed papers.

3.1 Human studies

To study genetic variance of PLIN5 gene, 468 patients with clinically suspected coronary artery disease were recruited. Four SNPs in PLIN5 were successfully genotyped in 466 of 468 patients using TaqMan assays. The four SNPs was selected based on an expected minor allele frequency >5%, no or weak linkage disequilibrium, and presumed potential effects on protein function (mediated by amino acid change) and protein concentration (mediated by mRNA stability and splicing pattern). The heart function of the patients was examined with myocardial perfusion scintigraphy and standard and stress echocardiography.

Myocardial perfusion scintigraphy is used to detect and localize perfusion defects. Myocardial perfusion images are acquired by injecting a radiotracer intravenously. The isotope is extracted from the blood by viable myocytes and retained there for a period of time. A gamma camera captures the photons and converts the information into digital data reflecting the magnitude of tracer uptake and location of the emission.[116] The resulting myocardial perfusion images show the presence, location, extent and severity of myocardial perfusion abnormalities. By comparing images acquired during rest and stress the defects can be determined to be either (1) reversible, reflecting stress-induced ischemia, or (2) irreversible, implying myocardial infarction. Patients with normal myocardial perfusion scans have a low rate (<1%) of future annual death or non-fatal myocardial infarction.[117] However, the greater the extent and severity of ischemic perfusion abnormalities the larger is the risk of adverse events.[118]

To assess abnormalities in wall motion of the heart, stress echocardiography can be used. 2D echocardiography images are obtained at rest and during

physical or pharmacological stress. The analysis and scoring of the regional wall motion are usually done using a 17 segment model of the left ventricle. In a normal response, a segment is normokinetic at rest and normal or hyperkinetic during stress. However, during ischemia the segment is normal at rest but displays an abnormal movement during stress. An infarcted area has abnormal movement both during rest and stress. Reduction in contractile function reflected by abnormal wall motion appears immediately during acute ischemia and infarction.[119]

3.2 *In vivo:* Animal models

3.2.1 *Plin5*$^{-/-}$ mice

A global *Plin5*$^{-/-}$ mice was kindly provided by K.T Dalen. Briefly, using homologous recombination, exons 4 to 6 of the *Plin5* gene were replaced with a hygromycin selection cassette flanked by FRT_5-sites and restriction sites. The Plin5-KO targeting vector was electroporated into embryonic stem (ES) cells and positive ES clones were injected into C57/Bl6 blastocysts. The obtained chimera was mated with a female 129/SvEv to confirm germ line in a pure 129/SvEv background. The mice were then backcrossed to a C57BL/6JBomTac background. Heterozygote breeding was used and WT littermates (*Plin5*$^{+/+}$) were used as controls.

The hygromycin selection cassette was not removed from the mutated PLIN5 gene in the *Plin5*$^{-/-}$ mice. A retained selection cassette has previously been reported to influence the gene expression.[120, 121] However, in a knockout model a truncated protein, or more often, no protein is produced. Thus, the retained selection cassette is of less importance in our *Plin5*$^{-/-}$ model.

Plin5 is globally deficient in our mouse model. The protein is expressed in highly oxidative tissues, such as the heart, liver and skeletal muscle. However, we are investigating the role of Plin5 in the heart. The Plin5 deficiency in the liver could influence the hepatic secretion of glucose and lipids into the circulation and hence affect the concentration of substrates available to the heart. However, when feeding the *Plin5*$^{-/-}$ mice a regular chow diet, the systemic levels of triglycerides, cholesterol, glucose and insulin did not differ between the WT and *Plin5*$^{-/-}$ mice. Thus, our experiments were conducted in mice fed a regular chow diet.

3.2.2 *Plin2*$^{-/-}$ mice

Whole-body *Plin2*$^{-/-}$ mice was kindly provided by K.T Dalen. To generate the *Plin2*$^{-/-}$ mice, the exons 4-6 were deleted using Cre-LoxP recombination. The mice were fed chow diet *ad libitum.* The mice were fasted 4 hours prior to experiments. Circulating levels of glucose, insulin, cholesterol and triglycerides did not differ between WT and *Plin2*$^{-/-}$ mice.

There are previously two Plin2 mutant models characterized. In the first model, exons 2 and 3 were deleted in the PLIN2 gene resulting in the absence of a full length Plin2 protein. In this *Plin2*$^{(\Delta 2,3/\ \Delta 2,3)}$ mouse, a bioactive large C-terminal variant of Plin2 was shown to be expressed in some tissues, however not in the liver.[91, 122] In the second Plin2 knockout model, *Plin2*$^{(\Delta 5/\ \Delta 5)}$, exon 5 was deleted and the mice had no detectable expression of the Plin2 protein. In both models, the Plin2 deficiency resulted in reduced hepatic lipid droplets following high fat diet. In agreement with our *Plin2*$^{-/-}$ model, the plasma levels of glucose, insulin, cholesterol and triglycerides was unchanged in the *Plin2*$^{(\Delta 2,3/\ \Delta 2,3)}$ mice on a chow diet.[93] In contrast, when fed a high fat diet, the *Plin2*$^{(\Delta 5/\ \Delta 5)}$ mice had reduced levels of insulin, fatty acids and triglycerides.[92]

3.2.3 Porcine model

Female pigs of a mixed Swedish, Pigham and Yorkshire race were used for an ischemia/reperfusion model. They were 3–4 months old and weighed 38–46 kg. The pigs were bred in Swedish farms and brought to the animal laboratory one week prior to the procedure.

3.3 Models of myocardial infarction

3.3.1 Induction of myocardial infarction in pig

To induce ischemia/reperfusion in pig, an angioplasty balloon was inflated in the left anterior descending artery distal to the second diagonal branch. After 40 min of occlusion, the balloon was deflated and the heart was reperfused. After 4 hours of reperfusion, the chest was opened and an angioplasty balloon was inflated at the same location as before. Evans blue was infused, followed by an injection of a lethal dose of potassium chloride.

3.3.2 Induction of myocardial infarction in mouse

The induction of myocardial infarction in mice is described in detail in the method section in Paper II. Briefly, an incision was made between the 4th and 5th ribs to reveal the upper part of the anterior LV wall and the lower part of the left atrium. A myocardial infarction was induced by ligating the left anterior descending coronary artery immediately after the bifurcation of the left coronary artery. After verification of the infarction, the chest was closed.

A comparison of the infarctions models

There are differences in our infarction models as the experimental procedure ought to be adjusted to the size and characteristics of the animal. The size of the pig makes it possible to perform a closed-chest protocol with an occlusion of the LAD with an inflated balloon. This resembles more closely the clinical situation were the artery are occluded by a plaque in vessel and the risk of external damages when ligating the vessel is eliminated. Further, the size of the pig heart enables separation of the area at risk (AAR), infarct area (IA) and the non-ischemic control area. Hence, it is possible to analyze lipid accumulation and the gene expression in every separate area (control, AAR and IA) of the infarcted heart. Another crucial difference between the models is the presence of reperfusion in the pig infarction protocol. This resembles more closely the optimal clinical situation where a patient with a myocardial infarction is revascularized within a short period of time.

The mouse is widely employed in studies of experimental myocardial infarction, in part because of the possibility of genetic manipulation. The mouse as a model has several other advantages, *e.g.* their small size and short life span makes them easy and less expensive to house. Further, in mice, the long-term remodeling of the heart and survival following a myocardial infarction can be studied.

Species differences of the cardiac vasculature

The rational for using a porcine model is the large homology with the human cardiovascular system. The heart of the pig is approximately the size of the human heart. More importantly, the coronary artery pattern and cardiac physiology in the pig heart is very similar to humans. Potential residual flow to ischemic tissue is an issue in animal models of myocardial infarction. Importantly, the low collateral circulation in pig resembles the human collateral flow during normal coronary conditions.[123-125]

The coronary arteries of the mouse are not characterized to the same degree as the human coronary vasculature. Mice have two major coronary arteries, both of which originate in or slightly above the aortic sinus. The left coronary artery generally crosses over the left ventricle and gives off variable branches.[126, 127] A branch has been described as LAD in mice because of its similarity to the LAD in human. However, in humans the LAD supplies approximately the anterior aspect of the LV wall and the anterior two thirds of the septum. In mice, ligation of the LAD gives rise to infarction of the free wall of the LV extending to the apex whereas the septum is unaffected.[128]

3.4 *Ex vivo:* Langendorff

We have used the Langendorff model system in order to study the intrinsic metabolism of the heart. This system enables the study of an isolated heart without confounding effects of other organ systems and exocrine control. Briefly, the mouse aorta was cannulated and perfused in a Langendorff mode at a constant pressure of 70 mm Hg. Hearts were perfused with Krebs-Henseleit buffer containing 11 mM glucose and 0.4 mM palmitate (bound to 1% fatty-acid free bovine serum albumin), gassed with 95% O_2 and 5% CO_2. To measure functional changes during the perfusion protocol, a fluid-filled balloon was inserted into the left ventricle, inflated to achieve an end-diastolic pressure of 5-10 mm Hg.

In the Langendorff methodology, the heart is perfused by cannulating the aorta. The perfusion buffer is hence flowing in opposite direction compared to the physiological blood flow. This pressure causes the aortic valve to close, and the column of buffer in the aorta causes the filling of the coronary artery vasculature via the two coronary ostia in the aortic root. The perfusion buffer then flows through the vascular bed before being drained through the coronary sinus in the right atria. The effluent is ejected through the pulmonary artery and is allowed to drip from the heart. Thus, the ventricles are not filled with perfusion buffer.[129]

3.5 *In vitro:* HL1 cells

The HL-1 cell line is a cardiac muscle cells line derived from the AT-1 mouse atrial cardiomyocyte tumor lineage. The HL-1 cells contracts

spontaneously and can, in contrast to primary cardiomyocytes, be serially passaged. The HL-1 cell line has been extensively characterized, and has been shown to have a gene expression pattern similar to adult atrial myocytes.[130, 131] The HL-1 cells was cultured in Claycomb medium supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 10 % fetal calf serum. Norepinephrine was added to the medium to enable the cells to contract.

Plin5 is expressed in HL-1 in a very low extent. This is consistent with other cell lines, such as the murine myoblast cell line C2C12.[105] For that reason, we have not used HL-1 cell line for studies regarding Plin5. However, the unphysiological expression of Plin5 might have an impact on the function and expression of Plin2 and other lipid droplet proteins. Thus, additional model systems, *i.e.* Plin2 deficient mice, are required to complement our studies on the function of Plin2.

4 RESULTS

4.1 Paper I

In this paper, we investigated the derangements of cardiac lipid metabolism in a porcine model of ischemia/reperfusion.

We investigated lipid accumulation in 7 pigs subjected to 40 min of ischemia followed by 4 h of reperfusion. The pig hearts was separated into the infarct area (IA) with irreversible injury and the area at risk (AAR) subjected to ischemia but with reversible injury. Non-ischemic biopsies from the lateral wall served as a control. Oil red O staining of cryosections from the heart biopsies showed an increase in lipid droplet accumulation in the AAR and IR. Interestingly, the triglyceride content was not altered in the ischemic areas whereas the cholesteryl ester content was highly increased. The VLDLr have previously shown to be up-regulated after an induced myocardial infarction in mice.[24] We analyzed the expression of VLDLr and did not observe an increase in this receptor. However, the elevated cholesteryl ester concentration led us to examine the expression of LDLr and LRP1 and we found that the expression of these receptors was greatly increased. Thus, our data indicates that LRP1 and LDLr mediate an increased uptake of cholesteryl esters in the porcine heart.

Elevated levels of bioactive lipids such as ceramides have been reported to cause impaired heart function. When analyzing the ceramide content we found increased levels in the IA but interestingly not in the AAR, suggesting that reperfusion normalize levels of ceramides in the viable area. Ceramides are synthesized *de novo* or from sphingomyelin. However, we found that sphingomyelin levels were decreased in AAR as well as in IA compared with control tissue.

Finally, we examined the inflammation and ER stress in our porcine model of ischemia/reperfusion. The cytokines IL-1b and IL-6 were dramatically increased in the AAR and the IA. Further, we found an increase in ER stress markers calnexin and GRP78 in the AAR and IA. Interestingly, the pro-apoptopic marker CHOP was upregulated only in the AAR.

In conclusion, we found that ischemia/reperfusion promoted cholesteryl ester accumulation mediated by the LDLr and LPR1 in the porcine heart. Further, we found increased levels of inflammation and ER stress in the AAR and IA. In addition, we found increased ceramide accumulation in the infarcted area of the heart. Thus, our results indicate that lipid accumulation in the heart is one of the metabolic derangements remaining after ischemia, even in the myocardium bordering the infarct area.

4.2 Paper II

In this paper, we investigated the role of Plin5 in myocardial lipid dynamics, cardiac function, and outcome after myocardial ischemia. Here, we studied the impact of polymorphism in the PLIN5 gene following myocardial ischemia in human subjects followed by mechanistic studies of the role of Plin5 in lipid metabolism and cardiac function in Plin5 deficient mice.

We could show that a single nucleotide polymorphism in the PLIN5 gene was associated with impaired heart function following myocardial ischemia, indicating that PLIN5 function is relevant to human cardiac physiology.

In the *Plin5*$^{-/-}$ mouse hearts, we found dramatically decreased levels of triglycerides and a small decrease in diglycerides compared to the WT. However, no other lipid species was affected. Interestingly, the cardiac fatty acid uptake was diminished in the *Plin5*$^{-/-}$ mice and the incorporation into triglycerides was almost abolished, suggesting a compensatory inhibition of the fatty acid uptake due to the decreased lipid storage capacity of the *Plin5*$^{-/-}$ hearts. Further, we could show an increased uptake of glucose in the *Plin5*$^{-/-}$ hearts and an increased utilization of glucose in isolated *Plin5*$^{-/-}$ hearts compared to WT. Thus, our data indicates that *Plin5*$^{-/-}$ hearts have altered substrate preference and shift from fatty acids to glucose utilization.

Plin5 have been shown *in silico* to possess a mitochondrial recruitment domain. In agreement with this, we observed *in vivo* that the distance between the lipid droplets and the mitochondria was increased in the *Plin5*$^{-/-}$ hearts compared to the WT hearts. In addition, we found that mitochondria isolated from Plin5 deficient hearts had a reduced membrane potential and a slightly reduced ATP production from succinate and pyruvate. Lipidomic

analysis of the isolated mitochondrial membrane from WT and *Plin5*$^{-/-}$ hearts revealed an altered fatty acid composition of PC and PE in the Plin5 deficient mice. Thus, the altered fatty acid length in the mitochondria of *Plin5*$^{-/-}$hearts may explain the reduced mitochondrial potential.

We assessed how the altered metabolism in the Plin5 deficient mice affects the heart function using ultrasound. The heart function during baseline conditions was close to normal in the *Plin5*$^{-/-}$ mice compared with the WT mice. However, when stressing the WT and *Plin5*$^{-/-}$hearts with the β-adrenergic agonist dobutamine, the heart function was severely reduced in the *Plin5*$^{-/-}$ mice. Further, deficiency of Plin5 resulted in a reduced survival after an induced myocardial infarction. Thus, our data indicates that *Plin5*$^{-/-}$ mice maintain a relatively normal heart function under baseline conditions, but their cardiac function is significantly reduced after hormonal or ischemic stress, resulting in increased mortality.

We hypothesized that the *Plin5*$^{-/-}$ mice are in a state of low substrate availability after myocardial ischemia. Therefore, we investigated the palmitate oxidation in isolated hearts in a model low flow and of high workload. We found a trend towards a decreased glycolysis in the *Plin5*$^{-/-}$ hearts in the model low flow. However, a model of high work load is more representative to our situation after an induced myocardial ischemia. In this model, we found that *Plin5*$^{-/-}$ hearts had slightly decreased substrate utilization from palmitate compared with WT hearts. In addition, when investigating the glycogen stores after myocardial infarction we found that the glycogen was almost abolished in the non-infarct areas in the *Plin5*$^{-/-}$ hearts. This suggests that the reduced substrate availability force *Plin5*$^{-/-}$ hearts to use the endogenous glycogen stores for energy. Consequently, our results indicate that *Plin5*$^{-/-}$ hearts have reduced substrate availability, resulting in inefficient energy utilization.

In conclusion, our results suggest that Plin5 regulates the metabolic flexibility of the heart and plays a key role in cardioprotection during myocardial ischemia.

4.3 Paper III

In this paper, we investigated the role of Plin2 in myocardial lipid metabolism, heart function and outcome after an induced myocardial infarction.

Here, we studied the lipid droplet protein Plin2 in HL1 cardiomyocytes and *Plin2*$^{-/-}$ mice. First, we analyzed the expression pf Plin2 after oleic acid treatment of HL1 cells. The mRNA expression of Plin2 was unchanged after oleic acid supplementation. However, the protein expression was increased after treatment. In agreement with previous studies, this indicates that the protein is stabilized by increased lipid accumulation.

Since deficiency of Plin5 resulted in dramatically reduced triglyceride content, we analyzed the lipid content in the hearts of the *Plin2*$^{-/-}$ mice. Surprisingly, we found markedly increased levels of triglycerides. One hypothesis was that the increased triglyceride accumulation was due to an elevated lipid uptake. However, the circulating levels of fatty acids and triglycerides were unaltered suggesting that the increased triglyceride content in the heart was not caused by an elevated availability of plasma lipids. Further, although we found increased expression of PPARγ, we did not observe a concomitant increase in the PPARγ regulated fatty acid transporters CD36 and FABP. We continued by analyzing the uptake of oleic acid, glucose and VLDL in control and Plin2 knockdown HL1 cells. However, our results showed that Plin2 deficiency does not alter the substrate uptake in HL-1 cardiomyocytes. Next, we wanted to repeat the fatty uptake studies *in vivo* using *Plin2*$^{-/-}$ mice. In agreement with the cell culture studies, we found that there were no differences in the uptake of fatty acids in the hearts of WT and *Plin2*$^{-/-}$ mice. There was also no difference in ability to incorporate palmitate into triglycerides. Our findings show that the increased amount of triglycerides in the *Plin2*$^{-/-}$ hearts is not due to increased uptake of fatty acids. Further investigations are needed to elucidate potential differences in lipid uptake, *e.g.* LPL mediated VLDL uptake.

Lipid droplets in the heart are also coated with Plin3, 4, and 5. Therefore, we analyzed the expression of these perilipins in the WT and *Plin2*$^{-/-}$ hearts.

Interestingly, the protein expression of Plin3 and Plin5 was significantly increased in the *Plin2*$^{-/-}$ hearts compared to WT. These findings indicate the Plin2 deficient heart compensates for the absence of Plin2 by upregulating the levels of other perilipins.

Finally, we examined whether the deficiency in Plin2 affected the heart function. We did not find any difference between the WT and *Plin2*$^{-/-}$ mice under baseline conditions or after dobutamine stress. However, the heart function was compromised after an induced myocardial infarction.

In conclusion, our results indicate that Plin2 is important for myocardial lipid storage and plays a role for cardiac function following myocardial ischemia.

5 DISCUSSION

In paper I, we showed that lipids accumulate in the porcine myocardium following myocardial ischemia mediated by LDLr and LRP1. In paper II and III, we proceeded to investigate the consequences of an altered lipid storage regarding lipid metabolism, heart function and outcome after myocardial ischemia. In order to investigate altered lipid storage capacity, we used two different models of genetically modified mice deficient in the lipid droplet proteins Plin2 and Plin5.

Key findings of this study as well as questions beyond the individual papers will be discussed in this section.

5.1 Lipid accumulation in the ischemic heart

Myocardial ischemia is associated with alterations in cardiac metabolism and has been reported to promote lipid accumulation in the heart.[24, 44]

We have shown that cholesteryl esters accumulate in the heart of our porcine model of ischemia/reperfusion. We found an increased expression of LDLr and LRP1 in the infarct area and the ischemic area bordering the infarct area (AAR). Whether the bulk of the cholesterol uptake is mediated via LDLr or LRP1, or a combination of both is a matter of speculation. LRP1 and cholesteryl esters have previously been shown to be upregulated in pig heart following 90 min of ischemia without reperfusion.[45] The LRP1 gene has been reported to contain two hypoxia responsive elements, indicating that hypoxia induced expression HIF1α are involved in the upregulation of LRP1.[132] In addition, we have analyzed the pig LDLr promoter for HIF1α binding sites and did identify one site in the LDLr promoter at position +10 (data not shown). However, the HIF1α site did not have a corresponding site in the promoters of mice and humans and the significance of this site is uncertain. Alternatively, the increased expression of LDLr in our model may be due to reperfusion of the heart following ischemia. Reperfusion induces inflammation and in agreement with this, we have reported markedly increased expression of IL-1β in the ischemic areas of the pig heart. IL-1β has previously been shown to disrupt the feedback mechanism that

downregulates the LDLr expression in response to high intracellular cholesterol levels.[133] This resulted in an excess of LDL uptake via the vascular smooth muscle cells. However, further studies *in vivo* are required to elucidate if inflammation is a mediator of increased cardiac LDLr expression. Altogether, HIF1α and inflammation could be important inducers of lipoprotein receptor expression following ischemia-reperfusion in pig heart, resulting in elevated levels of cholesteryl esters.

We see a similar increase in cholesteryl esters in mouse hearts compared to pig hearts following myocardial ischemia (data not shown). In contrast, increased cardiac content of triglycerides following myocardial ischemia was observed in mice but not in our porcine model. Accumulation of excess cholesteryl esters may be detrimental for the heart function. Excess of free cholesterol results in high membrane rigidity, which is toxic to the cell. The liver can eliminate excess cholesterol via the bile.[134] The heart lacks such effective elimination pathways and regulates the levels of free cholesterol mainly by uptake and esterification of cholesterol for storage in lipid droplet. Indeed, high cholesterol diet has been shown to result in an increased membrane cholesterol content and systolic and diastolic dysfunction.[135] Further, the accumulation of lipids, including cholesteryl esters, in the myocardium have been associated with dilated cardiomyopathy.[136] Thus, pharmaceutical targeting of cholesteryl ester accumulation in heart following myocardial ischemia is an interesting future research area.

In addition to accumulation of cholesteryl esters, we could show increased levels of ceramides in the infarcted area. Ceramides are considered as bioactive lipids and can cause cellular dysfunction and apoptosis following myocardial ischemia.[55] In agreement with our results, ischemia has been reported to increase levels of ceramides.[24, 55, 137] In contrast, ischemia with a subsequent reperfusion further increased ceramide accumulation in the heart.[55, 137] We did observe elevated levels of ceramides in the infarct area, but interestingly not in the ischemic area bordering the infarction (AAR). Whether ceramide levels were normalized after 4 hours of reperfusion or did not increase at all in the peri-infarct zone remains to be elucidated

Our results indicate that accumulation of cholesteryl esters and ceramides in the heart is one of the metabolic derangements remaining after ischemia.

Normalizing lipid levels in the myocardium after ischemia would likely improve myocardial function and should be considered as a target for treatment.

5.2 The role of lipid droplet proteins in cardiac metabolism

Neutral lipids are stored in lipid droplets which are coated with numerous proteins with various functions. In the heart, the lipid droplet proteins Plin2 and Plin5 are of importance for the function and protection of the lipid droplet. Genetic deletion of Plin5 and Plin2 in mice thus provided us with models for studying the influence of altered lipid storage capacity on lipid metabolism, heart function and outcome after myocardial ischemia.

Deficiency of Plin5 results in dramatically lowered levels of triglycerides in the myocardium. This result was expected, since one of the roles of Plin5 is to protect the lipid droplets against lipases. Indeed, the lipid droplets in the cardiomyocytes of the *Plin*5$^{-/-}$ mice were smaller and fewer compared to the lipid droplets in the WT. However, deficiency of Plin2 unexpectedly resulted in increased levels of cardiac triglycerides. In contrast, previous studies in *Plin*2$^{-/-}$ mice have reported decreased lipid accumulation in the liver.[92, 93] The increased triglyceride levels in the heart of our *Plin*2$^{-/-}$ mice was not the result of increased fatty acid uptake; the plasma levels of triglycerides did not differ between the WT and *Plin*2$^{-/-}$ mice and no increased fatty acid uptake was observed in *Plin*2$^{-/-}$ hearts. However, the protein expression of Plin5 and Plin3 was increased in the Plin2 deficient hearts. This finding is in agreement with previous reports showing that Plin5 protein is upregulated in the liver of *Plin2*$^{-/-}$ mice lacking leptin.[138] These results suggest that Plin3 and Plin5 are more protective against lipases than Plin2 in the heart. However, if and how additional Plin3 and Plin5 are recruited to the lipid droplet from the cytosol in the absence of Plin2 should be further studied. Also, the possibility of an increased lipid accumulation resulting in elevated levels of Plin3 and Plin5 cannot be excluded. A reduction in lipid oxidation or an increased uptake of other lipid sources than albumin-bound fatty acids *Plin2*$^{-/-}$ hearts would result in increased lipid content.

The *Plin*5$^{-/-}$ mice had decreased levels of cardiac triglycerides whereas the *Plin*2$^{-/-}$ mice instead displayed increased levels of triglycerides. However, the outcome following myocardial ischemia was similar. The *Plin*5$^{-/-}$ mice had severely reduced heart function after dobutamine stress and a reduced survival following myocardial infarction. We have shown that Plin5 facilitates the association between the lipid droplet and the mitochondria. This connection between the storage and utilization of lipids is of crucial in the heart that has a high and fluctuating energy demand. The *Plin5*$^{-/-}$ mice hearts have a diminished contact between mitochondria and lipid droplets combined with a reduced storage pool of lipids. Thus, our data suggest that the Plin5$^{-/-}$ mice have reduced substrate availability, resulting in increased mortality following myocardial ischemia. Plin2 deficiency resulted in a milder heart phenotype than the Plin5 deficient hearts with a normal heart function at baseline and after dobutamine stress. However, the *Plin*2$^{-/-}$ mice had a compromised heart function compared to the WT mice following myocardial ischemia. Levels of cardiac ceramide, triglycerides and cholesteryl esters were increased to similar levels in *Plin*2$^{-/-}$ and WT mice following ischemia, indicating lipotoxic aggravation of heart function to be less likely in the *Plin*2$^{-/-}$ mice.

Our data indicates that deficiency in Plin2 or Plin5 results in a compromised heart function although through different mechanisms. Although Plin5 is the most important lipid droplet protein in the normal heart, a balance between perilipins on the lipid droplet may be of importance for its proper function. A study in human skeletal muscle suggests a preferential utilization of Plin2 coated lipid droplets during moderate-intensity exercise.[139] It remains to be examined if this also occurs in the surviving part of the left ventricle after myocardial ischemia which experiences a high work load. Plin5 deficiency and a decreased triglyceride pool resulted in reduced lipid utilization and heart function at a high workload. The processes of storage and utilization of lipids are closely connected; therefore, lack of Plin2 on lipid droplets most likely also alters the flux and utilization of lipids and by that influences the heart function following myocardial ischemia

Altogether, our data shows that presence of lipid droplet proteins and a functional regulation of lipid droplets are crucial for the function of the ischemic heart.

5.3 Regulation of lipid uptake and storage

Cardiac lipid droplets play an important role in balancing the fluctuations in lipid availability and requirements of metabolic energy. However, little is known of the interplay between the lipid storage and lipid uptake.

The Plin5 deficient mouse heart displayed smaller and fewer lipid droplets compared to the WT mice and thus had decreased storage capacity. The first assumption would be that more fatty acids is shunted towards β-oxidation However, we could show that β-oxidation is unchanged. Interestingly, the fatty acid uptake is decreased in the Plin5 deficient hearts. This suggests that the heart of the *Plin*5$^{-/-}$ mice can adjust its fatty acid uptake in order to compensate for the decreased storage capacity. The mechanism to lower the fatty acid uptake remains to be elucidated. The altered lipolysis of the triglyceride pool in Plin5 deficient hearts likely changes the available amount and of fatty acids in the cardiomyocyte. These fatty acids can act as ligands to PPARs which in turn activates genes involved in lipid metabolism, including lipid uptake. Alteration of lipolysis by both deletion and overexpression of ATGL in mice has been shown to reduce cardiac expression of PPARα/δ target genes.[140, 141] Thus, decreased or increased activation of PPARs in the *Plin*5$^{-/-}$ mice compared to the WT may contribute to the decreased fatty acid uptake. In agreement with this hypothesis, the mRNA expression of CD36 is decreased in the myocardium of the *Plin*5$^{-/-}$ mice. The uptake of fatty acids is induced by a translocation of CD36 from intracellular compartments to the sarcolemma. Most stimuli that translocate of GLUT4 to the sarcolemma, such as contraction, AMPK and insulin signaling, similarly affect CD36 translocation.[33] Interestingly, we observed a reduced uptake of fatty acids in the hearts of *Plin*5$^{-/-}$ mice whereas the glucose uptake was increased. Similarly, a differential translocation of CD36 and GLUT4 was reported in a model of global ischemia in isolated hearts.[22] Thus, regulation of GLUT4 and CD36 translocation can occur by different mechanisms. This regulation may involve signaling pathways or the subcellular trafficking machinery involved in the translocation of CD36 and GLUT4. Cytoskeleton reorganization, v-ATPases (regulating endosomal acidification), Rab and SNARE proteins have been reported to be essential for the translocation process.[33] Further research is required to investigate how absence of Plin5 results in differential lipid and glucose uptake.

Interestingly, our results suggest that hearts with diminished capacity to store lipids can compensate by markedly decreasing fatty acid uptake. The lipid uptake may be regulated by signaling though fatty acids or by the subcellular trafficking machinery of CD36 translocation.

5.4 Survival following myocardial ischemia

The induction of a myocardial infarction by ligation of a coronary artery results in ischemia in the part of the heart normally supplied by the ligated artery. Thus, the infarction induces a substantial stress on the heart function.

Our Plin5 deficient mice had a reduced survival following myocardial ischemia compared to the WT mice. The infarct size after an induced myocardial infarction was comparable between the WT and *Plin5*$^{-/-}$ mice, indicating that Plin5 deficiency did not play an important role in the expansion and size of the infarct area. In agreement with this, we found that patients carrying a polymorphism in the PLIN5 gene responded worse to ischemia, but the genetic variation did not give rise to increased ischemia. Following a myocardial infarction, the ischemic and subsequent infarcted area is unable to contract resulting in a highly increased workload of the surviving left ventricle. The increased workload results in a higher energy demand of the heart. This is detrimental for the *Plin5*$^{-/-}$ hearts, because of their reduced energy substrate availability.

Thus, our data suggests that deficiency of Plin5 and the resulting reduction in substrate availability and ineffective substrate utilization in the non-ischemic left ventricle resulting in reduced survival.

5.5 Lipid utilization and storage

The healthy heart primarily uses fatty acids to produce ATP, but is able to shift substrate preference due to physiological and pathological challenges. For instance, the ischemic or failing heart shifts from fatty acid oxidation towards glucose utilization. A general consensus has considered this shift to be a beneficial oxygen-sparing mechanism for the heart. However, oxidation of fatty acids is far more energy efficient compared other energy substrates, thus, a large amount of glucose is needed to compensate for a decrease in

fatty acid oxidation. This suggests a shift from fatty acid oxidation to be less beneficial to the ischemic/failing heart.

We could show that Plin5 deficiency resulted in a reduced myocardial triglyceride pool. In baseline conditions, the *Plin5*$^{-/-}$ mice could compensate by increasing substrate utilization from glucose, and by that preserving the energy balance. However, during high workload the isolated *Plin5*$^{-/-}$ heart had a decreased utilization from fatty acids and a severely reduced heart function. There are mouse models investigating changes in fatty acid oxidation, *e.g.* *LCAD*$^{-/-}$ mice. Deletion of *LCAD* results in deficient mitochondrial long-chain fatty acid β-oxidation. In the fed state, these mice relied on glucose oxidation and had a normal energy status.[142] However, during fasting when the heart normally depends almost exclusively on fatty acid oxidation,[34] the *LCAD*$^{-/-}$ mice instead had a sustained glucose uptake compared to the fed state. However, this was insufficient to maintain the energy status resulting in reduced cardiac performance.[142] Together, this indicates that fatty acid utilization and a cardiac metabolic flexibility is crucial to maintain heart function.

Whether cardiac lipid accumulation is unfavorable for the heart is debated. Our Plin5 deficient mice have a reduced content of triglycerides and diglycerides and, interestingly, unaltered levels of ceramides. Heart function is severely reduced during stress in the *Plin5*$^{-/-}$ mice. There are other mouse models with altered lipid storage. Deletion of the lipase ATGL results in severe accumulation of lipids, reduced cardiac fatty acid oxidation, and lethal cardiomyopathy.[141, 143] On the other hand, overexpression of DGAT1, which catalysis the syntheses of triglycerides from diglycerides, also results in an increased content of triglycerides. However, the content of ceramides and diglycerides was reduced and the cardiac function was unaffected.[51] Thus, this indicates that the lipid dynamics and metabolism are important for the maintenance of cardiac function and not levels of cardiac lipids *per se*.

In conclusion, a maintained energy efficient fatty acid oxidation is favorable in stress conditions.

5.6 Clinical implication

Here, the clinical implications of our results will be discussed. We have studied the role of Plin5 in a human cohort showing that Plin5 influences cardiac functions in humans. This suggests that targeting of lipid droplet proteins could potentially be a strategy to develop novel pharmaceutical treatment. In addition, our studies on lipid storage and metabolism following myocardial ischemia in mice contribute to the development of future cardiac metabolic therapies for cardiovascular diseases.

Our results show that Plin5 influences cardiac functions in humans. A single nucleotide polymorphism in the PLIN5 gene was associated with impaired heart function following myocardial ischemia in patients with suspected coronary artery disease. The carriers of the minor allele of the SNP had slightly lower gene expression of Plin5. In the future, identifying low levels of Plin5 in patients with high risk of cardiovascular disease would represent a promising approach to decrease morbidity and mortality in this population.

The non-ischemic part of the left ventricle has an increased cardiac workload following myocardial ischemia, similar to the situation in the failing heart. Thus, our studies regarding storage and utilization of lipids in the non-ischemic parts following myocardial ischemia also contribute to the research field of heart failure. At present, pharmacological treatment of heart failure with neurohormonal antagonists, such as β-adrenergic blockers and angiotensin-receptor blockers, has successfully reduced heart failure mortality. However, the remaining disability and death rate remain high.[144] Because of the high energy consumption of the heart, even small variations in the efficiency of energy generation or utilization may have profound effect on cumulative energy levels in the cardiomyocyte. Thus, cardiac metabolic therapies represent promising targets for heart failure therapy.[144, 145]

The shift from fatty acid oxidation towards glucose utilization in the failing heart has been considered being a beneficial oxygen-sparing mechanism. Thus, metabolic therapies aiming to promote glucose oxidation have been tested. Enhancing glucose utilization in the ischemic and failing heart have been reported to improve cardiac function and symptoms of heart failure in the short term.[13] Dichloroacetate (DCA) promotes glucose oxidation by

inhibiting pyruvate dehydrogenase kinase (PDK). DCA treatment has been shown to improve recovery during reperfusion and also to improve cardiac function in right ventricular hypertrophy in multiple animal models.[146, 147, 148] However, human studies have reported inconsistent results regarding cardiac improvements of DCA treatment and long-term clinical trials have never been performed.[149]

On the other hand, reducing fatty acid supply to failing hearts seems to be harmful in spite of increased glucose uptake. The nicotinic acid derivative acipimox inhibits lipolysis in adipose tissue and by that decreases the circulating levels of fatty acids. Treatment with acipimox in patients with cardiomyopathic heart failure decreased fatty acid uptake by >80% and enhanced glucose uptake. However, this resulted in a reduction in cardiac work and efficiency.[150] Further, modulation of cardiac fatty acid utilization has been a target of metabolic therapy in heart failure. One target has been CPT1, the enzyme responsible for long chain fatty acid uptake in the mitochondria. Some studies have revealed beneficial effects of CPT1 inhibition in heart failure.[151] However, heterozygous CPT1b knockout mice showed an aggravated pressure overload-induced cardiac hypertrophy,[152] inducing concern about the safety and efficacy of CPT1 inhibition in heart failure patients. Instead, prevention of the metabolic switch toward glucose utilization in the hypertrophic mouse heart and a maintained fatty acid oxidation has been proposed to be beneficial to preserve myocardial energetics and cardiac function.[153]

Our study shows that a small triglyceride pool and reduced lipid utilization in the hearts of high workload is unfavorable to the heart function. Thus, our data suggests that pharmacological treatment strategies in order to maintain or increase lipid utilization are beneficial to the failing and post-ischemic heart.

6 CONCLUSION

In this study, I have investigated lipid accumulation in a porcine model if ischemia/reperfusion. Also, I have studied the consequences of an altered lipid storage regarding lipid metabolism, heart function and outcome after myocardial ischemia. I have reached the following conclusions:

- Ischemia/reperfusion in the porcine heart promoted cholesteryl ester accumulation mediated by the LDLr and LPR1.
- The lipid droplet protein Plin5 regulates metabolic flexibility of the heart and plays a key role in cardioprotection during myocardial ischemia.
- The lipid droplet protein Plin2 is important for myocardial lipid storage and plays a role for cardiac function following myocardial ischemia.

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