Myocardial metabolism in experimental infarction and heart failure

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A doctoral thesis at a university in Sweden is produced either as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarizes the accompanying papers. These papers have already been published or are in manuscript at various stages (in print, submitted or in manuscript).

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To my family

Abstract

The heart is an organ heavily dependent on exogenous lipids for the oxidative production of adenosine-triphosphate (ATP) and therefore maintenance of normal cellular energy homeostasis. However, high energy flux organs such as the heart must closely match lipid import and utilization or otherwise lipids will accumulate in the cardiomyocytes. Intracellular lipid accumulation has detrimental effects on cardiomyocyte function and viability and results in development of lipotoxic cardiomyopathy. Different pathophysiological states such as congestive heart failure (CHF), myocardial ischemia and hypertrophy are associated with myocardial lipid accumulation. The heart, however, produces and secretes apolipoprotein B containing lipoproteins (apoB), which enables the cardiomyocyte to export lipids. It has been proposed that apoB may be involved in cardioprotection by means of elimination of toxic intracellular lipids.

An important part of the patologic cardiac remodelling in CHF is disturbed myocardial energy metabolism. The failing myocardium contains low levels of creatine (Cr), phosphocreatine (PCr), and ATP. Cr depletion in the heart may result in disturbed energy production, transfer and utilisation of chemical energy and therefore compromised left ventricular function.

Growth hormone (GH) has been shown to exert numerous positive effects on the failing and remodelled heart suggesting that GH may be an additional agent in the treatment of CHF and myocardial infarction (MI).

The aims of this thesis were:

I. To investigate in vivo the effects of Cr depletion in mice on left ventricular function and morphology, energy metabolism and myocardial lipids.
II. To investigate importance of endogenous lipoproteins in the heart for cardiac function, morphology and survival in the settings of acute and chronic myocardial infarction and doxorubicine induced acute heart failure.
III. To investigate the effects of Growth hormone on arrhythmogenesis
IV. To evaluate the predictive value of native cardiac reserve on outcome after myocardial infarction in mice

Using a mouse model of chemically-induced Cr depletion we show in vivo that myocardial Cr depletion leads to disturbed energy metabolism, left ventricular dysfunction, pathologic remodeling and accumulation of intracellular triglycerides. These alterations are reversible upon the normalization of the creatine levels suggesting that creatine metabolism may be an important target for pharmacological interventions.

Using transgenic animals we show that myocardial apoB may be a cardioprotective system which is activated during ischemia, pathologic remodeling and heart failure and may be important for survival in myocardial infarction and heart failure.

We show that GH possess novel antiarrhythmic properties in the setting of acute MI which adds further evidence to the concept of GH as an additional pharmacological agent in the treatment of CHF and MI.

We demonstrate that native cardiac reserve is a predictor of post-MI survival.

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List of abbreviations

apoB	apolipoprotein B
ADP	adenosine-triphosphate
AGAT	L-arginine:glycine amidinotransferase
AMP	adenosine-diphosphate
ATP	adenosine-triphosphate
BGP	beta-guanidino proprionic acid
CHF	congestive heart failure
CK	creatine kinase
CO	cardiac output
Cr	creatine
CrT	creatine transporter
FA	free fatty acids
FS	fractional shortening
GAA	guanidinoacetate
GAMT	S-adenosyl-L-methionine:N-guanidinoacetate
HEP	high energy phosphometabolites
HPLC	high performance liquid chromatography
IR	ischemia reperfusion
LPC	lyso-phosphatidylcholine
LV	left ventricle
LVDd	left ventricular diameter in diastole
LVDs	left ventricular diameter in systole
LVEDd	left ventricular end-diastolic dimension
LVM	left ventricular mass
LVM/BW	left ventricular mass index
MI	myocardial infarction
MRS	magenetic resonance spectroscopy
MTP	microsomal transfer protein
NEFA	non-esterified fatty acids
NMR	nuclear magnetic resonance
PCr	phosphocreatine
PDE	phosphodiesters
P_i	inorganic phosphate
SCD	sudden cardiac death
SV	stroke volume

List of publications

This thesis is based on the following papers:

I

Lorentzon M., **Råmunddal T**., Bollano E., Waagstein F., Omerovic E

In vivo effects of myocardial creatine depletion on left ventricular function, morphology and energy metabolism in mice

Journal of Cardiac Failure, In print

II

T. Råmunddal, M. Lindbom, M. Scharin-Täng, P. Stillemark-Bilton, J. Boren, E. Omerovic

Overexpression of apolipoprotein-B improves cardiac function and increases survival in mice with myocardial infarction.

Submitted

III

Truls Råmunddal, Sigfus Gizurarson, Malin Lorentzon, Elmir Omerovic

Anti-arrhythmic effects of growth hormone- In vivo evidence from small-animal models of acute myocardial infarction and invasive electrophysiology.

Journal of Electrocardiology, In print

IV

Margareta Scharin Täng, **Truls Råmunddal**, Malin Lindbom and Elmir Omerovic

Native cardiac reserve predicts survival in acute post infarction heart failure in mice.

Cardiovasc Ultrasound. 2007 Dec 2;5(1):46

Introduction

The syndrome of heart failure

Heart failure is a syndrome which develops as a consequence of cardiac disease, and is recognized clinically by a constellation of symptoms and signs produced by complex circulatory and neurohormonal responses to cardiac dysfunction. Congestive heart failure (CHF) is the principle complication of all forms of heart disease. Between 1% and 2% of the adult population have heart failure, although it mainly affects elderly people; 6–10% of people over the age of 65 years have the disorder[1]. CHF imposes a heavy burden on health care resources, mainly because of the high costs of hospitalization[2]. Over the past decade, the rate of hospitalizations for CHF has almost doubled[3]. The prevalence of CHF is expected to double in the next decade mainly as a consequence of ageing population and increased survival in acute MI due to improved therapeutic interventions[4-6].The causes of heart failure are several and many different pathophysiological conditions may lead to the development of CHF. The etiology of CHF has shifted during the last 50 years. In the 1950s and 1960 hypertension was considered as the most important cause of CHF[7, 8]. However, data from randomized heart failure trials during the 1980s and 1990s show that ischemic heart disease has succeeded hypertension as the most prominent cause of CHF[9-13]. Other clinically important causes of CHF are valvular disease, idiopathic dilated cardiomyopathy, myocarditis and autoimmunity disorders, metabolic disorders, such as diabetes mellitus, and secondary cardiomyopaties due to toxic effects of e.g alcohol, cytostatics and others[1, 14, 15]. Although multiple clinical trials completed during the past two decades have unequivocally demonstrated decreased mortality and morbidity rates - thanks to the advances in pharmacological treatment with ACE inhibitors, β-blockers and aldosterone antagonists - CHF and myocardial infarction (MI) continue to be the most common threats to life and health[16]. Approximately three quarters of all patients hospitalized for the first time with CHF will die within 5 years – a survival rate far worse than for most types of cancer[17]. Sudden cardiac death (SCD) is the leading cause of mortality in heart failure and accounts for approximately 50% of all deaths from cardiovascular causes[18, 19]. Given that about 200 000 individuals in Sweden are afflicted with systolic heart failure (HF), that about 3000 new cases are diagnosed yearly[2], and that life expectancy is lengthening, the occurrence of HF and SCD will probably continue to rise in tandem. As medical therapy of heart failure has improved survival, the proportion of deaths that are sudden and unexpected has remained essentially unchanged, ranging from 30% to 50%[20]. The most common sequence of events leading to SCD appears to be the degeneration of ventricular tachycardia (VT) into ventricular fibrillation (VF) often followed by asystole or pulseless electrical activity. Preexisting coronary artery disease and its consequences (acute myocardial ischemia, scarring

from previous myocardial infarction, heart failure) are manifest in 80% of SCD victims [21]. Dilated nonischemic and hypertrophic cardiomyopathies account for the second largest number of SCDs, whereas other cardiac disorders, including congenital heart disease and the genetically determined ion channel anomalies (cardiac channelopathies), account for 5–10% of SCDs[19, 21, 22].

Despite the beneficial effects of modern medical therapy, and devices (ICD and CRT), many patients eventually progress to an advanced stage characterised by severely limiting symptoms, marked haemodynamic impairment, frequent hospitalisations and high mortality. Current medical therapies for HF are aimed at suppressing neurohormonal activation (e.g., angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists, β-adrenergic receptor antagonists, and aldosterone receptor antagonists), and treating fluid volume overload and hemodynamic symptoms (diuretics, digoxin, inotropic agents). These pharmacotherapies for HF can improve clinical symptoms and slow the progression of contractile dysfunction and expansion of LV chamber volume, nevertheless, there is still progression, and the prognosis for even the optimally treated patient remains poor [23, 24]. Thus there is a need for novel therapies for HF, independent of the neurohormonal axis that can improve cardiac performance and prevent or reverse the progression of LV dysfunction and remodeling.

Cardiac remodeling

Congestive heart failure is the final common pathway of various forms of cardiac disease. The development of CHF cannot be considered as a simple contractile disorder. The manifestation of the clinical syndrome of CHF is a result of a complex process leading to alterations of cellular and molecular components in the myocardium. This process of gradual transition from cardiac dysfunction into manifest CHF is referred to as “cardiac remodeling”. Cardiac remodeling can be defined as a continuous process of alterations in genome expression, molecular, cellular and interstitial changes that are manifested clinically as changes in size shape and function of the heart after any type of cardiac injury[25]. Myocardial infarction, chronic pressure overload (hypertension and aortic stenosis), volume overload (e.g. valvular regurgitation), inflammatory myocardial disease and idiopathic dilated cardiomyopathy (IDC) are the most common stimuli for cardiac remodeling, Although the etiology of these pathologic cardiac conditions varies broadly, several cellular, molecular, biochemical and mechanical events are common. The cardiomyocyte is the major cardiac structure involved in the remodeling process. Other components that are involved include the interstitium, fibroblasts, collagen and coronary vasculature[26-29]. Remodeling encompasses cellular changes including myocyte hypertrophy, necrosis, apoptosis, fibrosis,

increased fibrillar collagen and fibroblast proliferation[30-36]. Circulating and/or locally produced neurohormones such as catecholamines, angiotensin-II, cytokines and probably many others are thought to play a major role in change of the myocardial phenotype by altering gene expression via activation of second messenger systems[37-39]. At the organ level, the remodeling process results in increased LV mass and volumes and changes in cardiac geometry and the heart becomes more spherical and less elliptical. These changes have been shown to adversely influence the cardiac function[40-44]. Cardiac remodeling has been described both as adaptive and maladaptive, with the adaptive component enabling the heart to maintain function during the acute phase of cardiac injury (e.g. MI). The cellular rearrangements of the ventricular wall after MI helps maintain cardiac output in the short term, but this leads to structural dilatation of the left ventricle. The magnitude of this response relates to the degree of the injury. There are however, no experimental or clinical data to support the concept of a beneficial early adaptive remodeling in response to injury and loss of myocardial function. Progressive remodeling, the continuum of this early "adaptive" response to myocardial injury, however, can always be considered deleterious, leading to a progressive loss of myocardial function and finally development of overt CHF.
Modern therapies for CHF, such as angiotensin-converting-enzyme-inhibition, β-blockade and aldosterone antagonism, have all been shown to attenuate and even reverse cardiac remodeling in patients with CHF[30, 45-54]. Attenuation and interference with pathologic cardiac remodeling has emerged as a major goal in the modern pharmacological treatment of CHF.

Myocardial energy metabolism

Alterations in myocardial biochemical properties are integral part of the remodeling process, often referred to as biochemical remodeling. One of the most important consequences of this negative process is disturbed cardiac energy metabolism. The heart consumes enormous amounts of energy to fulfill its function. Each day, it beats about 100 000 times and pumps approximately 10 tons of blood through the body. To acquire the energy that is necessary to carry out its function, the heart converts chemical energy stored in fatty acids and glucose into the mechanical energy of the actin–myosin interaction of myofibrils. The heart cycles about 6 kg of ATP every day. Failure to produce an adequate amount of energy causes mechanical failure of the heart. Deprivation of cardiac energy plays a major role in heart failure and a large body of evidence supports this concept [55, 56]. Many different biochemical pathways are involved in the production, transfer and utilization of chemical energy in the cell which provides maintenance of its viability and functions. The main energy producing mechanism for ATP-synthesis is oxidative phosphorylation in mitochondria (Figure 1). This process is maintained by the production of reducing equivalents (i.e.

NADH) mainly through the tricaboxylic acid cycle from controlled combustion of the substrates, free fatty acids (FFA) and carbohydrates (glucose, lactate, pyruvate). The combined functions of the respiratory chain with the intervention of oxygen and ATPase (ATP synthase) allow the rephoshorylation of ADP to ATP. Adenine nucleotide translocase (ANT) controls the exchange of ATP and ADP between the mitochondrial matrix and cytosol. Several creatine kinases (CK) participate in the transfer of energy between ATP and phosphocreatine (PCr). The presence of CK specifically bound to mitochondria and to myofibrils creates a shuttle of energy from mitochondria to the sites of ATP utilization e.g. myofibrils and ion pumps. The main site of ATP dephosphorylation is the myofibrial ATPase, but other ATPases associated with different membranes (Na+/K+-ATPase, Ca++-ATPase, etc.) also participate in the expenditure and cleavage of ATP. Several intracellular compounds are proposed to play a role in the regulation of mitochondrial ATP production. These are phosphorylated compounds (ADP), redox state (NADH) or calcium (Ca^{++}). The production of ATP is also dependent on oxygen (O_2) supply. The main storage form of high energy phosphate is phosphorylated creatine (PCr). Cr and PCr are smaller and less negatively charged than ATP and ADP. Consequently, they can be stored in much higher concentrations and can be more easily transported to the different sites in the cell[57]. Cr can either be introduced by food intake or be synthesized in the kidney and liver. Tissues that contains CK, such as the as heart, skeletal muscle, brain or kidney take up Cr from the blood through a specific creatine transporter (CrT)[57, 58]. It has been found that the CrT can be either up- or down-regulated due to a number of different reasons. One of the consequences of CHF is down-regulation of the CrT in the cell membrane of cardiomyocytes[59]. In experimental studies, Cr supplementation leads to down-regulation of creatine transporter both in rats[60] and in cell culture[61, 62]. Cr depletion induced by the creatine analogue, β-guanidinoproprionic acid (BGP) in rats, leads to an increase in the CrT membrane availability[63].

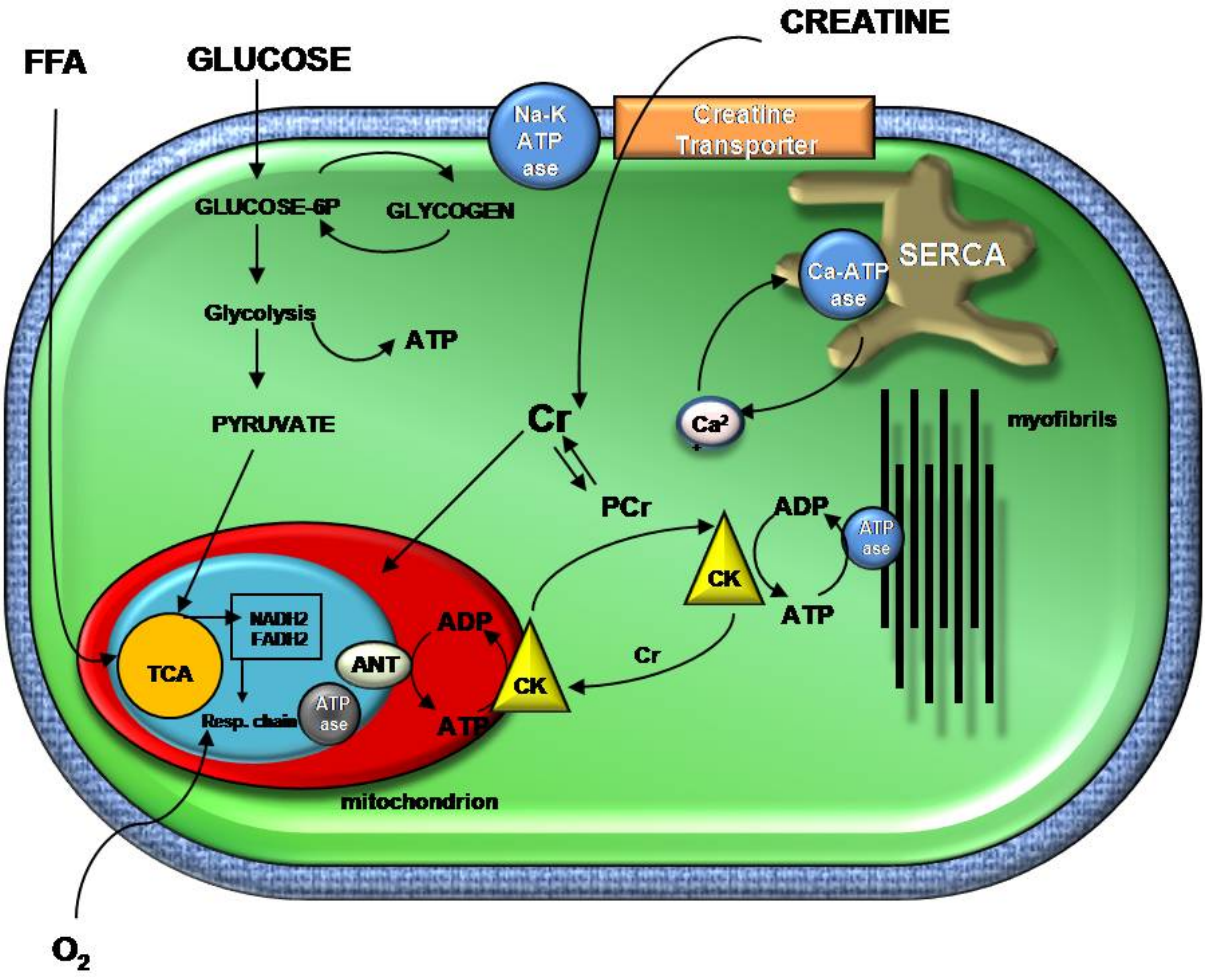


Figure 1: *Simplified overview over the most important parts of creatine and energy metabolism in the cardiomyocyte. Cr = creatine. ER= endoplasmatic reticulum, PCr = phosphocreatine, ATP=adenosine triphosphate. See the text for more explanations.*

The failing heart - an energy starved organ

There has been a longstanding and controversial debate about the hypothesis that the failing heart is an energy starving organ. This debate is partly due to the complexity of the cellular and molecular alterations involved in the pathogenesis of CHF but also to limitations in the methodology applied in the studies of myocardial energetics in the failing myocardium. Over a long period of time myocardial ATP concentration was considered to be the hallmark of the myocardial energy status. The introduction of whole organ magnetic resonance spectroscopy (MRS), particularly ^{31}P MRS, was a methodological breakthrough which has provided more accurate and comprehensive studies of myocardial energetics. ^{31}P MR spectroscopy is a powerful technique that allows non-invasive in vitro and in vivo measurements of myocardial high energy phosphate content. Today, there is compelling evidence, from both clinical and experimental studies, that the failing heart is characterized by disturbances in the myocardial energy metabolism[64-68]. It has been previously demonstrated that disturbances in myocardial energy

metabolism ensue early in the post-infarct period and that lowering of myocardial energy reserve correlates with parameters of LV systolic and diastolic dysfunction, as well as with LV wall stress. Failing and hypertrophied myocardium is characterized by several consistent changes in the cellular energetic system regardless of species. The size of the creatine pool is decreased in the failing heart and this decrease is cardiac specific[69] which is different in contrast to creatine deficiency syndrome[70]. High energy phosphate metabolites are decreased in failing[65, 67, 69, 71-74]and post-infarct remodeled hearts[75, 76] . PCr decreases early in the development of heart failure while ATP decrease occurs at the late-stage heart failure[67, 69, 77]. In many of these experiments, the decrease in PCr was larger than the decrease in ATP resulting in lower PCr/ATP ratio. The PCr/ATP ratio is a commonly used index of cellular energy status because it reflects the equation of cellular phosphorylation potential. The PCr/ATP ratio is clinically very useful as it can be measured noninvasively in vivo by means of ^{31}P MRS[72, 78, 79]. In CHF patients PCr/ATP ratio is reduced and correlates negatively with New York Heart Association class[66] and indexes of systolic[80] and diastolic[81] function. One study of 39 patients with dilated cardiomyopathy indicated that the phosphocreatine:ATP ratio might be a stronger predictor of both total mortality and mortality attributable to cardiovascular disease than functional or clinical indexes[82]. The total creatine pool, i.e. the sum of Cr and PCr, is decreased in the failing heart[55, 83-85]. Creatine depletion is now considered an inherent characteristic of the failing heart [86-89]. This has been proven in both human[55] and animal studies[89-92]. Decreases in PCr and Cr occur earlier than decreases in ATP. This could be the result of the heart's attempt to maintain normal free energy of ATP hydrolysis at the cost of decreasing intracellular Cr and thereby energy reserve. There is evidence for a decrease in the CK activity[65, 68, 93, 94] which results in compromised capacity of the CK system to rephosphorylate ADP into ATP leading to further decrease in the energy reserve. The loss of high-energy phosphates and creatine kinase activity causes a severe decline in ATP transfer[71, 95-97]. In other words, there is a severe decrease in energy flux within the cell and a reduction in energy delivery to the myofibrils by up to 70%[98]. The end-result is an energy starved heart[89]. Depletion of the creatine pool can also lead to LV hypertrophy, increased propensity for development of malignant arrhythmias and systolic and diastolic dysfunction[99, 100]. Down-regulation of CrT in the cell membrane is also a phenomenon found in the failing heart[89]. This is probably a negative phenomenon (if sustained over the long period of time) in the failing heart. Is the decrease in energy reserve a cause or a consequence of heart dysfunction? In recent years there has been numerous experimental studies performed in order to answer this question[69, 86, 101-104]. To study how disturbed myocardial energy metabolism affects function and morphology one can use various experimental approaches. A very simple in vivo animal model is chemical depletion of creatine by using a creatine analogue, beta-guanidinepropionic acid (BGP). BGP is an analogue

to creatine which can enter the myocyte through the creatine transporter[105] and thereby competitively inhibit Cr from entering the cell from the blood stream. Inside the myocyte, BGP can enter the mitochondria in the same way as Cr, and is used as a substrate for the CKmito to produce phosphorylated BGP and ADP[106]. However, compared to creatine, BGP functions very poorly as a substrate for the cytosolic CK. The activity of this enzymatic reaction is 3 orders of magnitude lower of its activity when using PCr as substrate[107]. This effectively inhibits the CK reaction[107] and creates an ATP deficiency in the myocyte. The model mostly used in BGP-induced creatine depletion is by administering BGP to the animals via food and water supply. The effect of BGP treatment is similar to what was observed in the CK-deficient mice[101, 108-110]. The CK deficient mice are only one of several knock-out/transgenic models used to study the influence of creatine metabolism on the heart. Others are GAMT deficient mice[88, 111], and mice overexpressing CrT[87].

Cardiac lipotoxicity

Normal cellular fatty acid homeostasis reflects a balance between processes that generate or deliver fatty acids and processes that utilize these molecules. In mammalian cells, free fatty acids (FFAs) are generated through the de-novo synthetic pathway and liberated when triglycerides and phospholipids are hydrolyzed by cellular lipases. FFAs can also be imported into mammalian cells by both protein- and non-protein-mediated mechanisms, either when cellular demand is high or when extracellular FFA concentrations are high[112]. FFAs derived from each of these processes can be utilized for energy production through β-oxidation, membrane biosynthesis, act as precursors of biologically active compounds such as prostaglandins and leukotrienes or may also act as natural ligands of nuclear factors like peroxisome proliferator-activating receptors (PPARs) enabeling FFAs to modulate the expression of cardiac enzymes involved in fatty acid metabolism. When cells accumulate more FFAs than are required for anabolic or catabolic processes, excess lipid is esterified and stored as triglyceride in lipid droplets. These single-membrane bound compartments are dynamic and fatty acids stored within may be mobilized through the actions of cellular lipases, in a process regulated by hormones and by droplet-associated proteins. Adipocytes have a unique capacity to store large amounts of excess FFAs in cytosolic lipid droplets. Cardiomyocytes, however, like other non adipose organs, have very limited capacity for storage of lipids. When this capacity is exceeded, the resultant process of cellular dysfunction or cell death is termed lipotoxicity[113].

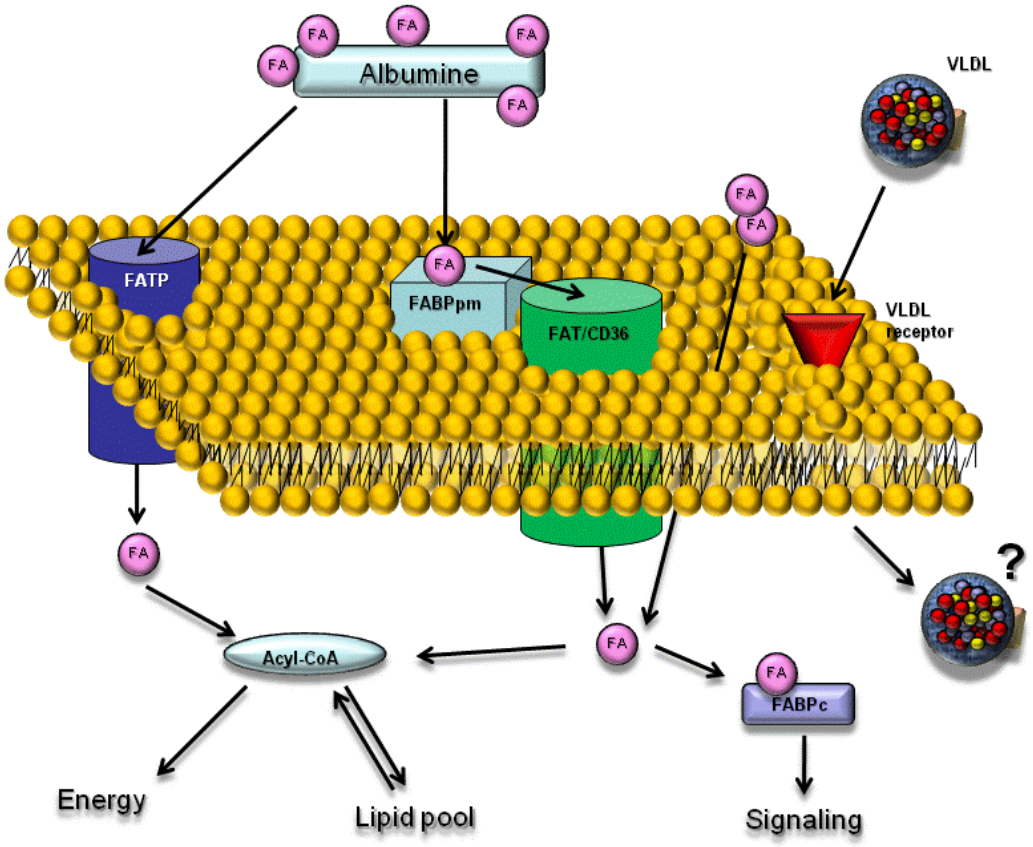


Figure 2. *Mechanisms of fatty acids tranport into cardiomyocytes*

Figure 2 schematically depicts the hitherto known mechanisms for transportation of free fatty acids (FA) into cardiomyocyte. FA may enter the cell directly or be transported by means of several membrane-associated mediators such as fatty acid transport protein (FATP), fatty acid binding protein/fatty acid translocator (FABP-FAT/CD36) or by receptor-mediated transportation through action of very low density lipoprotein receptor (VLDL). Once in the cytosol, FA enter different biochemical pathways. While transport mechanisms of FA into the heart are relatively well understood, the question whether the heart has developed mechanisms for export of FA and lipids (surplus, toxic) out of the heart is less investigated.

Lipid accumulation in non-adipose tissues occurs in disease states, such as diabetes and obesitas, with an excess availability of plasma FA and triglycerides (TG) causing a mismatch between lipid uptake and utilization[114, 115]. A second mechanism for lipid accumulation is observed in tissues, such as the heart, with high turnover/metabolism of FFAs when utilization of FFAs is impaired in the context of continued FFA import. High energy flux organs such as the heart are adapted to closely match energy substrate import and utilization. The heart is an organ heavily dependent on exogenous lipids for the oxidative production of ATP and therefore maintenance of normal

cellular energy homeostasis. While long-chain FFAs are the major source of energy in the normal adult mammalian heart, acquired disorders such as myocardial ischemia, heart failure and hypertrophy, but also inherited cardiac disorders are associated with a switch in energy substrate utilization from FFAs to glucose[116-120]. Decreased FFA oxidation in heart failure, hypertrophy and even myocardial ischemia is thought to be one reason for lipid accumulation. Moreover, in inherited fatty acid oxidation disorders, failure to utilize long-chain FFAs is associated with massive (up to 100-fold) increase in myocardial triglyceride content [121]. Similarly, pharmacological inhibition of β-oxidation in a rat model leads to intramyocellular[122] and myocardial [123] lipid accumulation, which is exacerbated in the setting of a high fat diet. In myocardial ischemia additional sources of lipids contribute to lipid accumulation besides the extracellular lipids. The fact that different lipid moieties accumulate in ischemic hearts in experimental settings, without lipids in the perfused medium indicate that intracellular endogenous esterified fatty acid pools, such as membrane phospholipids, contribute to the rise of lipids in ischemic cardiac tissue[124].

The detrimental effects of myocardial lipid accumulation during tissue ischemia and reperfusion have been recognized for a long time [124-127]. The concept of lipotoxicity has gained renewed appreciation in recent years especially due to the diabetes and obesity epidemic seen in western countries. The adverse effects of myocardial lipid overload are well documented in different animal models. A commonly used model of fatty heart is the ZDF rat. In the ZDF rat, a loss-of-function mutation in the leptin receptor [128] in the hypothalamic centers that regulate feeding behavior results in increased food intake, whereas in peripheral tissues, such as the pancreatic islets, it results in markedly increased lipogenesis. Consequently, the combination of increased caloric influx and a generalized increase in lipogenesis in tissues causes an accelerated steatosis in cardiomyocytes and other organs. Steatosis of the myocardium is associated with left ventricular hypertrophy and dysfunction that ultimately progresses to lipotoxic cardiomyopathy[129]. Several transgenic animal models of lipotoxic cardiomyopathy have been created. If hearts internalize excess lipid or have a defect in lipid oxidation, then lipid storage must increase. Augmentation of lipid uptake has been achieved through transgenic expression of a cell membrane anchored form of LpL[130], overexpression of (MHC)-long-chain acyl coenzyme A synthetase (ACS)1 [131], MHC-fatty acid transport protein (FATP)1 [132] and transgenic expression of PPARγ [133]. The lipid accumulation in these models is associated with the development of various degrees of cardiomyopathy with LV hypertrophy and dilatation, depressed cardiac systolic and/or diastolic function and with premature death in some models. Transgenic mouse models with specific defects in the mitochondrial fatty acid oxidation pathways have also been established[134-136]. These models all exhibit cardiac

lipotoxicity, although, these models display disparate cardiomyopathic phenotypes. For example, deletion of a fatty acid chain-length-specific dehydrogenase enzyme (VLCAD) shows increased susceptibility to ventricular tachycardia and arrhythmias without overt systolic dysfunction[134]. Myocardial lipid accumulation is present in many patients with inherited defects in FAO enzymes who die suddenly, suggesting that lipotoxicity may precipitate sudden myocardial dysfunction or arrhythmias[137, 138]. This suggests that not only lipid accumulation per see but also the profile of the accumulating lipid moieties are important. Malignant ventricular arrhythmias are the major cause of death during myocardial infarction and heart failure. It is suggested that activation of myocardial phospholipases during acute cardiac ischemia results in the generation of amphiphilic metabolites (lysophospholipids) that alter normal function of ion channels, receptors and gap junctions function and thereby precipitates lethal ventricular arrhythmias. Since myocytic electrophysiologic function is influenced by the physiochemical properties of the lipids surrounding ion channels, receptors and gap junctions, accelerated hydrolysis of sarcolemmal phospholipid constituents during acute ischemia could provide a foundation for the biochemical basis of ischemia-induced arrhythmias. Lysophosphatidylcholine (LPC), a hydrolysis product of phospholipid degradation by action of the phospholipases, accumulates in ischemic myocardium and this accumulation has been associated with the development of ventricular arrhythmias [139]. The LPC concentration in the myocardium increases during the first minutes of cardiac ischemia [140, 141]. LPC accumulation is also documented in ischemic human hearts [142 143]. LPC induces alterations in the action potential resembling those observed in ischemic myocardium in vivo (decrease in resting membrane potential, overshoot, Vmax of the rapid depolarization phase and action potential duration) [139, 144]. It has been also reported in anesthetized, LAD-occluded cats, that the severity of spontaneous ventricular arrhythmias is directly related to the increase of LPC during ischemia [145]. In addition, LPC increases free intracellular calcium concentration by increasing calcium uptake through a verapamil-insensitive pathway [146-148]. Abnormal cellular coupling through gap junctions may be a predominant factor in several cardiac arrhythmias [149, 150]. LPC, at concentrations measured in situ during cardiac ischemia, is a potent inhibitor of gap junction communications between cardiac cells. Impaired junctional communications due to LPC accumulation early during ischemia could decrease electrical conduction and contribute to the genesis of malignant arrhythmias[151].

Other proposed mechanisms responsible for the toxicity of accumulating lipids are several: direct toxic effects of neutral droplets or fatty acids on myofibrillar function[152], activation of apoptotic signaling pathways via ceramide-mediated processes[129, 153], reactive oxygen species generated as a toxic byproduct of lipid oxidation[154], mitochondrial dysfunction[155, 156], disturbed calcium handling[156], nitric oxide generation[157], detergent actions of NEFA and their CoA and carnitine

esters and phospholipids (e.g lysophospholipids) leading to membrane instability and dysfunction as mentioned above [158, 159]. Most data indicate that the triglycerides themselves serve primarily a storage function with toxicity deriving mainly from NEFA and their products such as ceramides, diacylglycerols and CoA and carnitine esters of FA that accumulate either as a result of failure of esterification or breakdown of the triglycerides[160].

Pathophysiological states such as myocardial ischemia, heart failure and cardiac hypertrophy are associated with myocardial accumulation of different lipid moieties in the heart.[161-166] While the adverse effects of lipid accumulation are relatively well documented in experimental settings, it is less known to which extent lipotoxicity contributes to the pathogenesis of MI, CHF and cardiac hypertrophy in a clinical setting. We need to develop methods to control myocardial lipid accumulation by means of pharmacological interventions in order to study the importance of lipotoxicity in clinical settings.

Myocardial lipoproteins – an endogenous protective system?

Several approaches to prevent or treat lipotoxicity have been proposed. Strategies that divert excess lipids away from non-adipose tissues such as treatment with PPARγ agonist has been shown to reduce lipotoxicity, by decreasing ectopic deposition of lipids and presumably increasing adipose tissue accumulation[167]. Lipid content may also be decreased by increasing metabolism of excess lipid within the myocardium[168]. Studies in which inhibition of specific metabolic or signaling pathways decreases lipotoxicity provide evidence for the importance of these mechanisms in lipotoxic disease and suggest potential therapeutic targets, such as pharmacological interventions to prevent nitric oxide production or to block ceramide production[157, 169].

During the last years surprising data have been reported showing unequivocally that the heart synthesizes apolipoprotein B containing lipoprotein particles (Figure 3) [170, 171]. This phenomenon has been confirmed in different species including humans[170]. The fact that both humans and mice - two species separated by millions of years of evolution - have preserved the biochemical machinery for myocardial production of lipoproteins suggests an important physiological and/or pathophysiological regulatory role. Indeed, the myocardial apoB do regulate myocardial triglyceride content. Transgenic mice, overexpressing human apoB have substantially (75%) lower triglyceride content in the myocardium compared to wild-type mice. On the other hand, heart specific MTP knock-out mice that lack the ability to produce the apoB containing lipoprotein particle in the heart, show increased levels (~20%) of triglycerides in the heart[172]. ApoB and microsomal transfer protein (MTP) plays critical roles in the formation and secretion of triglyceride-rich lipoproteins from cells. ApoB forms the structural backbone of the triglyceride-

rich lipoproteins acquiring lipids during its translation and translocation into the lumen of the endoplasmatic reticulum (ER). As newly synthesized apoB is translocated into the lumen of the ER, it co-translationally associates with lipid to form a small nascent, lipid poor, lipoprotein particle. MTP is crucial for the transfer of lipid to apoB as the polypeptide is translocated into the ER lumen. Although MTP preferentially transports triglycerides and cholesteryl esters, it will transport a wide variety of lipids and phospholipids[173]. The concentration of MTP in the ER is a critical determinant of lipoprotein secretion.

Due to the crucial role of MTP in lipoprotein assembly, variations in that gene may influence efficiency of the lipoprotein assembly and secretion. In accordance with this possibility, Karpe et al. [174] have sequenced the promoter region of the MTP gene and have identified the −493G/T polymorphism associated with changes in the plasma LDL-C and LDL-TG concentrations. Functional studies have revealed that this particular polymorphism influenced cell's transcriptional activity regulating MTP expression. The less-common T variant has been associated with reduction of plasma LDL cholesterol levels.

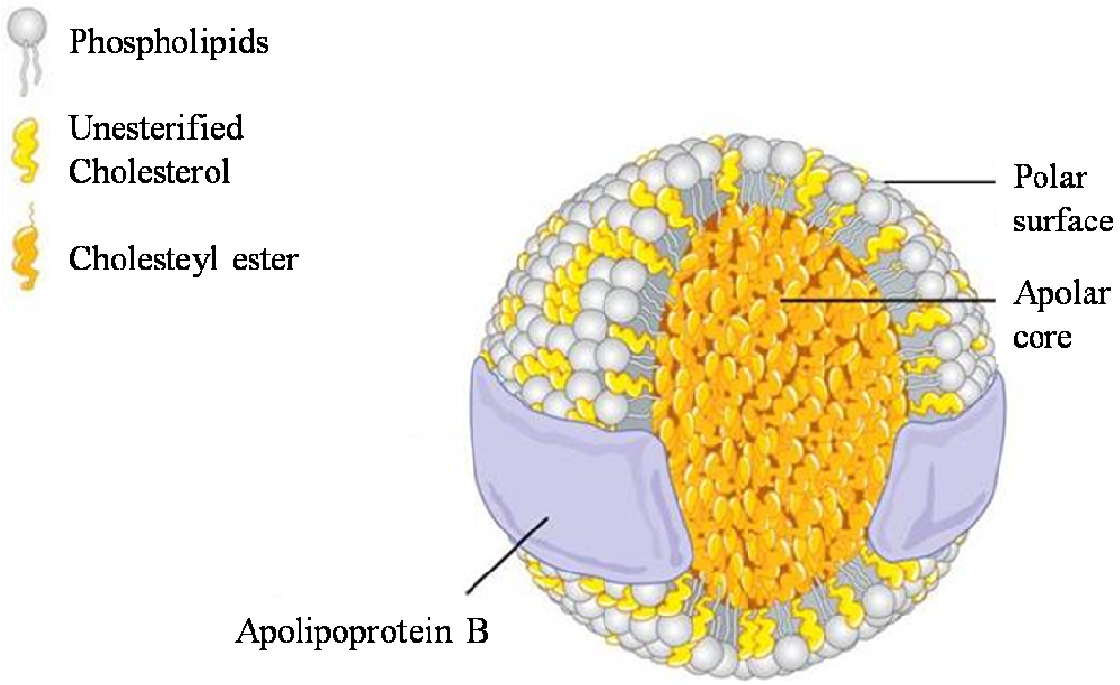
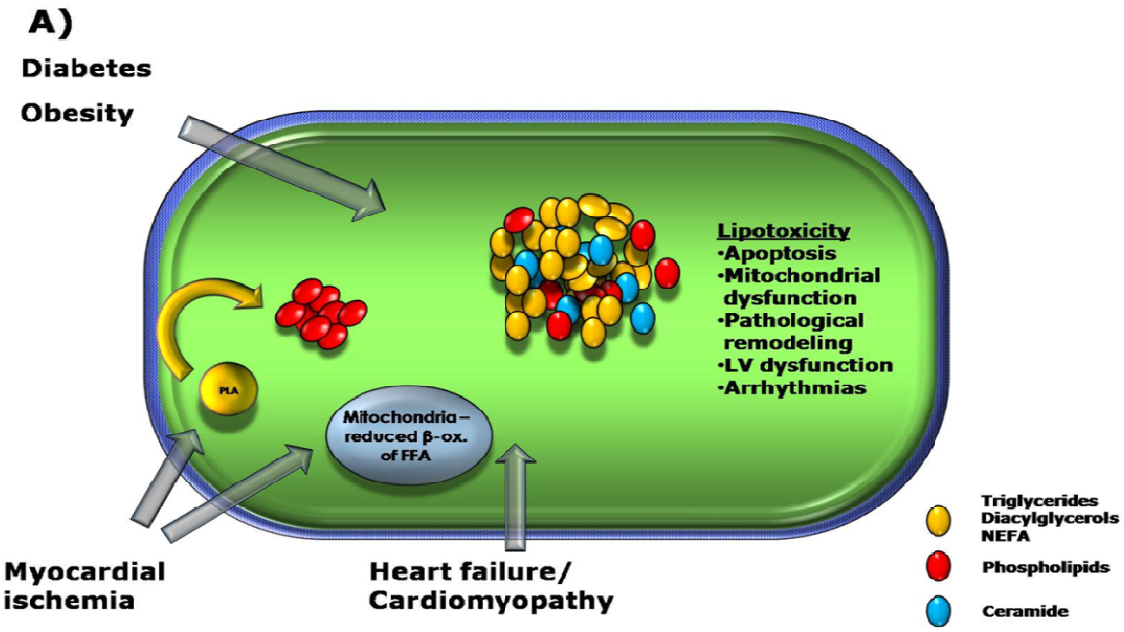
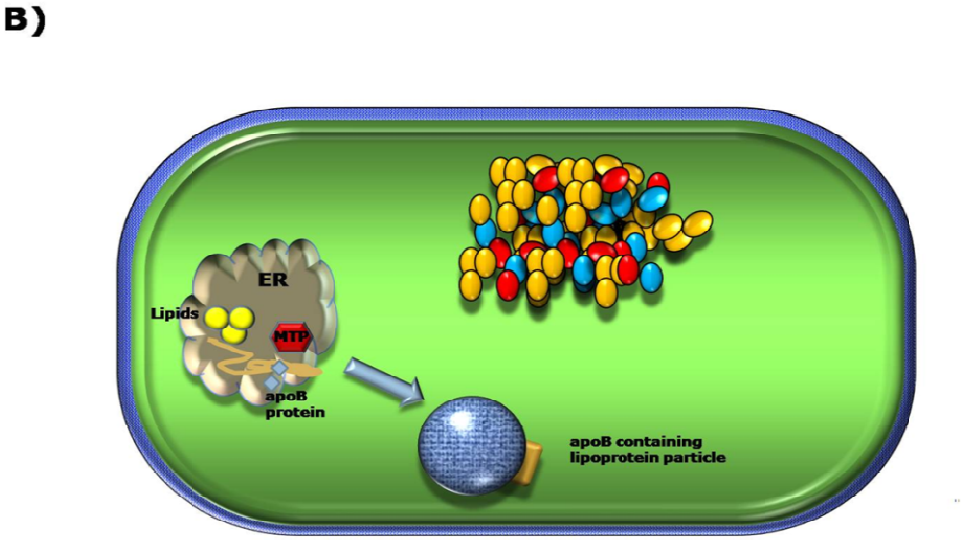


Figure 3. *Apolipoprotein B containing lipoprotein. The apoB lipoprotein forms the structural backbone of the lipoprotein particle. Phospholipids form the polar surface. The core of the lipoprotein particle contains predominantly triglycerides and cholesteryl esters but can transport a wide variety of lipid moieties.*

Recently, Ledmyr and colleagues[175] reported an increased CHD event rate among carriers of the MTP-493T variant despite that these carriers had lower total plasma cholesterol. These findings confers that the MTP 493 T-variant is a plasma lipid independent risk factor for CHD events. In an attempt to explain these results it is speculated that decreased myocardial MTP expression might attenuate lipid export from the cardiomyocytes and thereby increase the vulnerability upon ischemic damage and increased susceptibility to fatal ventricular arrhythmias.

What we know from experiments performed in transgenic animals supported by human data is that myocardial apoB production is probably not important for maintenance of normal cardiac function and structure. Both MTP knock-out mice and humans with the rare genetic defect resulting in inability to express MTP and consequently in abetalipoproteinemia have normal cardiac structure and function. Accordingly, the research focus during the last years was oriented toward defining the role of myocardial apoB in the pathophysiological-disease settings. It has been demonstrated that apoB may be involved in cardioprotection by means of elimination of toxic intracellular lipids [176]. While it has been reported that apoB overexpression may prevent or inhibit development of cardiomyopathy due to excessive intracellular lipid accumulation [176], the importance of this biochemical system for the heart during acute myocardial infarction (MI) and acute heart failure (HF) has not been studied.


A)
Diabetes
Obesity
Lipotoxicity
·Apoptosis
·Mitochondrial dysfunction
·Pathological remodeling
·LV dysfunction
·Arrhythmias
PLA
Mitochondria – reduced β-ox. of FFA
Myocardial ischemia
Heart failure/ Cardiomyopathy
Triglycerides
Diacylglycerols
NEFA
Phospholipids
Ceramide


B)
ER
Lipids
MTP
apoB protein
apoB containing lipoprotein particle

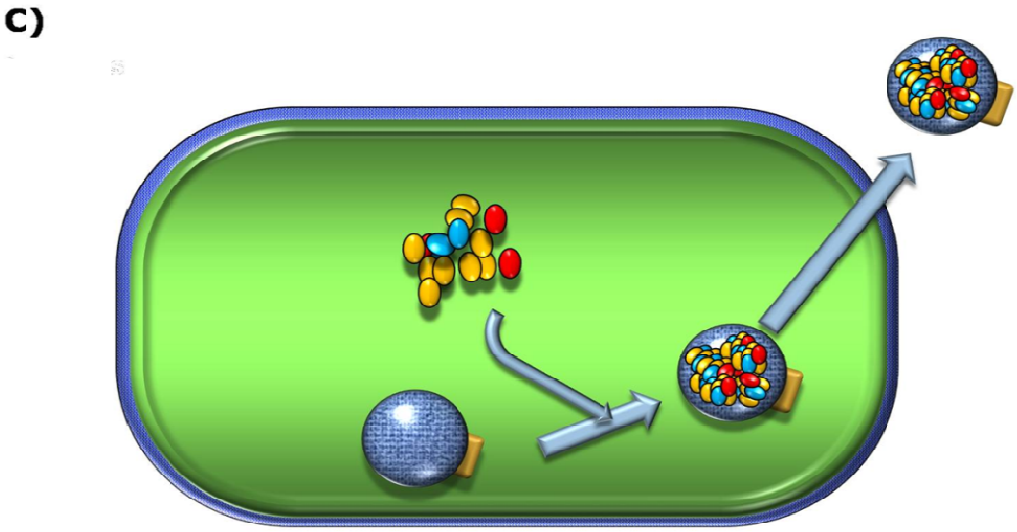

C)

Figure 4. *Hypopthetical view of the protective role of apoB lipoproteins in the hea*rt*. A) Diabetes and obesity causes intracellular lipidaccumulation due to excess plasma availability of lipids. In myocardial ischemia, heart failure and cardiomyopathy lipid accumulation is partly explained by reduced capability to utilize FA by decreased β-oxidation. In addition, during myocardial ischemia intracellular lipid pools (such as sarcolemma phospholipids) contribute to lipid accumulation through activation of intracellular phospholipases (PLA). B) Microsomal transfer protein (MTP) transfers lipids onto the growing apoB protein as the polypeptide is translated into the lumen of the endoplasmatic reticulum and forms an apoB containing lipoprotein particle. C) Intracellular* (*toxic*) *lipid moieties are first sequestrated by the apoB containing lipoprotein partiles and than exported out of the intracellular environment.*

Our hypothesis is that apoB isolates and exports toxic lipids from cardiomyocytes and therefore plays an important role in maintenance of normal membrane function of organelles such as mitochondria, sarcoplasmatic reticulum and sarcolemma (Figure 3). Functional disturbance of these cellular units results in development of cell death and/or electrophysiological instability. In clinical terms these events would translate into development of congestive heart failure (CHF), malignant ventricular arrhythmias and sudden death. The myocardial apoB production constitutes an endogenous cardioprotective system, which is mobilized during MI and CHF to counteract lipotoxicity by isolating and exporting toxic lipids (NEFA, triglycerides, diacylglycerols, phospholipids, ceramide etc). Effective export of intracellular lipids accumulated during ischemia is essential for recovery of normal function of sarcolemma and other membrane-associated organelles. This improves myocardial function, attenuates pathologic remodeling and reduces malignant arrhythmias.

Growth hormone and the heart

The interaction between GH and the cardiovascular system has attracted interest of experimental and clinical scientist for a long time. This interest is based on the fact that both states of GH excess[177] and deficiency[178-180] are associated with profound alterations of the cardiovascular system. Increasing knowledge from basic research about the actions of the GH/IGF-I axis on the cardiovascular system have given rise to the concept of using GH as an adjunctive treatment for CHF and myocardial infarction (MI). Several experimental studies have shown positive effects of growth hormone on cardiac function and remodeling in animal models of MI and CHF[181-185]. Additional attention to the concept of GH in the treatment of CHF was created when the first clinical study was published[186]. In this uncontrolled open study 7 patients with moderate to severe CHF due to idiopathic dilated cardiomyopathy (IDC) were treated with GH during 3 months. The authors reported increase in myocardial mass, reduction of the LV size, improvements in LV

function and hemodynamics and improvements in cardiac energy efficiency after the treatment. However, the of the following clinical trials[187-195] of GH treatment in patients with IDC or ischemic cardiomyopathy have shown inconsistent results . In these trials conflicting results were obtained for several cardiac parameters of cardiac remodeling and function. However, most of these trials included small numbers of patients, raising the possibility that nonsignificant results were related in part to inadequate statistical power. A recently published meta-analysis[196] of the existing clinical trials the authors included 12 trials of which , four were open studies, and eight were blinded, randomized, placebo-controlled trials, including a total of 195 patients. This meta-analysis suggests that GH treatment improves several relevant cardiovascular parameters in patients with CHF, such as improved systolic function (LVEF), reduction in systemic vascular resistance, reduction in left ventricular dimensions (LVEDd), increased left ventricular wall thickness and improved functional capacities (improvement in NYHA class, exercise duration, VO2max). However, these results needs confirmation in large placebo controlled clinical trials. Although the results from the clinical trials are inconclusive, the massive body of experimental evidence provides ethical and scientific imperative to continue with preclinical and clinical studies in order to define the settings in which GH may be used in MI and CHF. Attenuation and interference with pathologic cardiac remodeling has emerged as a major goal in the modern pharmacological treatment of CHF. Indeed, GH has been shown to exert powerful antiremodeling properties. A number of animal studies have consistently documented the efficacy of GH and IGF-I in attenuating LV remodeling and improving myocardial energetics and function in experimental MI[184, 197-200]. The earlier investigations[185, 199] showing improvement of cardiac function, focused on treatment of chronic, well-established CHF, studied when most of the dynamic remodeling processes, including attendant gene activation, LV dilation and hypertrophy of the noninfarcted myocardium, have already occurred in the rat model used. Cittadini at al.[200] have addressed this issue in the rat model of MI and were able to show that early administration of GH, started on the day of induction of MI, attenuated the early pathologic LV remodeling and improved LV function. More recently, Jin at al.[201] demonstrated that early administration of GH to rats with MI significantly reduced the infarct size and survival at 52 weeks. In the same study it was shown that GH administration attenuated the cardiac expression of atrial natriuretic factor (ANF), β-myosin heavy chain, α-smooth muscle actin, collagen I, collagen III and fibronectin. These findings are consistent with earlier studies showing increased ventricular expression of genes encoding for the fetal phenotype (ANF, β-myosin heavy chain, α-smooth muscle actin) during ventricular remodeling following MI in rats. [181, 202, 203] Furthermore, the known increased expression of extracellular matrix genes following acute MI[203, 204], was found to be attenuated by administration of GH. Progressive loss of cardiomyocyte due to apoptosis has been proposed to play an

important role in the progression of cardiac dysfunction[31] and has been demonstrated to occur at an increased rate following ischemia, reperfusion and MI[33] . IGF-I and GH have been shown to possess powerful anti-apoptotic properties [205-207]. Cittadini at al. demonstrated recently that GH treatment post MI, prolonged survival of rats with experimental CHF, which was associated with marked attenuation of cardiomyocyte apoptosis and pathologic interstitial remodeling[208].

It is well known that GH has profound influence on the regulation of carbohydrate, protein, and fatty acid metabolism[209, 210]. However, little is known about the specific metabolic effects of GH at the level of the heart. Our laboratory has provided evidence that treatment with GH in the early post infarct period improves myocardial energy status[211]. This improvement is accociated with up-regulation of creatine translocator (CrT) in the heart[212]. It has been hypothesized that GH, by increasing the expression of CrT, may, exert protective effects on myocardial energetics by preventing or attenuating the progressive course of PCr and Cr depletion in the remodeling and failing myocardium[212]. Activation of the sympathetic nervous system has long been recognized as an integral part of acute coronary syndrome and CHF. During and after myocardial ischemia, a dramatic increase in sympathetic activity has been observed[213]. Noradrenaline is known to exert direct pathologic effects on the myocardium in high concentrations[214]. The deleterious effects of excess sympathetic activity in CHF are underlined by reductions in morbidity and mortality in large clinical trials achieved with β-blockers in CHF[52, 215].There is compelling evidence for the existence of a tight relationship between the autonomic nervous system and sudden cardiac death[216]. The interest in this correlation has been focused primarily on the electrophysiologic mechanisms involved and on the evidence that VF could be enhanced by sympathetic[217] and antagonized by vagal activity[218]. Clinical implications of these concepts have been successfully applied. The most obvious example is the widespread use of β-blockers post-MI[219] and in CHF. In the large clinical trials with β-blockers (eg, MERIT-HF and CIBIS-II), sudden death was reduced by approximately 40%[215, 220]. There is solid evidence that GH interacts with the autonomic nervous system. In patients with GH efficiency, there is an increase sympathetic nerve activity in muscle[221]. Growth hormone administration to patients with dilated cardiomyopathy reduces the myocardial NA release in response to physical exercise[222]. Our laboratory demonstrated that both myocardial and plasma NA content is markedly decreased in rats treated with GH early post-MI[211] as well as in transgenic mice with cerebral GH overexpression[223]. With this background, and with the additional knowledge that GH may influence important cellular mechanism involved in the maintenance of electrophysiologic stability of the heart, such as fatty acid metabolism, energetic balance, calcium kinetics, myocardial hypertrophy, activity of ion channels, and others[211, 224] we hypothesized that GH administration in the setting of an acute MI may reduce the incidence and severity of ventricular tachyarrhythmias

Aims of the thesis

I.

To investigate in vivo the effects of creatine depletion on left ventricular function and morphology, energy metabolism and myocardial lipids

II.

To investigate the importance of endogenous lipoproteins in the heart for cardiac function, morphology and survival in the settings of acute and chronic myocardial infarction

III.

To investigate the effects of growth hormone on cardiac arrhythmogenesis

IV.

To investigate wheather native cardiac reserve predicts survival after myocardial infarction

Methodological considerations

General descriptions of material and methods are given in each individual paper. In this section specific consideration and in some cases more detailed descriptions of some of the methods are discussed.

Myocardial injury models

In this thesis we have used several different models of myocardial injury.

- Myocardial infarction in rats and mice (papers II, III and IV)
- Ischemia reperfusion (IR) injury in mice (paper II)
- Doxorubicine induced heart failure (paper II)

Induction of myocardial infarction (Paper II, III, IV)

The induction of myocardial infarction in rats and mice were principally performed in the same manner. The animals were anesthetized with isoflurane, orally intubated and connected to a small animal ventilator. The animals were kept ventilated and maintained on 2% isoflurane mixed with oxygen and room air, during the operation (Figure 5 and 6). Electrodes were placed on the extremities and connected to an ECG device in order to observe the cardiac rhythm during surgery. The chest was shaved. Left thoracotomy was performed between the 4^{th} and 5^{th} ribs in order to expose the left ventricular wall (Figure 5 and 6). The pericardium was removed and the branch of the left coronary artery was ligated proximally by positioning a suture between the pulmonary artery outflow tract and the left atrium. The efficacy of the procedure was immediately verified by characteristic ECG pattern changes, and akinesis of the left ventricular wall. If these changes were not seen, an additional ligature was done. After induction of MI was verified, the lungs were hyperinflated, positive end-expiratory pressure was applied and the thorax was closed by means of 3-4 sutures. All animals received postoperative analgesia with buprenoprin 0.05 mg/kg s.c. and 0.6 mg/100 ml in the drinking water and were placed in cages with temperature control for spontaneous recovery. This procedure aims to induce a large anterior wall myocardial infarction involving ~40% of the LV, by placing the ligature as proximally as possible on the LAD.

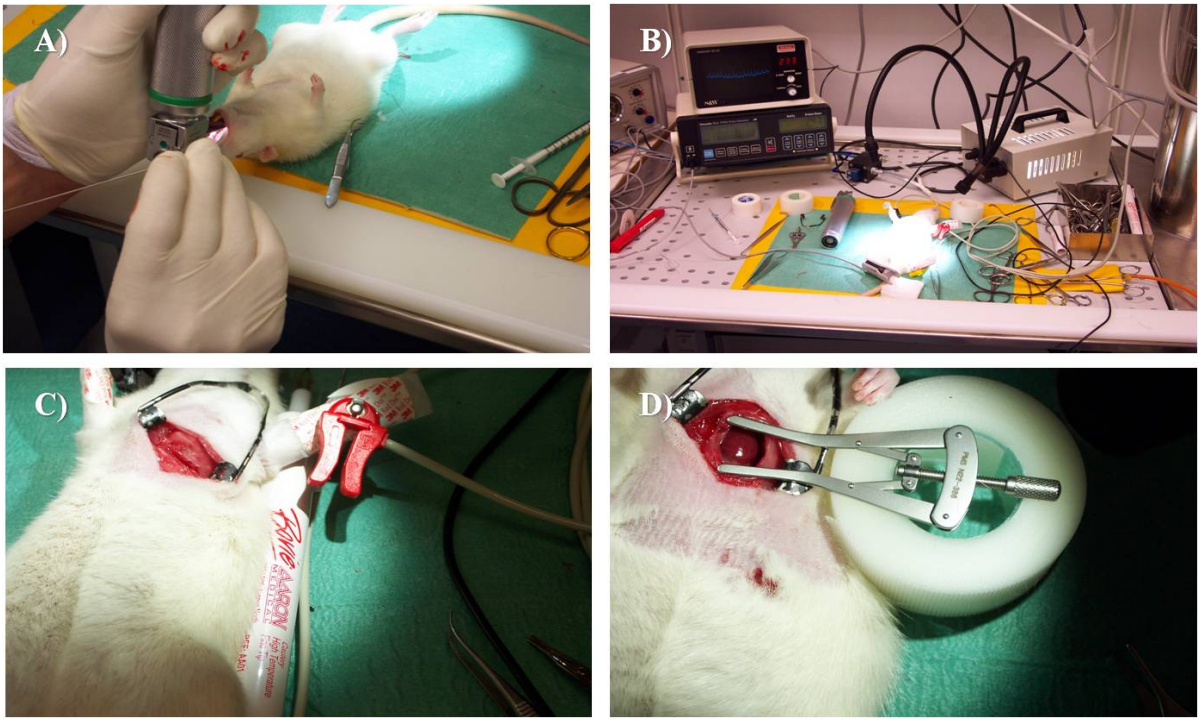


Figure 5.

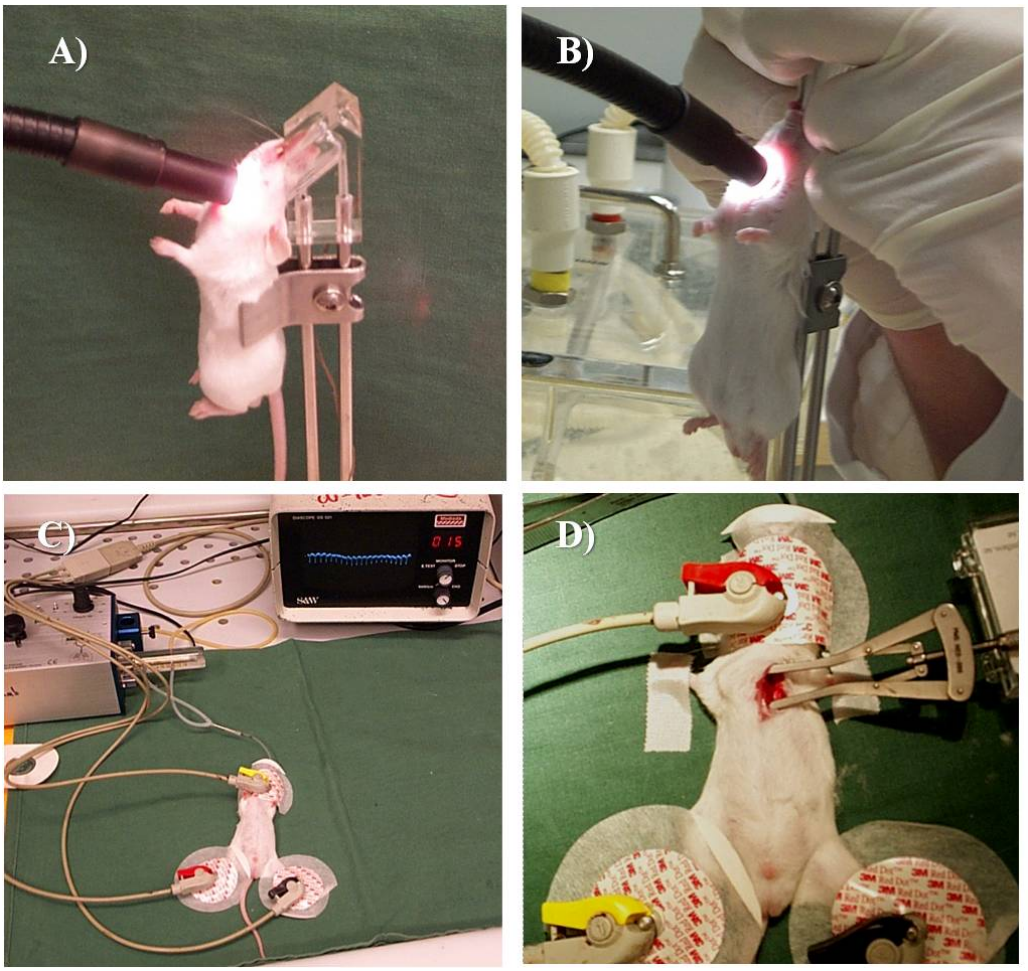


Figure 6.

Figure 5. *Rat model of myocardial infarction. A) Oral intubation of the anesthetized rat. B) Experimental set-up. Artificial respiration with the respirator. Online ECG monitoring with electrodes on the extremities and oxygen saturation monitoring with a sensor on the tail.C and D) Left sided thoracotomy exposing the anterior wall of the left ventricle and the left atrium.*

Figure 6. *Mouse model of myocardial infarction. A and B) Oral intubation. The anesthetized mouse is fixed into an isoflurane filled chamber with the device especially designed for oral intubation of the mouse. A small tube is placed in the trachea under guidance of an external light source. C) Artificial respiration with the respirator. Online ECG monitoring with electrodes on the extremities and neck. D) Left sided thoracotomy exposing the anterior wall of the left ventricle and the left atrium.*

Ischemia-reperfusion injury (Paper II)

IR injury was performed according to the previously described protocol[225]. The same protocol was applied both for rats and mice. Briefly, the animals were anesthetized with isoflurane, orally intubated and connected to a ventilator (SAR -830 small animal ventilator, GENEQ inc., Montreal, Canada) for artificial ventilation with room air and oxygen. Left thoracotomy was performed as described above. An 8-0 ethilon suture was passed under the proximal segment of the left anterior descending artery (LAD). Both ends of the suture were the passed through a small, short PE tube. The PE tube was then pressed gently against the LAD by pulling on both ends of the suture, thereby inducing temporary occlusion of the vessel verified by immediate akinesis of the LV anterior wall as well as with characteristic ECG pattern with ST-T elevation and increased R-wave amplitude. The animals were subjected to 30 minutes of ischemia and thereafter the vessel was reperfused, the chest was closed, and the animals were extubated and placed in temperature controlled cages for recovery. The animals were sacrificed and the hearts were collected at different periods of time after reperfusion.

Doxorubicine-induced acute heart failure (Paper II)

Mice were injected with a single intraperitoneal bolus dose (15 mg/kg) of doxorubicine (Meda AB, Solna, Sweden) (DOX) known to cause acute HF with high mortality rate [226].
Echocardiography was performed 24 hours after DOX injection according to a prespecified protocol (see below) to confirm the presence of heart failure.

Invasive hemodynamics (Paper III)

In order to monitor left ventricular pressure (LVP) and arterial pressure (AP), we cannulated the left carotid artery and the left ventricle through the apex (Figure 7). We used commercially available fluid filled pressure sensors and registered LVP and AP along with ECG. The data were displayed and analyzed off-line using Biopac acquisition software. Following hemodynamic parameters were derived: systolic and diastolic blood pressure, mean arterial pressure, left ventricular systolic pressure, left ventricular end-diastolic pressure, dP/dT max and dP/dt min.

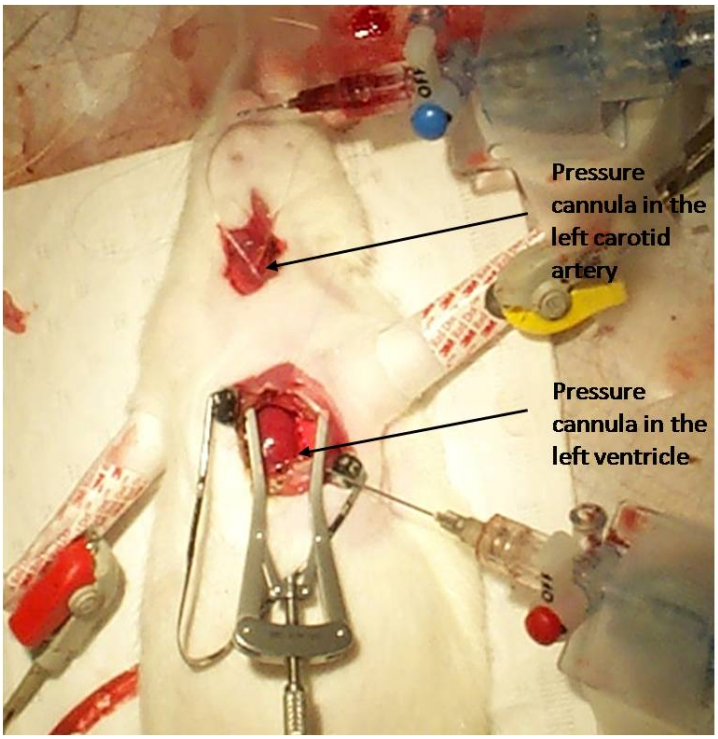


Figure 7. Invasive hemodynamics. The left carotid artery and the left ventricle are cannulated for registration of pressure (AP, LVP). ECG electrodes are attached to the extremities.

Echocardiography (papers I, II and IV)

The animals were anesthetized with isoflurane (0.4–0.6%) and mixture of O2 and N2O 2:1 using a nose mask. The anterior chest was shaved and ECG leads were placed on extremities. A warming pad was used to maintain normal body temperature. Commercially available ultrasonograph (HDI 5000 ultrasound system, ATL, Philip Medical System, Best) was used for noninvasive evaluation of the heart applying the methods previously validated [227, 228]. A 15 MHz linear transducer was used to obtain two-dimensional parasternal short axis imaging close to the papillary muscles. (Figure 8) This served as a guide for M-mode tracing. For pulsed-wave Doppler recordings, the minimum sample size was used and the pulse frequency of 5 MHz to record the estimated peak left ventricular outflow tract velocity and the mitral inflow velocities. The same protocol was used

for the dobutamin-stress echocardiography. Five minutes before stress protocol, the mice were injected with 1µg/g BW dobutamine intraperitoneally. All tracings were recorded at a sweep of 200 mm/s and were stored in magnetic optical discs for off-line measurements. Off-line measurements were done using image analysis system (Echo Pac 5.4, Ving Med) by one blinded observer. M-mode measurements of LV internal diameters and wall thickness in diastole and systole were made by using the leading-edge method (Figure 9). End diastole was defined at the onset of the QRS complex, and end systole was defined at the peak inward of interventricular septum (IVS) motion. At least four beats were averaged for each measurement. LV fraction shortening (FS) was calculated as follows. (LVIDd-LVIDs)/LVIDd x 100 where LVIDd and LVIDs are respectively LV internal diameters in diastole and systole. Ejection fractions (EF), relative wall thickness (RWT), velocity of circumferential shortening (Vcf) were calculated by formulas described elsewhere[229].

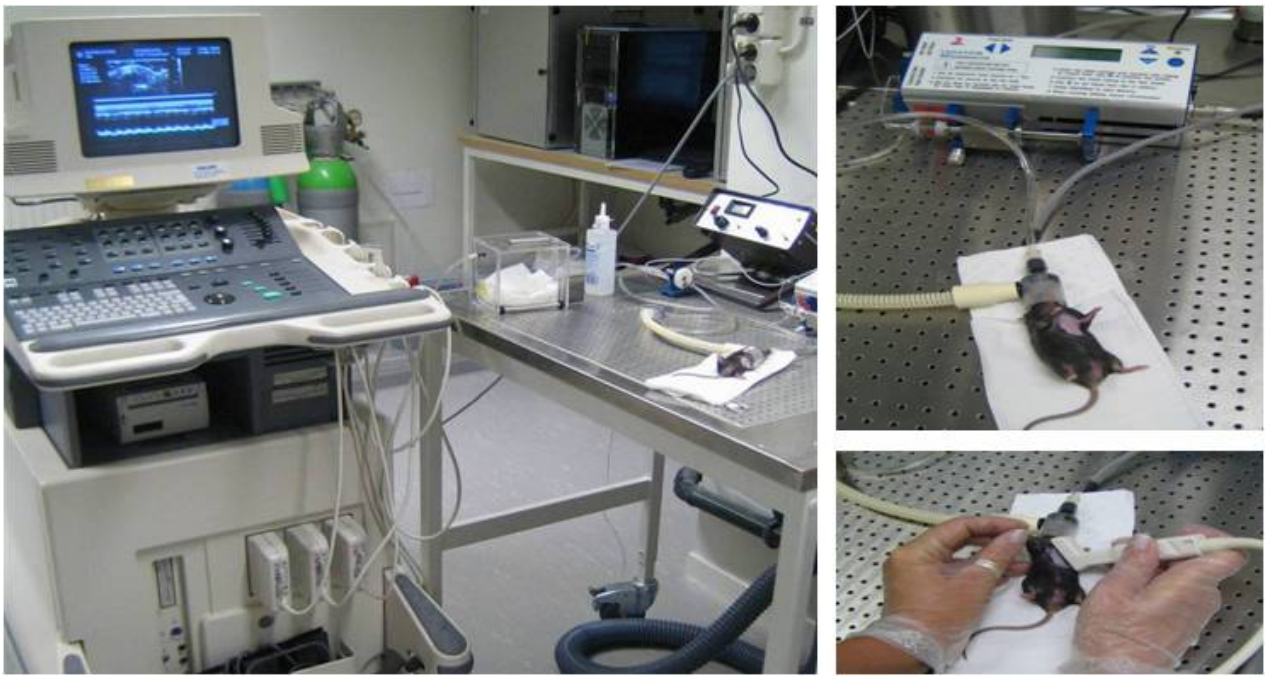


Figure 8. *Mouse echocardiography. Experimental set-up*

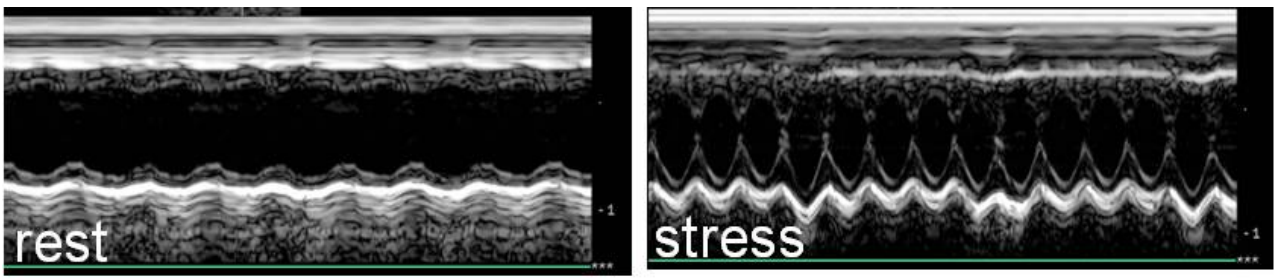


Figure 9. *Example of M-mode echocardiography in the mouse during rest and stress conditions*

Arrhythmia analysis and programmed electrophysiological stimulation (paper III)

A computerized electrocardiographic (ECG) tracing and invasive hemodynamics including intraventricular and arterial pressure were obtained continuously before induction of MI and up to 60 minutes post-MI (Figure 10). The 60-minute cutoff is based on our and others observation that most of deaths after induction of MI in this model occur within 60 minutes postinfarct [230, 231].

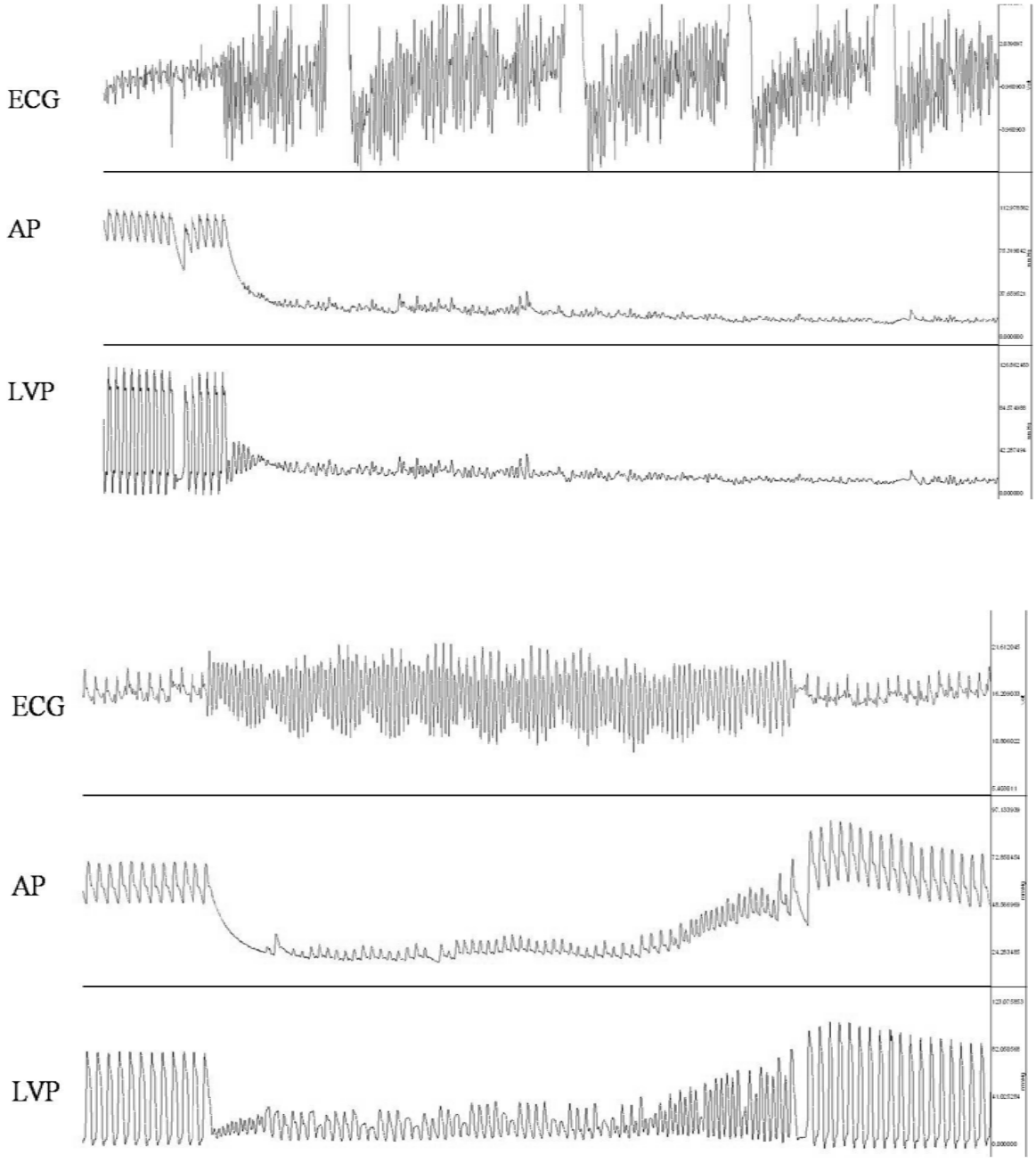


Figure 10. *Terminal ventricular fibrillation post MI in the rat (top)*
Non-sustained ventricular tachycardia post MI in the rat (bottom). Simultaneous registration of ECG, left ventricular pressure (LVP) and arterial pressure (AP)

Qualitative as well as quantitative variables of ventricular tachyarrhythmias were analyzed according to a 10-point arrhythmia score[232, 233]. The acquired single-lead ECG tracings were displayed and analyzed off-line using Biopac acquisition software in a semiautomatic fashion. All arrhythmic events were classified by the observer according to the guidelines provided by the Lambeth Convention[234]. Ventricular tachycardia (VT) was defined as more than 4 consecutive ventricular premature beats. Ventricular fibrillation (VF) was defined as a signal that changed from beat to beat in rate and configuration and was discerned from VT on the basis of arterial and LV pressure pattern (pressure drop) during the arrhythmic period[235].

Table 1. *Arrhythmia score adapted from Johnston et al. ref.* [232]

Score	Event
0	PVC 0 - 49
1	PVC 50 - 499
2	>500 PVC/ 1x VT/VF
3	> x 2 VT/VF total duration < 60 sec
4	VT/VF total duration 60 - 120 sec
5	VT/VF total duration > 120 sec
6	Terminal VT/VF > 15 min postinfarction
7	Terminal VT/VF 4 - 15 min postinfarction
8	Terminal VT/VF 1 - 4 min postinfarction
9	Terminal VT/VF < 1 min postinfarction

Several parameters must be included in the analysis of arrhythmias, such as premature ventricular complexes (PVCs), ventricular tachycardia (VT) or ventricular fibrillation (VF), number of episodes of VT/VF, total arrhythmia duration and time from MI to onset of VT/VF. This makes the analysis of arrhythmias problematic because most of these parameters certainly describe the burden of arrhythmias after an ischemic event, but leaves out the aspect of severity of the arrhythmias. For example, a non-lethal VT over a longer period would seemingly be a more severe arrhythmia than a short, but lethal VT when analyzing only the duration of the arrhythmias. In

addition, most of the parameters describing arrhythmias are non-parametric. To overcome these problems we used the arrhythmia score system as seen in Table 1. The arrhythmia score system summarizes the history of arrhythmic events and includes the aspect of arrhythmia severity in the grading system in logical manner from a clinical point of view. This previously validated score system[232, 233] also transform non- parametric parameters of the arrhythmia analysis into values suitable for parametric statistical tests.

Supraventricular and ventricular arrhythmia inducibility with programmed stimulation

These experiments were performed to investigate the effects of GH on inducibility of ventricular and supraventricular arrhythmias in normal rats using an invasive electrophysiology protocol. This method was previously described elsewhere in small animals (for review, see reference[236]). The electrophysiologic study was performed on healthy male Sprague-Dawley rats (approximately 300 g). The animals were randomized into 3 groups. Growth hormone treated rats (n = 6) received 6 mg/kg of human GH. The control group (n = 16) received saline. Amiodarone treated rats (n = 6) were injected with 25 mg/kg. All animals received a single intraperitoneal injection (1 mL) 6 hours before experiment. The animals were anesthetized with isoflurane (2%), intubated, and ventilated with a respirator. The right atrium and right ventricle were catheterized via the right internal jugular vein with a 1.1F 8-polar catheter for small animal electrophysiology (Millar Instruments, Texas, USA; Figure 11 and 12).

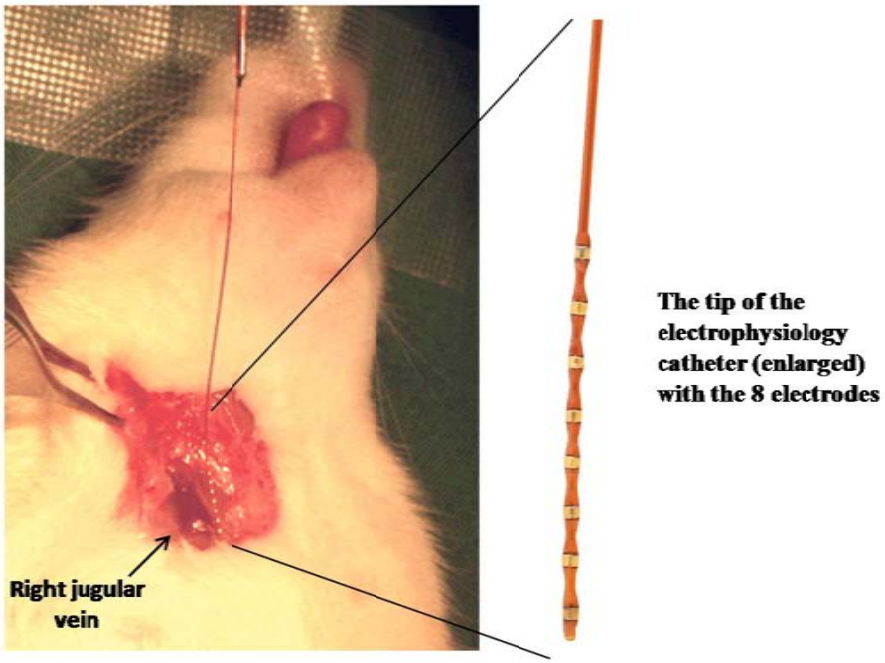


Figure 11. *The 1.1F 8-polar electrode for small animal electrophysiology.*

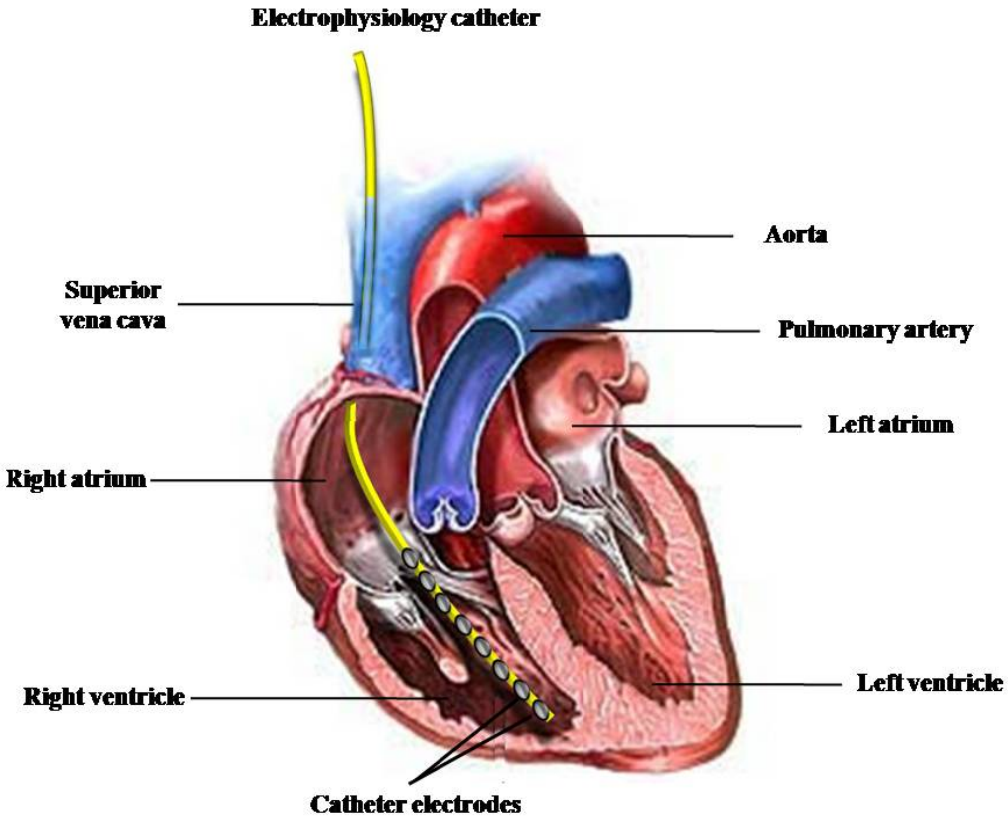


Figure 12. *Schematic view of the 8-polar electrode inserted through the right jugular vein and the superior vena cava into the right atrium and right ventricle. The electrodes are used for pacing or ECG recording at the level of atrial or ventricle.*

Ventricular stimulation

The threshold potential for stable pacing was assessed at a cycle length of 100 milliseconds. The stimulation study was then initiated at 2 times the threshold. Ventricular effective refractory period (VERP100) was assessed by introducing a single extra stimulus (second stimulus [S2]) after a train of 8 paced beats (stimulus [S1]), with an S1 to S1 at 100 milliseconds. The S2 was set at 60 milliseconds and successively lowered stepwise by 2 milliseconds until occurrence of loss of capture. This was defined as VERP100. The third stimulus (S3) was then introduced into the pulse train. The coupling interval of both S2 and S3 were set at VERP100 + 20 milliseconds. The pacing protocol continued with gradual lowering of S3 by 2 milliseconds until the occurrence of loss of capture and was subsequently set at the shortest coupling interval with maintained S3 capture. Thereafter, S2 was lowered 2 milliseconds at a time until the occurrence of loss of S2 capture but with preserved S3 capture. The stimulus (S4) was then introduced at the same coupling interval, that is, VERP100 + 20 milliseconds. Similarly, S4 was lowered until the occurrence of loss of capture and was set at the shortest coupling interval with maintained S4 capture. The S3 was lowered in the same manner, maintaining the capture of S4. At last, S2 was lowered with the same objective. This protocol was performed at baseline and after stimulation with a low dose of isoprenaline administered subcutaneously (0.02 mg/kg) to increase heart rate (HR) by approximately 10% to 20% from baseline. Evaluation of arrhythmias was performed using a binary system (inducible VT = yes or no). VT of less than 6 beats was considered not significant, whereas a VT of more than 6 beats was considered significant (Fig. 5). No discrimination was made between monomorphic and polymorphic VT (Figure 13).

Atrial stimulation

After stimulation protocol in right ventricle, we tested inducibility of supraventricular arrhythmias. The catheter was withdrawn into the right atrium, and a new pacing threshold was determined. Then a 10-second burst pace at 5 times the threshold with 1-millisecond pulse width and S1 to S1 of 10 milliseconds (100 Hz) was applied. The total time of atrial arrhythmia was determined based on the intraventricular ECG recording (Fig. 4). No discrimination was made of atrial tachycardia based on P-wave morphology. Evaluation of inducibility of atrial arrhythmias was performed using a binary system (inducible arrhythmia = yes or no; Figure 14).

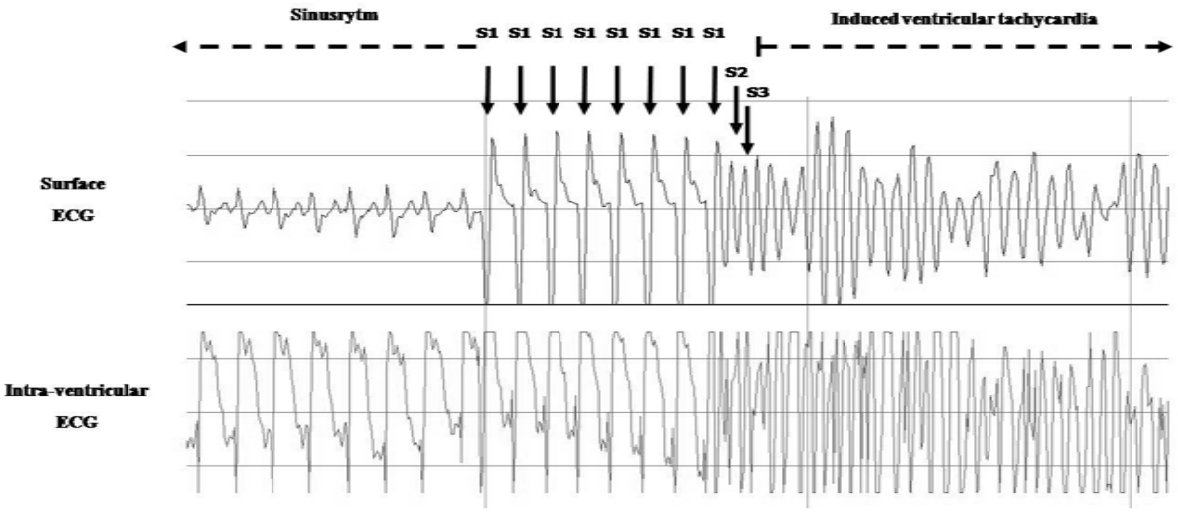


Figure 13. *Programmed ventricular stimulation protocol with induction of ventricular tachycardia*

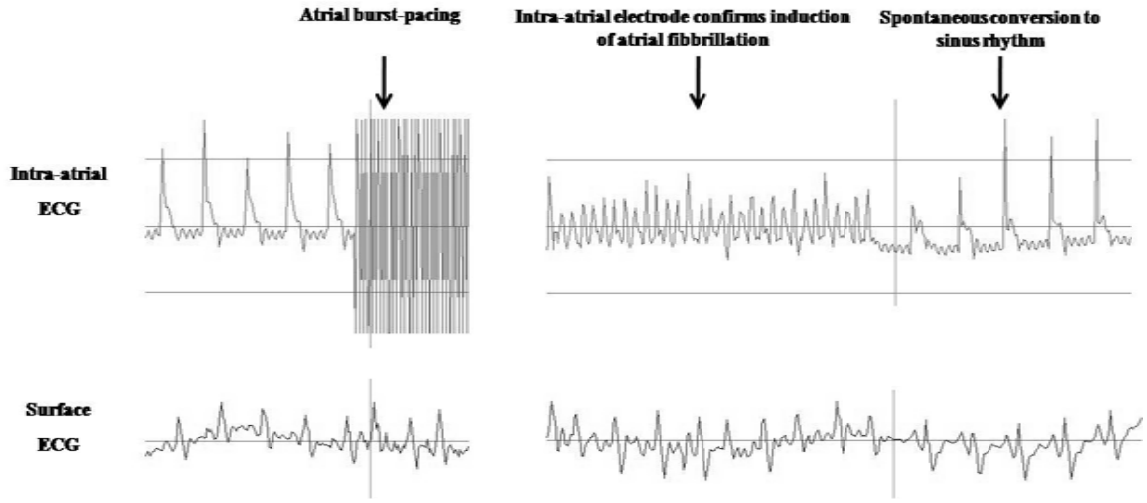


Figure 14*. Programmed atrial stimulation protocol with induction of atrial fibrillation.*

Biochemical analysis of creatine and adenine nucleotides (paper II)

The myocardial content of high energy metabolites was of high interest in these studies. Total creatine (TCr), which is the sum of Cr and PCr, and total adenine nucleotides (TAN), which is the sum of ATP, ADP and AMP, were measured in LV tissue. The reason why we present our data as TCr and TAN instead of the respective values for each compound is that the breakdown of ATP and PCr in particular, is so rapid that reliable values cannot be obtained using biochemical analysis[237]. Since TAN and TCr is the sum of ATP, ADP, AMP and Cr and PCr respectively, these values are less sensitive to degradation of ATP and PCr[89]. Standard HPLC method was used for

these measurements. Pieces of freeze-clamped tissue were homogenized on ice, in 0.4M perchloric acid. Aliquots of the homogenate were taken for protein determination. The rest of the homogenates were neutralized with 1M potassium hydroxide, centrifuged for 6 minutes in 5500 rpm at 4° C. Then the supernatants were filtrated on ice, using a syringe filter (0,22μm) and thereafter immediately injected into the HPLC (Smart system) to be analyzed. The column used for this analysis was a Luna 5u C18(2) column (Phenomenex). The high energy metabolite content was related to total protein content of each sample. The total protein content was determined using a BCA protein assay reagent kit.

Metabolic labeling and analysis of labeled lipoproteins (Paper I)

Quantification of myocardial apoB was performed using the method of metabolic labelling with [^{35}S] methionine according to the previously described protocol [171]. Heart tissue samples was minced, placed in an Eppendorf tube, and metabolically labeled with [^{35}S]methionine. After several hours, the medium was fractionated by sucrose density gradient. Apo B was immunoprecipitated from each fraction and examined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and autoradiography. The myocardial content of apoB in the samples from injured hearts was related to the levels of apoB in the control hearts at different time points i.e. at 0h, 3h, 6h, 12h, 24h, 48h and 120h in the IR study in mice, at 8 weeks post MI in mice, at 30 minutes of reperfusion in the IR study in rats as well as at 24h and 48h post doxorubicin-induced HF. The apoB content was expressed as percent of the control value that was arbitrary set to 1.

Results

Paper I

An effective way to deplete creatine in experimental models is to use the creatine analogue β-guanidinoproprionic acid (BGP), previous studies, mostly in vitro, has shown that introduction of BGP in the system competitively inhibits Cr from entering the cardiomyocytes, thereby reducing myocardial content of total creatine. This inhibits the creatine-kinase (CK) reaction and results in compromised systolic and diastolic function. The aim of this study was to investigate the effects of myocardial BGP-induced creatine depletion on LV function and morphology in mice. We also aimed to evaluate effects of creatine depletion on lipid metabolism. Another specific goal was to investigate whether alterations in myocardial structure, function and biochemistry were reversible upon normalization of the creatine content. The novelty of this study is induction of BGP-induced creatine depletion in mice, assessment of lipid metabolism and test of reversibility. After four weeks of BGP treatment the total myocardial Cr pool was decreased by 40% compared to controls (Figure 15).

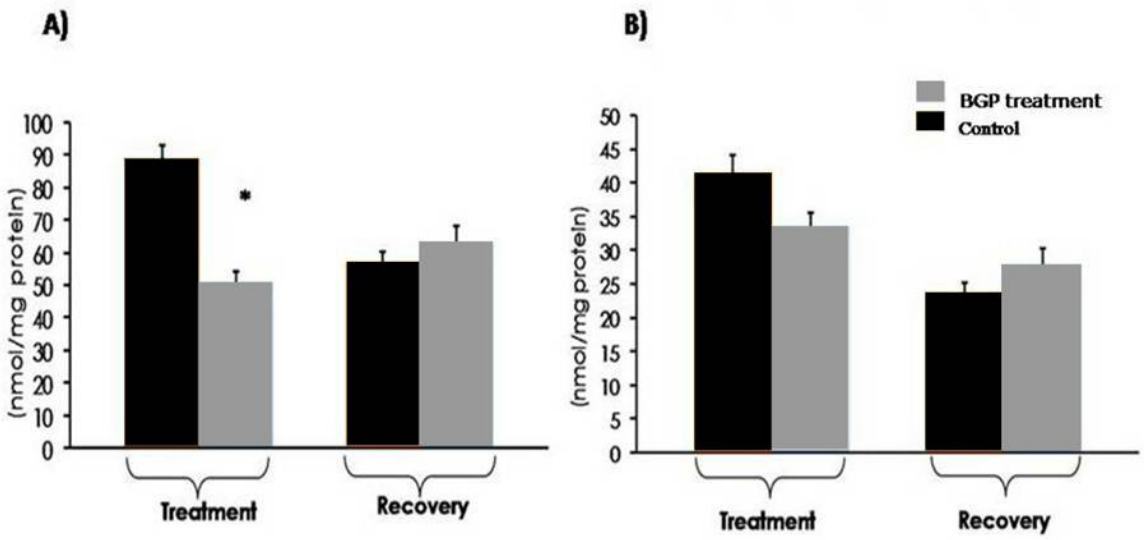


Figure 15. *(A) Cr (total creatine) and (B) TAN (total adenine nucleotides) in the heart. Cr was decreased by ~ 40% in the BGP treated animals after four weeks ($p < 0.001$). B) No significant difference was seen in TAN. No differences were found in Cr or TAN between the groups 4 weeks after discontinuation of BGP treatment.*

LV systolic function was decreased in the BGP treated mice. LV dimension both in systole and diastole were increased compared with controls, indicating LV dilatation. LV mass was also elevated in the BGP treated animals suggesting presence of myocardial hypertrophy.

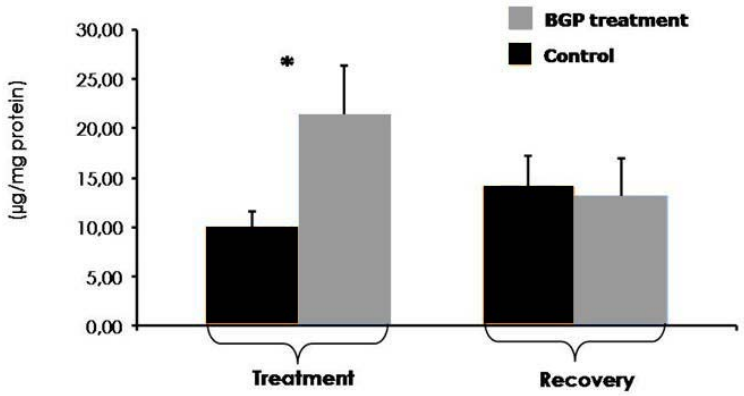


Figure 16. *Myocardial levels were elevated in the BGP group after four weeks. After discontinuation of BGP triglyceride levels had normalized (* $p < 0.05$).*

There was a 2-fold increase in the myocardial content of triglycerides in the BGP treated animals after four weeks (Figure 16). No significant differences were found in the other lipid compounds analyzed. Four weeks after discontinuation of the BGP treatment all of these functional, morphological and metabolic disturbances (except for the BW) were completely reversed. The BW was increased in both groups but was still significantly lower in the BGP treated group compared to the control group.

Paper II

In this study there were two main aims, 1) to investigate if cardiac apoB-containing lipoprotein is activated in response to ischemic injury and doxorubicin (DOX) induced acute heart failure and 2) to investigate the effects of apoB overexpression on myocardial function and survival after MI.

The myocardial apoB content in mice was increased both in the ischemic anterior wall as well as in the remote non-ischemic posterior wall, compared with the normal hearts after 0, 3, 6, 24 and 48 hours of reperfusion following the 30 minutes of ischemia. After 120 hours of reperfusion the difference was no longer significant compared to the controls. There was no difference in apoB content between the anterior and posterior wall at any of the given time-points of reperfusion. Surprisingly, eight weeks post-MI, there was a marked decrease in apoB content down to 16% of the value measured in the controls (Figure 17).

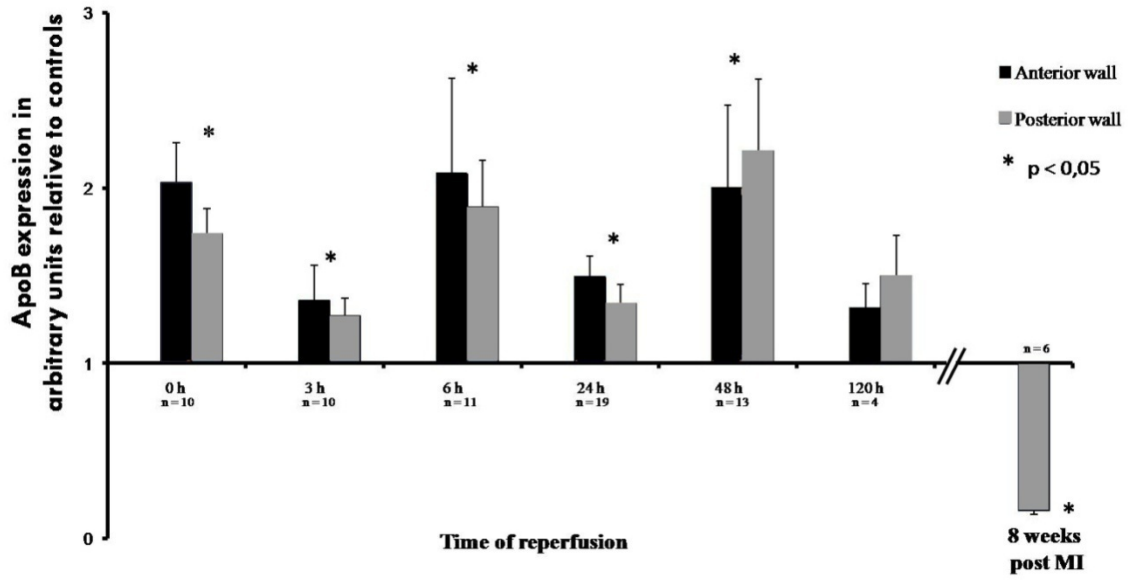


Figure 17. *Myocardial apoB in the anterior and the posterior wall compared to control hearts at the different time points of reperfusion. The apoB expression in the control hearts is set to 1.*
(= p < 0.05 v. controls.)*

In the rats the apoB content was increased ~2-fold in the ischemically-damaged part of the myocardium compared to the controls, at 24 hours of reperfusion after ischemia, but there was no such increase in the remote non-injured region. This suggests a species specific apoB response to myocardial injury. Furthermore, DOX-induced heart failure in rats, resulted in a ~70% reduction of myocardial apoB content compared to controls. No upregulation of apoB was detected in the mice with DOX induced acute heart failure. An important finding of this study was a difference in myocardial apoB response in rats compared to mice (Figure 18).

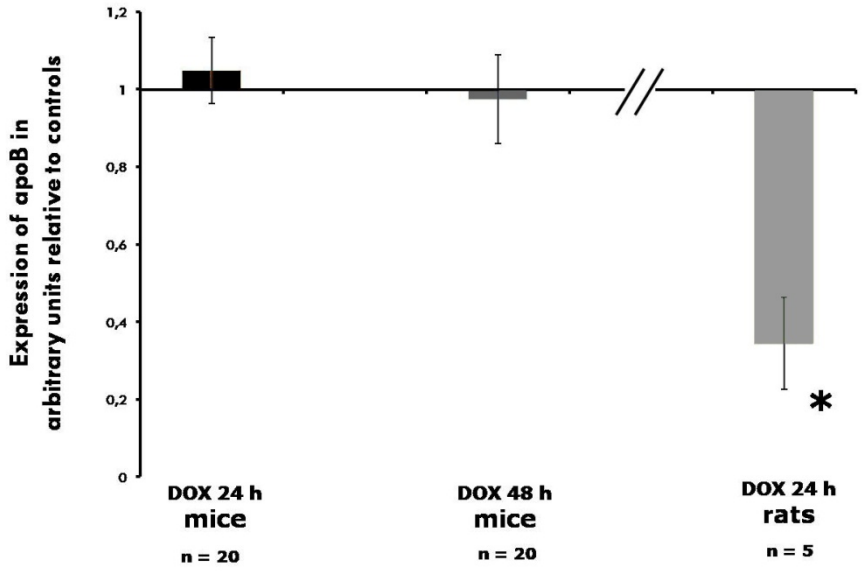


Figure 18. *Expression of myocardial apoB in mice (24 and 48 hours after DOX injection) and rats 24 hours after DOX injectionas compared to the controls. The apoB expression in the control hearts is set to 1. (* = $p < 0.05$ v. controls)*

Echocardiographic examinations revealed important differences between the mice with apoB overexpression and wild-type mice. The apoB mice had a thinner posteriors wall and tended to have a lower heart rate compared to the wild-type mice at baseline. However, there were no differences in parameters of systolic or diastolic function of left ventricular dimensions between the groups, either at rest or during stress conditions. There was no difference in LV dimensions between the groups post-MI. At 2 and 4 weeks post MI the apoB mice had better systolic function at rest. This beneficial effect was not sustained at 6 weeks post MI. We found no differences in infarct size between the apoB mice and the wild-type mice at six weeks post MI. BW was similar between the groups at the end of the study. However, LV weight was lower in the apoB group.

The apoB transgenic mice showed a two-fold better survival at 6 weeks post MI compared to wild type control mice .The largest part of the mortality occurred acute post MI i.e. within the first 24 hours post infarction (Figure 19).

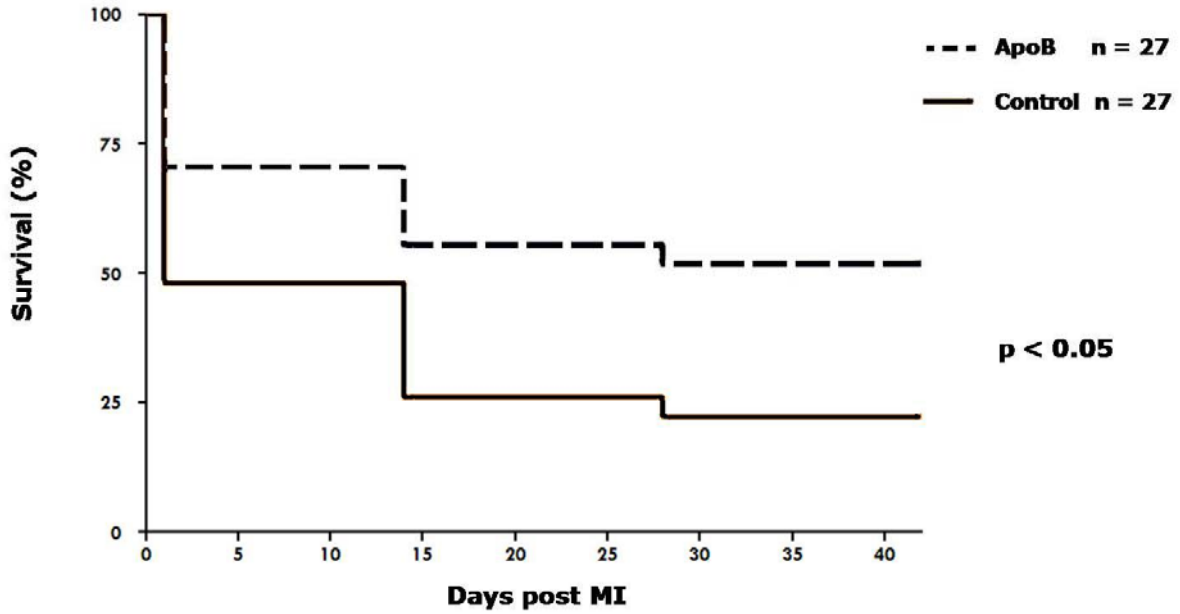


Figure 19. *Kaplan-Meier analysis of survival following acute MI. ApoB transgenic mice have shown two-fold improvement in the survival rate. The survival curves separate early, i.e. already at 24 hours post-MI and remained separated over the 6 weeks period.*

Paper III

In this study we aimed to evaluate the effects of GH on 1) ventricular arrhythmias during acute MI and 2) inducible ventricular and supraventricular arrhythmias during programmed ventricular and atrial stimulation in normal rats.

The rats were randomized into 3 groups. 1) Amiodarone group, 2) GH group and 3) Saline group (Control). All animals were injected with a single bolus of Amiodarone, GH or saline 6 hours prior to induction of MI in the first substudy and programmed stimulation protocol in the second substudy. The heart rate was lower in the amiodarone and GH groups before induction of MI (control, 411 ± 26 beats per minute; GH, 333 ± 10 beats per minute; amiodarone, 329 ± 16 beats per minute; $p < 0,05$). There was a tendency for a lower increase in HR over the follow-up period in the GH group after induction of MI. There was no difference between the groups regarding other relevant hemodynamic parameters, such as left ventricular end-diastolic pressure, left ventricular systolic pressure, dP/dt max, dP/dtmin, systolic and diastolic blood pressure at baseline or during the post MI period.

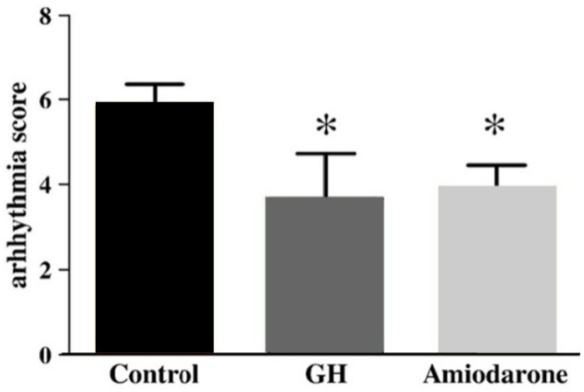


Figure 20. *Arrhythmia score. In the setting of MI, pretreatment with GH resulted in marked decreases in incidence and seriousness of ventricular arrhythmias (* $p < 0.05$ v. control)*

The GH and amiodarone treated animals displayed a significant lower arrhythmia score during the first 60 minutes post MI as compared to the controls (Figure 20). There were no significant difference in arrhythmia score between the GH and amiodarone treated animals. The number of deaths at 60 minutes in the control, GH, and amiodarone group was 6, 5, and 4, respectively. Mortality in the control group was related to sudden death due to malignant arrhythmias. In the GH and amiodarone groups, 60% and 50% of deaths, respectively, were caused by progressive heart failure.

During the invasive electrophysiologic stimulation protocol we could demonstrate that the incidence of inducible ventricular arrhythmias was lower in the GH (33%) and the amiodarone (33%) groups compared with the controls (81%, $p < 0,05$; Figure 21). There was no difference in the inducibility of AF (atrial fibrillation) between the GH (83%) and the control group (93%), whereas the inducibility of AF was significantly lower in the amiodarone group (33%, $p < 0,05$; Figure 21).

Duration of PQ and corrected QT time did not differ between the groups, whereas the QRS complex tended to be lower in the GH group.

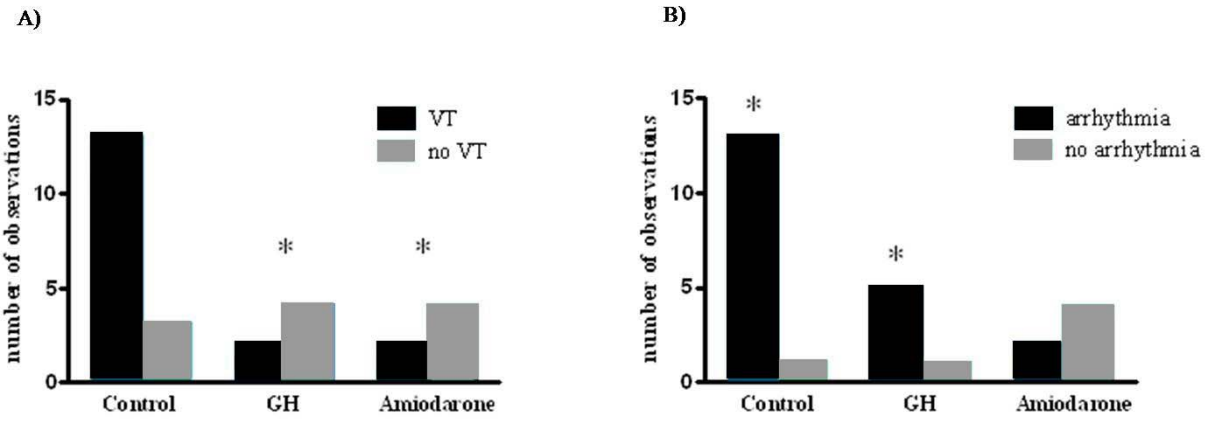


Figure 21. *A) Pretreatment with GH resulted in decreased inducibility of ventricular tachycardia,(* p < 0.05 v. control B) Pretreatment with GH had no effect on inducibility of atrial arrhythmias suggesting selective anti-arrhythmic effects of GH at the level of ventricle, * p < 0.05 v. amiodarone*

Paper IV

In this study we aimed to investigate if native cardiac reserve can predict the outcome after myocardial infarction.

We examined 27 healthy C57Bl6 mice with echocardiography both at rest and during pharmacological stress induced by dobutamine (1 μg/g body weight i.p.). The day after the echocardiography examination, a large anterior wall MI was induced and mortality was registered up to 2 weeks post MI. Two weeks after induction of MI, 7 of the 27 mice were alive (26%). We compared the native cardiac function (echocardiography) of the surviving (n=7) and the deceased (n=20) animals at rest and during dobutamine stress. The only statistically significant difference in resting values between survivors and deceased animals, was a slower heart rate in the surviving animals (R-R interval 183 vs. 138 ms p=0.02; Table 2). Evaluation of cardiac reserve (stress-rest), revealed that the survivors had increased systolic performance (ΔLVESd, p=0.02), increased cardiac reserve (ΔFS, ΔCO p=0.02 respectively) and increased chronotropic reserve (ΔR-R interval p< 0.01) during dobutamine challenge in comparison with non-survivors (Table 3).

Table 2. *Echocardiographic data during resting conditions prior to induction of MI*

		All (n=27)	Survivors (n=7)	Deceased (n=20)	p-value
REST	LPWd (mm)	0.56±0.07	0.57±0.09	0.56±0.06	0.61
	LVEDd (mm)	4.22±0.32	4.25±0.43	4.21±0.28	0.74
	LVESDd(mm)	2.73±0.56	2.97±0.59	2.65±0.54	0.19
	FS (%)	35.7±9.9	30.7±8.7	37.4±9.9	0.13
	CO (ml/min)	14.9±3.3	12.9±3.6	15.6±3.0	0.07
	R-R (ms)	150±44	183±70	138±24	0.02

Left ventricular posterior wall dimeter (LPWd), left ventricular end-diastolic diameter (LVEDd), left ventricle end-systolic diameter (LVESd), fraction shortening (FS), cardiac output (CO), R-R interval (R-R), survivors (S) and deceased (D). Data shown as mean ± SD.

Table 3. *Delta values between rest and stress echocardiographic parameters prior to induction of MI.*

	All (n=27)	Survivors (n=7)	Deceased (n=20)	p-value
ΔLVEDd (mm)	-0.62±0.32	-0.77±0.38	-0.57±0.29	0.15
ΔLVESd(mm)	-1.43±0.64	-1.86±0.76	-1.28±0.53	0.04
ΔFS (%)	28.2±12.5	37.4±14.4	25.0±10.3	0.02
ΔCO (ml/min)	-0.01±0.38	0.27±0.48	-0.10±0.29	0.02
ΔR-R (ms)	-32±43	-68±68	-19±21	<0.01

Left ventricle end-diastolic diameter (LVEDd), left ventricle end-systolic diameter (LVESd), fraction shortening (FS), cardiac output (CO), R-R interval (R-R), survivors (S) and deceased (D). Data shown as mean ± SD

Discussion

Creatine metabolism - a future target in the treatment of heart failure? (Paper I)

The failing heart is characterized by multiple and pronounced disturbances in many aspects of myocardial structure, function and metabolism[26, 238]. In this context, energy metabolism has gained increasing attention during last years as researchers are concentrating their efforts to define novel pharmacological targets for metabolic interventions with aim to improve functional efficiency and structural integrity of the failing heart[90]. Myocardial energetics and particularly creatine metabolism were addressed in numerous experimental works during recent years given the fact that Cr depletion is an obligatory characteristic of the pathologically remodeled and failing heart[86-89]. This fact has been established in different animal models as well as in humans[55, 89-92]. Several major consequences of myocardial Cr depletion are known. These include decreased PCr/ATP ratio which together with decreased velocity of CK reaction creates the condition of energetic deficit in the heart[89]. Furthermore, there is a development of LV hypertrophy, increased arrhythmia propensity as well as precipitation of systolic and diastolic dysfunction[100, 239, 240]. To our knowledge, no previous studies have addressed the simple question, namely, whether these pathologic alterations in cardiac structure and function are permanent or reversible upon Cr normalization in this model.

In this study we have demonstrated that Cr depletion in a mouse model results in compromised cardiac function, development of pathologic LV remodeling and accumulation of intracellular triglycerides. Interestingly, all morphological and biochemical alterations of Cr depletion was completely reversible upon normalization of myocardial Cr levels.

We have also investigated whether negative consequences of the Cr depletion on LV function, morphology and metabolism are reversible. Cr depletion and disturbances in the myocardial energy metabolism are generally regarded as an inherent part of the pathological remodeling in CHF. However, some scientists argue that Cr depletion may be a compensatory mechanism activated to preserve normal value of ΔG of ATP hydrolysis and that any measures to increase Cr in the failing heart may be deleterious [55, 64, 90]. Our data speaks against this argument since the spontaneous normalization of Cr, following the cessation of BGP treatment, lead to complete reversal of the pathologic remodeling with normalization of cardiac function and metabolism. There has been a previous attempt to increase myocardial Cr in the chronically infarcted and failing rat heart by means of exogenous Cr supplementation[241]. Despite marked increase in the availability, i.e. high

plasma Cr concentration, this intervention ultimately failed to increase myocardial Cr suggesting that normalization of Cr levels in the infarcted and remodeled heart may be a difficult pharmacological task. Our data are to some extent in conflict with these observations since a simple measure of BGP discontinuation was sufficient to achieve spontaneous Cr normalization in the mouse heart within four weeks. The reason for the discrepancy is not clear but it could be simply related to the difference in the animal model, i.e. the absence of post-MI heart failure in our experimental model. However, other plausible mechanisms may be involved as well. One of the known consequences of CHF is down-regulation of a specific Cr transporter (CrT) situated in the cell membrane of heart muscle cells[89]. This phenomenon is regarded as the main cause of Cr depletion in the failing heart. The CrT provides an active transport for intracellular Cr enrichment and maintenance of a steady Cr pool that is readily accessible to phosphorylation by CK enzyme reaction for production of PCr and ATP. In this way the levels of high energy metabolites i.e. PCr and ATP are maintained within narrow physiological boundaries. It has been shown that CrT is down-regulated by increased availability of its agonist i.e. extracellular Cr[62]. Indeed, this may be the reason for failure of the previous attempt since increased availability of Cr in plasma may further down-regulate CrT. We have not measured CrT during BGP treatment and after its discontinuation in this mouse model. However, others have demonstrated that BGP-induced Cr depletion leads to five-fold increase in CrT membrane availability in the rat model [63] and it is plausible to assume that the mouse heart behaves in a similar way upon exposure to BGP. Therefore, the presumably up-regulated myocardial CrT may have created optimal settings for rapid Cr recovery upon discontinuation of its competitor (i.e. BGP). Taken together, the available evidence suggests that interventions directed toward normalization of myocardial Cr in the failing heart should aim primarily to increase CrT's transport capacity in cardiomyocytes as well as to increase Cr availability in plasma. In this direction, our group has previously demonstrated that growth hormone (GH) may increase expression of CrT and normalize PCr/ATP ratio in the failing rat heart[212]. It would be interesting to explore the possibility that Cr supplementation together with GH may result in rapid normalization of myocardial Cr and improvement of cardiac function, structure and energy reserve in the post-MI failing heart. Transgenic models of both the depletion of myocardial creatine[88] and the excess of creatine[87] have been reported. These models have contributed to our knowledge of the creatine system and its role in myocardial energy metabolism, but they also have limitations. For example the knocking of GAMT resulted in decreased Cr levels in both serum and heart, but had no obvious effect on the structure and morphology of the heart. These results are in conflict with

the result from both this study and our previous study in rat[240] using the BGP induced Cr depletion. The differences could be explained by the fact that in the genetically altered animal model, there could be other biochemical mechanisms activated to compensate for the faulty metabolic pathway.

Conditions as ischemia, pathologic remodeling and heart failure are associated with myocardial accumulation of intracellular lipids. Excessive lipid accumulation in the heart is damaging to the cellular function and structure, and leads to the development of lipotoxic heart disease[166]. Our study demonstrates that left ventricular dysfunction and pathologic remodeling induced by Cr depletion is sufficient to give rise to pronounced intracellular accumulation of triglycerides. It is possible that the triglyceride accumulation itself contributed to the developed abnormalities in LV function and structure. The discontinuation of BGP followed by normalization of the Cr levels in the heart, lead to a complete normalization of both triglyceride levels and LV function and morphology. The study provides the evidence for an interaction between Cr and TG metabolism. The mechanisms behind this is not clear, but some previous studies have demonstrated that Cr is directly involved in the regulation of mitochondrial function, which is mediated by a direct stimulatory effect of Cr on Krebs cycle and mitochondrial respiration[57, 242]. The Cr depletion in this model reduces the stimulatory effect on mitochondria. This may cause decreased fatty-acid utilization, which in turn could lead to triglyceride accumulation. The hypothesis that Cr may play a role in the regulation of mitochondrial function is supported by the existence of a specific mitochondrial CrT and a mitochondrial Cr/PCr pool. [58]. Future studies should look into the mechanisms behind the association between Cr depletion and triglyceride accumulation.

Cardiac apoB lipoproteins - an endogenous cardioprotective system (Paper II)

There is experimental evidence to support the hypothesis that myocardial apoB system may play an important role in cardioprotection by counteracting excessive intracellular lipid accumulation which prevents/attenuates development of the lipotoxic heart disease[176, 243]. This evidence is mainly based on the data from transgenic models in which the genetic manipulation have resulted in myocardial lipotoxicity and development of cardiomyopathic HF. When the initial genotype was crossed with cardiac overexpression of apoB, it resulted in a rescue of cardiomyopathic phenotype and was associated with improved cardiac function and survival[176]. However, the possible importance of myocardial apoB for survival,

preservation of cardiac function and structure as well as the effect on ventricular arrhythmias in the setting of acute MI and HF has not been previously studied.

I this study we demonstrate the involvement of the myocardial apoB in the response to ischemic injury. We observed a biphasic pattern of myocardial apoB response with early up-regulation and late depletion after ischemic injury. Interestingly, in DOX-induced acute HF the apoB expression was either unchanged or down- regulated. The most intriguing finding in the study is however, the marked decrease in the mortality rate following MI in mice with apoB overexpression.

Studies of heart biopsies from patients undergoing cardiac surgery due to the presence of ischemic heart disease have shown increased MTP expression in hypoxic areas of left ventricle. Importantly, MTP expression correlated negatively with cardiac triglyceride storage in this study[243]. Our results are congruent with these human data. Indeed, they show that myocardial apoB response elicited by ischemia is prompt and sustained in the early post-MI phase. The apoB response to an ischemic injury seems to be species specific. In mice we observed up-regulation of apoB in the ischemic area but also in the non-ischemic remote area of the heart whereas in rats apoB was only up-regulated in the ischemic region. Other studies have shown that lipids accumulate rapidly in the peri-infarct area as well as in the remote, viable myocardium post-MI[244]. The reason for the species difference in apoB response is not known, but it may be related to known species-dependent differences in the myocardial metabolism[245, 246].

One of the most surprising findings was the dramatic decrease in apoB in the setting of chronic post-MI HF. The mechanisms and reasons for such a divergent time-course in the apoB response are unclear. Although we have not measured the apoB between five days and eight weeks post-MI, it is plausible to assume that the initial increase in the apoB expression reaches plateau at five days and then steadily decreases to the subnormal levels suggesting the development of exhaustion in the apoB response. These findings are congruent with the mortality rate as well as with the functional data in our survival substudy conducted in the transgenic mice. The excessive mortality in the control group was most notable within the first 24 hours post-MI, the phase during which apoB is upregulated ~ 2 times the normal level in the native mice. This early post-MI phase is also characterised by development of acute HF due to the presence of a large MI. The exact reason for the improved survival in the apoB mice may not be determined by the presented data. We speculate that myocardial overexpression of apoB may have resulted in a better and more prompt activation of compensatory mechanisms for maintenance of cardiac function. Furthermore, increased

myocardial apoB may exert protective effects against malignant ventricular arrhythmias by isolating and exporting pro-arrhythmic lipids accumulated during and after IR period[247, 248]. Although intracellular lipid accumulation has been shown to exert pronounced pro-arrhythmic effects[145, 249] we did not observe any difference in the incidence of arrhythmias between the two groups within the first 45 minutes post-MI. We believe, however, that this finding should be interpreted with caution since the incidence of arrhythmias in the setting of MI is generally low in mice as compared to other animal models of experimental MI[250, 251]. There are also important differences between different mouse strains in regard to arrhythmia susceptibility[252]. Future studies using other models and experimental settings should address the question whether apoB provides anti-arrhythmic protection. The fact that the systolic function was better in the apoB mice early post-MI and the observation that incidence of arrhythmias was no different between the groups strongly suggests that reason for the increased survival in the apoB mice is to be found in the better ability to cope with the hemodynamic consequences of acute HF. One explanation may be that transgenic apoB hearts are remodelled in terms of function, structure and biochemistry in a way that sum of these alterations provides better ability for prompt adaptation during life-treating condition such as a large MI and acute HF. Further studies are needed to explain in detail the reasons and mechanisms for these and possibly other important differences in cardiac phenotype that are consequence of cardiac apoB overexpression.

ApoB overexpression had no apparent effect on the infarct size measured at six weeks post-MI. The finding of similar infarct size but lower heart weight suggests an anti-remodelling effect of apoB. Post-MI remodelling is the process that is responsible for transition from compensated LV dysfunction to over heart failure and is an important focus of current research in experimental as well as in clinical cardiology[25, 26, 253]. Previous studies have demonstrated the close relationship between myocardial lipid metabolism and hypertrophy[116, 117, 254]. Improved systolic function was not sustained at the later post-MI phase while the survival benefit remained. It is tempting to speculate that the biphasic time-dependent post-MI apoB response may be related to these findings. Exhaustion of apoB may be part of pathologic biochemical remodelling contributing to the phenomenon of transition from compensated LV dysfunction to overt HF.

Regulation of the apoB synthesis in the liver and intestine is relatively well studied[255], however, the details about regulation of endogenous apoB in the heart are largely unknown. It is possible that mechanisms similar to those in the liver and intestine are operating in the heart as well. Giving the fact that neurohormonal, mechanical and metabolic processes are of

great importance for determination of cardiac function and structure after injury, it will be important to explore how these affect the regulation of apoB in the heart post-MI and in HF. Furthermore, we should study how myocardial regulation of apoB is affected by pharmacological agent used for the treatment of MI and HF such as beta-blockers, ACE inhibitors, statins and other.

The finding that DOX-induced acute HF did not induce myocardial apoB synthesis in mice and was actually decreased in the rat heart is rather intriguing. It suggests that the mechanisms of myocardial damage and/or the nature of the noxious stimuli may be important for apoB activation. DOX-induced cardiotoxicity is a well described model of experimental HF[226, 256].This agent induces myocardial injury through several mechanisms including severe mitochondrial dysfunction, disturbed energy metabolism, lipid accumulation and DNA damage (for review see[257, 258]). We should explore the possibility that DOX - due to the DNA damage - may disrupt the genetic message involved in the synthesis of MTP and apoB molecules. Indeed, DOX although being one of the most effective cytostatic agents for treatment of different malignancies, may cause special form of toxic cardiomyopathy and HF[259, 260]. This leads to the question whether DOX-induced cardiomyopathy at least in part may be mediated by its ability to suppress the apoB response.

Anti-arrhythmic properties of growth hormone (Paper III)

It has been established previously that both GH and amiodarone exert acute effects, upon administration of a single dose, on several physiologic and pathophysiologic variables that may be important for development of arrhythmias both in humans and in animals[261-264]. GH exerts numerous effects on the heart in the setting of MI, such as reduction of infarct size, hypertrophic response in the injured myocardium, improved energy metabolism which contributes to preservation of LV size and function[197, 201, 208, 261, 265, 266]. Early post MI GH treatment has also been shown to reduce mortality in the rat model of MI. GH may influence important cellular mechanism involved in the maintenance of electrophysiologic stability of the heart.

In the present study we show that GH exerts pronounced antiarrhythmic properties in the setting of acute MI in rats and reduces the susceptibility to develop ventricular, but not atrial tachyarrhytmias during the invasive programmed electrophysiologic stimulation protocol. We speculate that the antiarrhythmic effect of GH may be mediated by inhibition of the sympathicus at the organ level, for example, in the brain and/or heart. This speculation is at

least partly supported by the fact that the resting HR was lower in animals receiving GH pretreatment. However, lower HR was not sustained, raising the question whether repeated or higher GH doses might have elicited a more persistent effect. On the other hand, the observed antiarrhythmic effects of GH may have been mediated by other yet-unknown mechanisms independent of interaction with the sympathicus or vagus area. Indeed, our results from invasive electrophysiology protocols in normal rats support the hypothesis that antiarrhythmic effects of GH may be mediated by mechanisms independent of sympathicus inhibition. It is plausible to assume that several different mechanisms are involved, such as sympathetic activity, fatty acid metabolism, energetic balance, calcium kinetics, activity of ion channels, and others[197, 222, 224, 267, 268]. The favourable haemodynamic effects of GH administration reported previously [189, 266, 269] may reduce LV wall stress and decrease stretch-induced VTs[270]. Acute GH administration may induce coronary vasodilation[271], resulting in increased oxygen supply to the border zone and, thereby, to decreased arrhythmogenesis[272]. Interestingly, the GH effect during the electrophysiological study was confined to the ventricle and was not effective in preventing tachyarrhythmia at atrial level. The reason for this is not clear, and further studies should elucidate the mechanisms behind these intriguing findings. The important limitation of the study is the relatively low number of observations and that no data are provided regarding the MI size. Both incidence of ventricular tachyarrhythmias and the severity of LV dysfunction may depend on the extent of myocardial ischemia as well as MI size. However, the groups have shown similar LV hemodynamics after MI induction, suggesting the presence of comparable myocardial damage. Neither GH nor amiodarone has decreased the overall mortality, but both have significantly altered the timing and the mode of death (early sudden death due to ventricular arrhythmias vs late death due to progressive heart failure). The most likely explanation is that the single dose of GH and amiodarone probably did not alter underlying size and severity of MI.
We believe that this study, demonstrating novel antiarrhythmic properties of GH, adds further evidence to the concept of GH in the treatment of MI and heart failure.

Native cardiac reserve predicts outcome after acute infarction in mice (Paper IV)

The chronotropic reserve in humans has been shown in several studies to be a predictor of mortality and morbidity. A Finnish study[273] has shown in healthy middle-age men that survivors had a lower resting heart rate but a higher maximal heart rate than individuals who died of cardiovascular mortality during a follow up of 11 years. Similarly, Cheng et al[274] has

shown in healthy men (27 459) that chronotropic reserve was inversely associated with cardiovascular mortality. Especially, low chronotropic reserve in healthy younger men seems to be associated with future cardiovascular events. Dobutamine stress echocardiography has been shown to be an independent prognostic predictor of all-cause mortality and hard cardiac events in elderly patients. On the other hand, 47% of these patients showed signs of ischemia during the dobutamine stress echocardiography and therefore this population cannot be defined as a normal healthy population[275]. However, cardiac reserve as described in this study has not previously been evaluated in healthy individuals for prediction of mortality.

In this study we demonstrate that native inotropic and chronotropic reserve in mice may predict survival after acute myocardial infarction. To our knowledge this is the first study to demonstrate a direct correlation between cardiac reserve in healthy animals and survival after acute myocardial infarction.

Although it may seem logical that a better cardiac reserve prior to a large MI would predict a better outcome, this study does not provide any explanation for the observed results and we can only speculate about the mechanisms involved.

While humans are characterised by general variability in the coronary artery anatomy, inbreed mice (C57BL6) have a relatively uniform coronary tree anatomy[276]. Ligation of the LAD immediately after the bifurcation results in an extensive infarction of the free wall extending down to the apex, with sparing of the septum. Salto-Tellez et al could also show that ligation of the LAD ~1mm after the bifurcation results in a consistent size of the infarction[276]. Although, the infarct size of the deceased animals in this study is unknown, all survivors had large MI with comparable reduction in systolic function. In our experience, using the same ligation technique the study groups develop approximately the same mean infarct size. We believe that explanation for our results may be found in the heterogeneity of the normal myocardium. It has been shown previously that even genetically homogenous groups of animals with seemingly uniform macroscopic characteristics of the heart show substantial physiological heterogeneity of protein expression, energy turnover and flow [277].

We believe the explanation for our results showing that the surviving animals have a better native cardiac function may be found in the heterogeneity of the normal myocardium. This heterogeneity might affect several aspects of normal cardiac physiology that could be important for the ability of the heart to respond to stress. This may include several metabolic pathways but also receptor function. An interesting question is whether a better native cardiac reserve predicts better post MI outcome simply due to a better ability to cope with the hemodynamic consequences of a large MI, or if the native cardiac reserve depicts the

involvement of cardioprotective pathways that may affect final infarct size and improve post MI survival. It would be of interest to evaluate the possible prenatal influence on native myocardial function and heterogeneity. Some animal studies have already addressed this issue in regard to other physiological and pathophysiological variables [278, 279]. It is also possible that a reduction in the β-1 adrenoceptor density or sensitivity could account for a reduced inotropic and/or chronotrophic reserve. Independently of the mechanisms involved, the predictive value of cardiac reserve is not diminished. Because different mechanisms may be involved in the improved survival in the presence of higher cardiac reserve further studies are needed to clarify this novel observation.

Conclusions

Congestive heart failure and myocardial infarction are major global health problems and will continue to be major threats to life and health in the coming decades. New therapeutic strategies are needed in order to alter the detrimental course of these diseases and decrease morbidity and mortality in our patients. The search for new interventional targets will continue to depend on the knowledge about basic pathophysiological mechanisms based on relevant preclinical models. This thesis, while exploiting some aspects of myocardial metabolism in experimental heart failure and infarction, adds a small contribution to that "bank of knowledge".

In conclusion, myocardial creatine depletion leads to disturbed energy metabolism, left ventricular dysfunction, pathologic remodeling and accumulation of triglycerides. These alterations are reversible upon the normalization of the creatine levels suggesting that creatine metabolism may be an important target for future pharmacological intervention in order to increase myocardial efficiency and maintain structural integrity of the failing heart.
We have demonstrated that the myocardial apoB is mobilized during pathophysiological conditions such as ischemia, pathologic remodeling and may protect the heart from the detrimental effects of lipotoxicity.
We have shown that growth hormone possess antiarrhythmic properties in the setting of acute MI which adds further evidence to the concept of growth hormone as an additional pharmacological agent in the treatment of CHF and MI.
Finally, we have demonstrated that native cardiac reserve is a predictor of post-MI survival.

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