Myocardial creatine metabolism in experimental infarction and heart failure

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Abstract

The failing heart is characterized by changes in its structure, function and metabolism. An important part of this negative remodelling process is disturbed myocardial energy metabolism. The failing myocardium contains low levels of creatine (Cr), phosphocreatine (PCr), adenosine-triphosphate (ATP) and accumulates intracellular lipids. Cr depletion in the heart muscle may result in disturbed energy production, transfer and utilisation of chemical energy and therefore compromised left ventricular function. The heart depends on exogenous lipids for the oxidative production of ATP but it also synthesizes and releases lipids in the form of apolipoprotein-B containing lipoproteins (apoB). It has been proposed that apoB may be involved in cardioprotection by means of elimination of toxic intracellular lipids.

The aims of this thesis were:

- To investigate whether measures of intensive cardiac care applied to rats with acute myocardial infarction would reduce mortality in this small animal model.
- To investigate *in vivo* the effects of Cr depletion in rats on left ventricular function and morphology, energy metabolism, catecholamines and incidence of malignant ventricular arrhythmias during acute myocardial infarction.
- To investigate *in vivo* the effects of Cr depletion in mice on left ventricular function and morphology, energy metabolism and myocardial lipids.
- To investigate importance of endogenous lipoproteins in the heart for cardiac function, morphology and survival in the settings of acute and chronic myocardial infarction.
- To investigate acute and chronic effects of complete heart block on cardiac function, morphology and energy metabolism in a rat model.

Using small animal models (rat and mouse) of chemically-induced Cr depletion we show *in vivo* that myocardial creatine 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 future pharmacological interventions. We provide experimental evidence that the biochemically remodeled heart is prone to malignant ventricular arrhythmias and to rapid progression to acute heart failure when subjected to myocardial infarction.

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

Using a rat model of complete heart block we demonstrate that long-term bradycardia leads to development of pronounced eccentric hypertrophy with preserved energy metabolism and no signs of heart failure – a possible model for future studies of mechanisms behind the beneficial cardiac remodeling.

List of abbreviations

2, 3-DPG	2, 3-diphosphoglycerate
^{31}P	Phosphorous
apoB	apolipoprotein B
ADP	adenosine-triphosphate
AGAT	L-arginine:glycine amidinotransferase
AMP	adenoseine-diphosphate
ATP	adenosine-triphosphate
BGP	beta-guanidino proprionic acid
CHB	complete heart block
CHF	congestive heart failure
CK	creatine kinase
CO	cardiac output
Cr	creatine
CrT	creatine transporter
FFA	free fatty acids
FS	fractional shortening
GAA	guanidinoacetate
GAMT	S-adenosyl-L-methionine:N-guanidinoacetate
HEP	high energy phosphometabolites
HPLC	high performance liquid chromatography
ISIS	image selected in vivo spectroscopy
LV	left ventricle
LVDd	left ventricular diameter in diastole
LVDs	left ventricular diameter in systole
LVM	left ventricular mass
LVM/BW	left ventricular mass index
MI	myocardial infarction
MRS	magenetic resonance spectroscopy
MTP	microsomal transfer protein
NMR	nuclear magnetic resonance
PCr	phosphocreatine
PDE	phosphodiesters
P_i	inorganic phosphate
SV	stroke volume

List of publications

This thesis is based on the following papers:

I

Råmunddal T, Lorentzon M, Omerovic E.
Decreased mortality in a rat model of acute postinfarction heart failure.
Biochem Biophys Res Commun. 2006; 341(2):459-63

II

Lorentzon M., Råmunddal T., Bollano E., Soussi B., Waagstein F., Omerovic E.,
In vivo Effects of Myocardial Creatine Depletion on Left Ventricular Function, Morphology and Energy Metabolism – Consequences in Acute Myocardial Infarction.
J Card Fail. 2007; 13(3):230-7.

III

Lorentzon M., Råmunddal T., Camejo G., Waagstein F., Omerovic E.
In vivo effects of myocardial creatine depletion on left ventricular function, morphology and energy metabolism in mice
(Submitted)

V

Råmunddal T, Lindbom M, Stillemark-Bilton P., Scharin-Täng M, Boren J, Omerovic E.
Overexpression of apolipoprotein-B improves cardiac function and increases survival in mice with myocardial infarction.
(Submitted)

IV

Gizurarson S., Lorentzon M., Råmunddal T. Waagstein F., Bergfeldt L., Omerovic E.
Effects of complete heart block on myocardial function, morphology and energy metabolism in rats.
Europace. 2007; 9(6): 411-6.

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Introduction

The syndrome of heart failure

Myocardial infarction (MI) and congestive heart failure (CHF) are the two leading causes of morbidity and mortality in Sweden. In spite of the therapeutical success during recent years with new drugs and devices the prognosis for patients with MI and CHF remains poor[1-3]. Almost half 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. Patients living with CHF endure a worse quality of life than those with most other chronic diseases. CHF also imposes a heavy burden on health care resources, mainly because of the high costs of hospitalization. Sudden cardiac death is the most common cause of death in CHF patients and survivors of MI. Over the past decade, the rate of hospitalizations for CHF has almost doubled[4]. 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[5-7]. Having this in mind, the necessity for development of new therapeutic interventions for patients with CHF becomes obvious.

There are various definitions of congestive heart failure (CHF). Probably the most used definition of CHF is the one defined by Eugene Braunwald. According to the definition, CHF is a pathophysiological condition where the heart is unable to pump sufficient amounts of blood to satisfy the metabolic needs of the tissues and organs of the body.[8] CHF is a complex syndrome and may be elicited by various pathophysiological mechanisms such as ischemic heart disease, hypertension, valve disease, myocarditis, autoimmunity, toxins, different cardiomyopathies and others.

Cardiac remodeling

As heart disease progresses towards CHF, the myocardium undergoes profound alterations in its structure and function at several levels. As the size of the organ increases and function deteriorates, there is gradual transformation from a compensated to a decompensated condition during which clinical symptoms of CHF become visible (dyspnea, peripheral edema, increased heart rate, decrease in physical activity etc.). The complex process responsible for this gradual deterioration leading to development of mechanical (pump failure) and electrophysiological dysfunction (arrhythmias) is called pathologic cardiac remodeling. It can be defined as the sum of all changes at molecular, cellular and at organ level which manifests as change in size, shape and function of the heart. The cardiac cell that is most affected by cardiac remodeling is the myocyte although other cells are affected as well i.e. fibroblast end endothelial cells[9].

Pathologic cardiac remodeling is a consequence of different types of heart disease or injuries. The process that is of greatest interest for this thesis is remodeling caused by extensive ischemic damage i.e. post-infarction remodelling[9, 10]. The cardiac remodeling after MI begins immediately after the injury[11-13]. During the first hours post-infarction, myocyte death (necrosis, apoptosis) causes development of inflammation and edema in the infarcted region of the heart. After this initial (inflammatory) phase, the formation of scar tissue (replacement fibrosis) continues for up to several months after infarction in the human heart[14]. The process

of scar formation is much faster in rodents (mice, rats) and is completed typically within 2-3 weeks. Pathologic cardiac remodeling is responsible for development of decompensate CHF and its most important clinical consequences, i.e. decreased quality of life and premature death[10]. To improve symptoms and increase survival in patients with CHF it is very important to find ways to reduce and prevent ventricular remodelling[9, 10]. This could be achieved by limiting the infarct (damage) size, reduce or prevent infarct expansion or enhance myocardial reparation-regeneration processes. To succeed in this goal in the future we will continue to depend on relevant animal models[10].

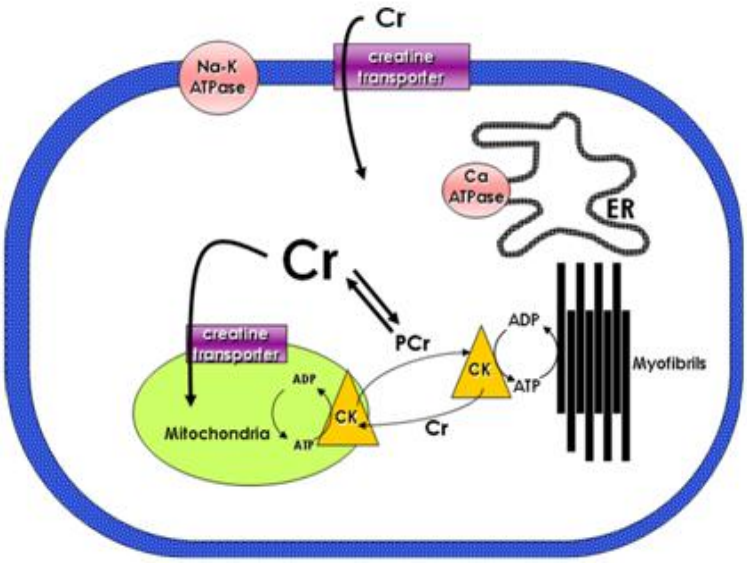
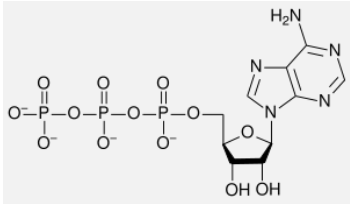


Figure 1 *Simplified overview for the most important parts of creatine and energy metabolism in the cardiomyocyte. Cr = creatine. ER= endoplasmatic reticulum, PCr = phosphocreatine, ATP=adenosine triphosphate. See the text for more explanations.*

Myocardial energy metabolism

An integral part of pathologic cardiac remodeling is alteration in myocardial biochemistry - biochemical remodelling[15]. One of the most important consequences of adverse biochemical remodeling is the disturbed myocardial energy metabolism given the importance of chemical energy for normal heart function. There is large body of evidence to support the argument that the failing heart is an energy starved organ[16, 17]. The energy starvation is due to disturbances in production, transport and utilization of chemical energy in form of ATP (adenosine triphosphate)[17-19]. At a cellular level, ATP is the energy source for most biological systems. The heart has intricate biochemical machinery for production, transport and consumption of chemical energy (Figure 1). The ability of the heart to develop a propelling pressure to cause the blood to flow in our vessels by its "indefatigable" contractions is a process that requires an enormous amount of energy in uninterrupted supply. Myofibrils use the energy stored in the ATP molecule producing degradation products ADP (adenosine-diphosphate) and inorganic phosphate (P_i). ATP can be produced in several different ways: de novo synthesis, phosphoryl transfer from phosphocreatine (PCr) catalyzed by creatine kinase (CK), oxidative phosphorylation of ADP. For the heart to function normally it is necessary that the myocardial ATP is maintained within narrow narrow boundaries of "physiological" concentration. The heart has developed a robust "biochemical machinery" in order to fulfill this important prerequisite even if the energy demand characteristically varies in the mammal heart due to different workload. Myocardial oxygen consumption is directly proportional to the rate of the oxygen that is needed for ATP synthesis by oxidative phosphorylation. With increasing oxygen consumption due to higher workload, the healthy heart will increase its synthesis of ATP in order to match the increased energy demand and maintain [ATP] constant.

The main energy producing mechanism in the heart is the ATP synthesis by oxidative phosphorylation. This takes place in the mitochondria, the respiratory chain, supply of oxygen and the ATPase taken together, rephosphorylate ADP into ATP.



ATP is required for many essential reactions in the myocytes for example; in the myofibrilar cross-bridge ATPase reaction, the Ca^{2+} -ATPase reaction in the endoplasmatic reticulum (ER) and many energy-demanding sodium-potassium pumps in the cell membrane. In order to describe the energy state of the cell we need to define relationship between energy demand and supply. This can be achieved by providing different biochemical indexes such as phosphorylation potential, PCr/ATP ratio, free energy of the of ATP hydrolysis and others. Phosphorylation potential is defined as: [ATP]/ [ADP] [P_i]. High [ATP]/ [ADP] [P_i] ratios inhibits ATP synthesis and stimulates chemical reactions that use ATP as substrate and low [ATP]/ [ADP] [P_i]ratios results in the opposite[20]. In a normal, well perfused heart the typical values for ATP, ADP and P_i concentrations are 10mM, 30μM and 1mM respectively. Even very modest increases in ADP and P_i concentrations at essentially constant ATP concentrations results in a huge difference in the phosphorylation potential, thus making it a very sensitive marker of the energetic state of the cell[20]. The phosphorylation potential has vast influence on the free energy of the of ATP hydrolysis, $\Delta G_{\sim P}$. The free energy of ATP hydrolysis defines the driving force for all ATP utilizing reactions in the cell, it is expressed as : $\Delta G_{\sim P} = \Delta G_{\sim P}^{\circ}$ - RT *ln* [ATP]/ [ADP] [P_i], where $\Delta G_{\sim P}^{\circ}$ is the standard free energy change of ATP hydrolysis, R is the gas constant and T is the absolute temperature in Kelvin. $\Delta G_{\sim P}$ is often described as its absolute value, $|\Delta G_{\sim P}|$. For an ATP utilizing reaction to take place, the driving force ($|\Delta G_{\sim P}|$) of the cytoplasm of the cell must be greater that the threshold value for that particular reaction. One way to describe the difference between $|\Delta G_{\sim P}|$ for the cell and the $|\Delta G_{\sim P}|$ for a specific ATP utilizing reaction is energy reserve[20]. So it is important for the cell to maintain high phosphorylation potential and $|\Delta G_{\sim P}|$. One way to maintain a high phosphorylation potential is via the creatine kinase (CK) reaction.

The CK reaction rapidly resynthesize ATP by transferring a phosphoryl group from phosphocreatine (PCr) to ADP, and thereby it also keeps the [ADP] low[20].

ATP utilization: $ATP + H_2O \rightarrow ADP + P_i + H^+$

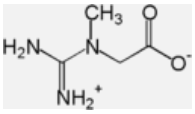
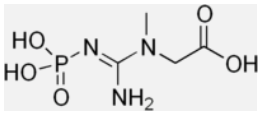
ATP resupply: $PCr + ADP + H^+ \leftrightarrow Cr + ATP$

There are different kinds of CK enzymes, the mitochondrial CK (CK_{mito}, which comes in two different forms ubiquitous and sarcomeric), the cytosolic M-CK (M = muscle) and B-CK (B = brain). The CK enzymes catalyze the reversible transfer of a phosphate group from ATP to the guanidine group of creatine (Cr) to yield ADP and PCr[16, 20-22].

The presence of one CK enzyme both in the mitochondria and in the cytosol creates a shuttle of energy from the mitochondria, where it is produced, to the sites of energy utilization, e.g. the myofibrils and ion pumps. After ATP synthetisation in the mitochondria, a phosphate group from ATP is transferred by CK to Cr to produce ADP and phosphocreatine (PCr). The ADP released in this reaction is directly transported back and rephosphorylated. PCr on the other hand, leaves the mitochondria and diffuses through the cytosol to the sites of ATP

consumption. At these sites, CK enzymes uses PCr to rephosphorylate ADP into ATP after which Cr diffuses back to the mitichondria[21].

Creatine and creatine transporter

Cr  and PCr  are smaller and less negatively charged than ATP and ADP. Consequently, they can be stored in a much higher concentrations and can be more easily transported to the different sites in the cell[21]. PCr is therefore considered as the main energy storage in the myocyte. Cr can either be introduced by food intake or be synthesized in the body. Cr synthesis begins in the kidney with the transfer of the amidino group from arginine to glycine which produces guanidinoacetate (GAA) (Figure 2). This reaction is catalyzed by L-arginine:glycine amidinotransferase (AGAT). GAA is then transported through the blood to the liver where it is methylated to Cr. The methylation step is catalyzed by S-adenosyl-L-methionine:N-guanidinoacetatetransferase (GAMT). The Cr leaves the liver and is transported through the bloodstream to tissues that require Cr[21]. Cr uptake from the blood, into tissue that contains CK, as heart, skeletal muscle, brain or kidney is enabled by a specific creatine transporter (CrT)[21, 23]. 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[24]. In experimental studies, Cr supplementation lead to down-regulation of creatine transporter both in rats[25] and in cell culture[26, 27]. Cr depletion induced by the creatine analogue, β-guanidinoproprionic acid (BGP) in rats, lead to an increase in the CrT membrane availability[28].

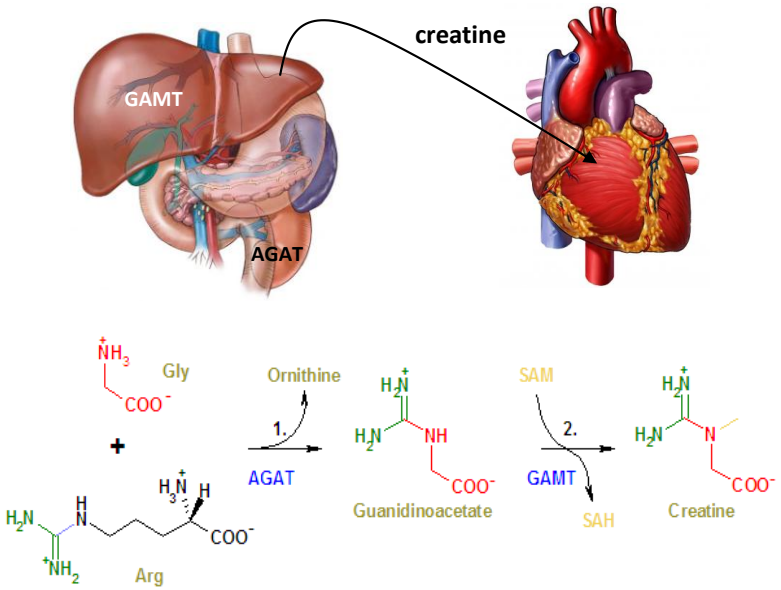


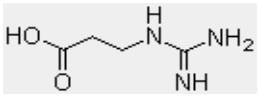
Figure 2 *Synthesis of creatine.*
Arg - Arginine; AGAT - L-arginine:glycine amidinotransferase*; GAMT* - Guanidinoacetate N-methyltransferase*; Gly - Glycine; Met - Methionine; SAH - S-adenosyl homocysteine; SAM - S-adenosyl methionine.*

Myocardial energy metabolism in the failing heart

There has been a longstanding and controversial debate on the hypothesis of energy starvation of the failing heart[17, 29, 30]. The debate is in part due to the complexity of the cellular and molecular alterations in the pathogenesis of heart failure and partly due to limitations in the methodology for studying myocardial energetics. 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 NMR spectroscopy was a methodological breakthrough in the study of myocardial energetics, gaining new insights in to the field of myocardial energetics in the failing heart. ^{31}P NMR spectroscopy allows in vitro and in vivo measurements of myocardial high energy phosphate content simultaneously with measurements of contractile function. This technique allows dynamic measurements of reaction velocities of the ATP synthesis pathways.

Today, there is compelling evidence, from both clinical and experimental studies, that HF is characterized by disturbances in the myocardial energy metabolism[20, 31-34]. It has been previously demonstrated that 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. In CHF, the decrease in Cr is cardiac specific[35], in difference to in creatine deficiency syndrome[36]. High energy phosphate metabolites are decreased in failing[31-33, 35, 37-39]and post-infarct remodeled hearts[40, 41] . PCr decreases early in the development of heart failure while ATP decrease occurs at the late-stage heart failure[33, 35, 42]. 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 Magnetic resonance spectroscopy (MRS)[32, 43, 44]. The total creatine pool, i.e. the sum of Cr and PCr, is decreased in the failing heart[16, 22, 45, 46]. Creatine depletion is now considered a characteristic of the failing heart muscle[24, 47-49]. This has been proven in both human[16] and animal studies[24, 50-52] Decreases in PCr and Cr occurs 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[31, 34, 53, 54] which results in compromised capacity of the CK system to rephosphorylate ADP into ATP leading to further decrease in the energy reserve. The end-result is an energy starved heart[55]. Depletion of the creatine pool can also lead to LV hypertrophy, increased propensity for development of malignant arrhythmias and systolic and diastolic dysfunction[56, 57]. Down-regulation of CrT in the cell membrane is also a phenomenon found in the failing heart[24]. 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[35, 47, 58-61]. 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 the creatine by creatine analogue, BGP.

BGP  is an analogue to creatine which can enter the myocyte through the creatine transporter[62] 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[63]. But there is one major difference of BGP's function in the myocyte compared to the function of creatine, it functions very poorly as a substrate for the cytosolic CK to produce ATP. The activity of this reaction is 3 orders of magnitude lower of its activity when using PCr as substrate[64]. This effectively inhibits the CK reaction[64] and creates an ATP deficiency in the myocyte. The model mostly used in BGP-induced creatine depletion is by distribution of BGP to the animals via food and water supply. The effects of BGP feeding, are similar to what is observed in CK-deficient mice[58, 65-67]. 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[49, 68], and mice overexpressing CrT[48].

Lipotoxicity in myocardial infarction and heart failure - importance of endogenous lipoproteins for cardiac function, structure and arrhythmias.

The heart is an organ heavily dependent on exogenous lipids for oxidative production of ATP, which is essential for maintenance of normal cellular energy homeostasis. During the last years surprising data have been reported showing unequivocally that the heart besides being dependent on exogenous lipids also synthesizes its own endogenous lipids[69, 70]. These lipids are produced and secreted in the form of apoB-containing lipoproteins (apoB) - which are structurally much alike plasma low-density lipoprotein particles. This phenomenon has been confirmed in several different species including humans. The fact that both humans and mice - two species parted by million years of evolution - have preserved the biochemical machinery for myocardial production of lipoproteins suggests an important physiological and/or pathophysiological regulatory role. 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 knock-out mice and humans with the rare genetic defect resulting in inability to express MTP (microsomal transfer protein – initial step in the synthesis of apoB) and consequently in abetalipoproteinemia, have normal cardiac structure and function. What we don't know is the function of myocardial apoB in the heart under pathologic conditions. Myocardial apoB could be an important cardioprotective system mobilized during pathophysiological conditions such as ischemia, pathologic remodeling and heart failure. These conditions are associated with accumulation of intracellular lipids (free fatty acids, triglycerides, ceramides, lysophospholipids etc.) in the heart. Excessive accumulation of lipids is damaging to cellular function and structure and results in development of lipotoxic heart disease. It has been demonstrated that excessive accumulation of lipids in the heart leads to[71-74]:

1. Reduction in mitochondrial electron transfer activity.
2. Uncoupling of oxidative phosphorylation.
3. Reduction in activity of ATPases.
4. Induction of cardiac hypertrophy.
5. Induction of mitochondrial death and apoptosis.
6. Systolic and diastolic dysfunction.

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. 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.
Are endogenous lipoproteins important for preservation of myocardial function, structure and survival during MI and CHF? Intracellular lipotoxicity during MI and CHF causes cell dysfunction, cell necrosis and apoptosis. Given the previous statement we propose the following hypothesis**:** The heart has a protective system- apoB - which is mobilized during MI and CHF to counteract lipotoxicity by isolating and exporting toxic lipids (FFA, triglycerides, oxidized lipoproteins 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.

Aims of the study

- To investigate whether measures of intensive cardiac care applied to rats with acute myocardial infarction would reduce mortality rate in this small animal model.

- To investigate *in vivo* the effects of Cr depletion in rats on left ventricular function and morphology, energy metabolism, catecholamines and incidence of malignant ventricular arrhythmias during acute myocardial infarction.

- To investigate *in vivo* the effects of Cr depletion in mice on left ventricular function and morphology, energy metabolism and myocardial lipids.

- 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.

- To investigate acute and chronic effects of complete heart block on cardiac function, morphology and energy metabolism in the rat model.

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

The rat model of myocardial infarction is widely used in experimental cardiology for preclinical studies. It has been a very valuable tool in this research field because of its similarity with the major pathophysiological events occurring in patients with MI and heart failure[75, 76]. However, this model has one large disadvantage. In order to induce postinfarction heart failure, one needs to create large infarction which results in a high mortality rate.
In paper I we aimed to address the question if it was possible to lower the mortality rate using some of the therapeutical interventions used in the every-day-care of patients in coronary care units. So we randomly divided the animals into two groups; the conventional care (CC) group and the intensive care (IC) group. The intensive care group was pre-treated with an injection of amiodarone to lower the incidence of arrhythmias. The anesthesia used in this group was isoflurane instead of injection anesthesia used in the CC group. This enabled us to control the grade of anesthesia during the surgery, and allowed for a quick recovery post-surgery. After surgery the IC group animals were given continuous respiratory support until they showed signs of spontaneous recovery. The IC group was monitored with ECG during the entire surgical procedure and during the recovery period. If the animals in the IC group suffered any arrhythmias, they were treated with cardioversion using a home-made defibrillator for small animals, with delivery of electroshocks in the range of 2-6 Joule.

Induction of myocardial infarction (papers I, II, IV)

The animals were anesthetized with isoflurane, intubated and connected to a small animal ventilator. The animals were kept ventilated and maintained on 2% isoflurane mixed with oxygen and room air, all through the operation. 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 using an electrical clipper. Left thoracotomy was performed between the 4th and 5th ribs in order to expose the left ventricular wall. 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.

Ischemia-reperfusion injury (paper IV)

The animals were anesthetized with isoflurane, intubated and connected to a small animal ventilator. The animals were kept ventilated and maintained on 2% isoflurane mixed with oxygen and room air, all through the operation until they spontaneously recovered after surgery. 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 using an electrical clipper. Left thoracotomy was performed between the 4^{th} and 5^{th} ribs in order to expose the left ventricular wall. After pericardiotomy, a suture was passed under the proximal part of the branch of the left coronary artery that corresponds to the LAD in humans. Both ends of this suture were then passed through a short plastic tube, by pulling on both ends of the suture the plastic tube was gently pressed down on the artery producing a temporary occlusion of the vessel. This was verified in the same way as for the permanent MI, by akinesis of the left anterior ventricular wall and characteristic ECG changes. 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.

In vivo ^{31}P MRS (paper II)

Quantitative analysis of high-energy phosphometabolites (HEP) in tissues has been traditionally provided with the freeze clamping technique which involves extraction of HEP from rapidly frozen tissue samples. High performance liquid chromatography is then used to quantify the levels of HEP (PCr, ATP) and inorganic phosphate (Pi) as well as other metabolites. This technique is still continuously used in experimental settings. HPLC (High performance liquid chromatography) is regarded as reliable analysis but it is performed on tissue samples *in vitro* and does not allow repeated *in vivo* investigations of the study object. Volume-selective ^{31}P MRS is a unique non-invasive tool for *in vivo* measurements of cellular energetic. Using this method, one can produce a ^{31}P spectrum that contains information about tissue phosphometabolites. Couple of years ago Omerovic et al. were first to establish in vivo volume-selective ^{31}P MRS in small animals (rats and mice)[77, 78] using ISIS localization method. A typical ^{31}P MR spectrum of the normal rat heart is given in the Figure 3. In this spectrum one can clearly discern several separate resonance areas. The origin of the ^{31}P signals are phosphorus containing substances in the myocardium of rat heart obtained *in vivo* at 2.35 T performed at our laboratory. The most prominent resonance is a signal from PCr. To the right of PCr there are three resonances that originate from the α, β and γ phosphorus atoms of ATP molecule. To the left of PCr there are resonances originating from phosphodiesters (PDE) and inorganic phosphate (Pi). Spectra obtained *in vivo* usually contain an additional signal close to or overlapping with the signal from Pi. This signal originates from blood 2, 3-diphosphoglycerate mainly contained in erythrocytes. Besides concentration of phosphorus metabolites, additional information may be derived from ^{31}P MR spectrum. Since the position of Pi in regard to PCr is dependent on intracellular pH, one can calculate intracellular pH in tissue of interest according to the equation. Furthermore, from the distance[79] or the ratio[80] between β- and α-ATP resonances one can indirectly calculate the intracellular Mg^{2+} concentration. Another great advantage of the technique is the possibility to calculate enzyme kinetics of the creatine kinase reaction using the method of magnetization transfer[81-83], i.e. one can calculate the velocity of the transfer of the phosphate group from PCr to ATP and vice versa. For evaluation of the myocardial energy status *in vivo* it is generally

accepted to use different ratios as indicators of energetic state. The two most frequently used ratios are, PCr/ATP and PCr/Pi ratios[84-86]. In this thesis we have used PCr/ATP ratio as an indicator of myocardial energy status.

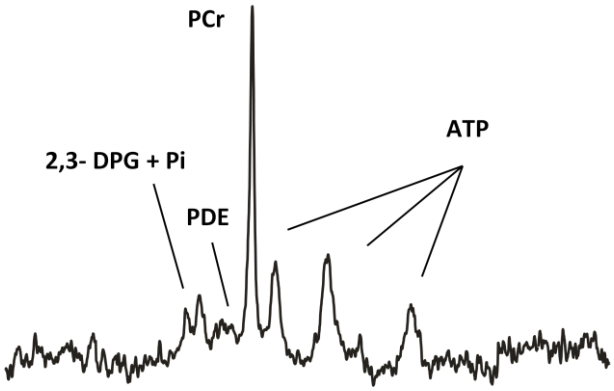


Figure 3 *^{31}P MR spectrum obtained in vivo from the normal rat heart.* PCr = phosphocreatine; ATP = three ^{31}P atoms of the ATP molecule; PDE = phosphodiesters; 2, 3-DPG = 2, 3-diphoshoglycerate, Pi = inorganic phosphate

Echocardiography (papers II, III, IV, V)

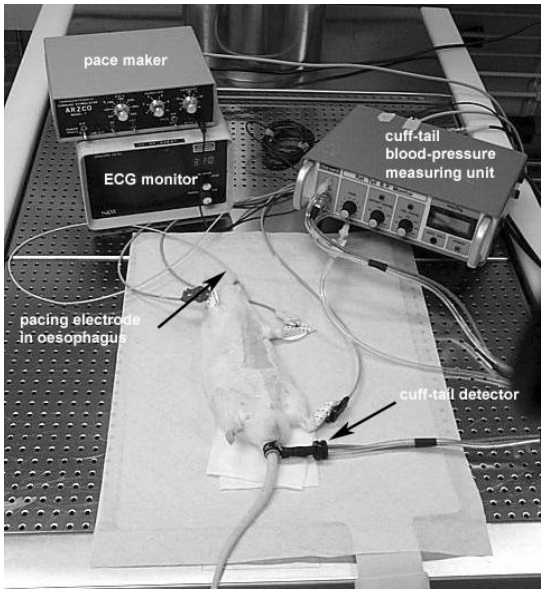


Figure 4 *Pacing-induced stress echocardiography in the rat model.*

We used transthoracal echocardiography for non-invasive evaluation of cardiac function and morphology in rats and mice. The previously validated two-dimensional, M-mode and Doppler techniques were used. Ventricular function was evaluated both during rest and stress conditions. Stress was induced either by esophageal pacing (Figure 4)[87] (paper II) or pharmacologically by means of dobutamine injection (1μg/g BW) (paper III, IV). The investigations were performed with a 15-MHz linear transducer connected to a HDI 5000 ultrasound system (ATL, Philips Medical Systems). The parameters assessed were: left ventricular diameter in diastole (LVDd), left ventricular diameter in systole (LVDs), fractional shortening (FS), posterior wall thickness (PWT), intraventricular septum thickness (IVST), relative wall thickness (RWT), left ventricular mass (LVM), and left ventricular mass index (LVM/BW). LVM was estimated using the formula: $LVM = 1.05 \times [(IVST + LVDd + PWT)^3 - (LVDd)^3]$.

Biochemical analysis of creatine and adenine nucleotides (paper II, III, IV)

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[88]. 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.

Summary of the results

Paper I

Here we wanted to apply standard treatment methods from clinical patient care onto rats in order to see if we could decrease the mortality rate in the experimental myocardial infarction model.

Two different clinical outcomes were seen in the animals that died acutely; sudden death due to malignant ventricular arrhythmias (MVA) and cardiogenic shock due to progressive heart failure. The majority of the animals developed showed signs of some form of MVA within 5 minutes after occlusion of the coronary artery. The incidence of MVA declined over time. The animals in the IC group showed a decrease in the severity and duration of MVA compared to the CC group. Bradycardia was more frequently occurring in the CC group. All rats that experienced bradycardia had decreased pulse oximetry values and signs of dyspnea which indicated that they suffered from progressive heart failure-cardiogenic shock. The mortality rate in these animals were 100%, and interventional treatments, such as continuous i.p. infusion of dobutamine or atropine, additional bolus of diuretics, transesophageal pacing, and prolonged respiratory support, did not improve their survival.

Taken together the intensive care methods applied to the rats randomized to the IC group, resulted in a 3.5-fold reduction in acute (24 hours) mortality.

Paper II

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 evaluate the in vivo effects of BGP-induced creatine depletion in rats. Furthermore we evaluated the effects of Cr depletion on mortality and occurrence of ventricular arrhythmias in the setting of acute MI. We also used a novel technique in the administration of the creatine analogue. Traditionally, BGP has been administered by supplementation in the food and water. But this is very costly and it is also difficult to assess if each animal received the same dose of BGP. We chose instead to administer the BGP by means of subcutaneously implanted osmotic minipumps containing 1M BGP. This subcutaneous delivery of BGP was applied over a period of 4 weeks. No local

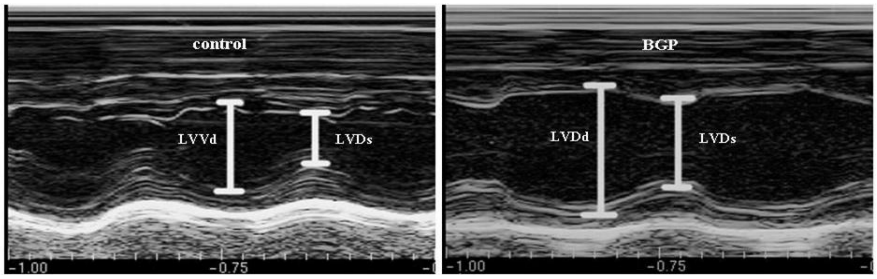
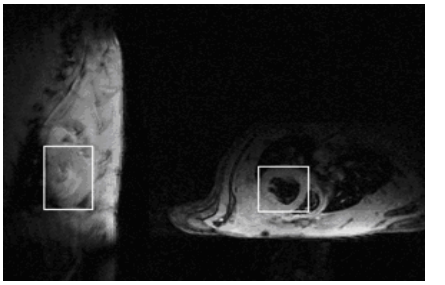


Figure 5 *Echocardiographic measurements of left ventricular diameters in systole and diastole. There was evidence of LV dilatation and impairment of LV function in the BGP group compared to the control group.*

adverse skin effects were found, suggesting good tolerance for this new BGP administration technique.

The results showed that the animals in the BGP treated group had decreased BW and increased left ventricular (LV) mass indicating myocardial hypertrophy. LV diameters in diastole and systole were increased both during rest and pacing-induced stress in the BGP treated animals (Figure 5). LV systolic function measured as FS was disturbed during both rest and stress. In order to evaluate LV energy status the animals were investigated with in vivo volume-selective ^{31}P MRS. We were unable to calculate PCr/ATP in the treated animals due to the overlapping of the resonance areas of PCr and phosphorylated BGP (P-BGP) at magnetic 2.35 T field strength. However if calculated together, the PCr + P-BGP/ATP was decreased by 39% compared to controls, which suggests that the PCr/ATP was decreased at least by ~40%, possibly more, in the BGP treated group (Figure 6). Similar overlapping between Cr and BGP and PCr and P-BGP was seen in the HPLC analyses. When calculated together, the total Cr was reduced by 50% in the BGP group. This finding suggests that in the BGP treated animals, the total creatine content in the myocardium was decreased by at least 50%. We found a tendency towards lower myocardial noradrenaline (NA) content in BGP group. No difference was observed in the plasma catecholamine content.



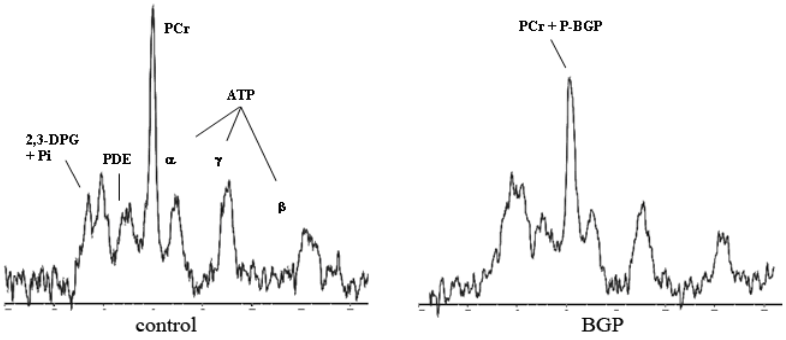


Figure 6 *Volume-selective in vivo ^{31}P MRS of the rat heart. PCr/ATP ratio was reduced by ~40% in the BGP group*

As for the effects of creatine depletion in the settings of acute MI, there were two sets of clinical courses in the animals that died acutely: Their mode of death was either sudden due to ventricular arrhythmias (ventricular fibrillation), or protracted due to progressive heart failure and cardiogenic shock characterized by development of sustained bradycardia and severe hypoxia. There was a distinct difference in mortality rate between the groups. In the BGP treated group 93% of the rats died within 60 minutes post-MI, compared to 46% in the control group. There was also a difference in a cause of death. In the control group 100% of the deaths were due to arrhythmias, compared to 78% in the BGP group. The remaining 22% of deaths in the BGP treated group were due to worsening heart failure and cardiogenic shock. The BGP treated animals demonstrated a higher arrhythmia score suggesting that Cr-depleted heart is more prone to develop malignant ventricular arrhythmias (Figure 7) during the course of acute myocardial infarction.

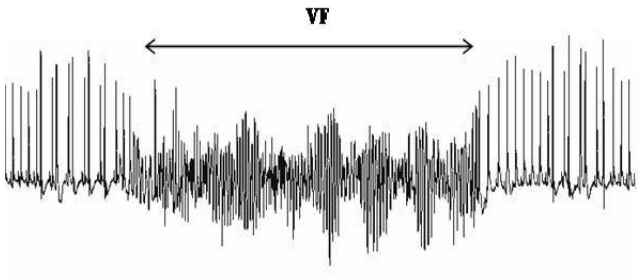


Figure 7 *ECG tracing from a rat with acute MI with spontaneously terminating ventricular fibrillation (VF)*

Paper III

Similarly to paper 2 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. Similarly to the rat model, after four weeks of BGP treatment the total myocardial Cr pool was decreased by 40% compared with controls.

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.

There was a 2-fold increase in the myocardial content of triglycerides in the BGP treated animals after four weeks. 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 IV

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 the 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. No upregulation of apoB was detected in the mice with DOX-induced acute heart failure.

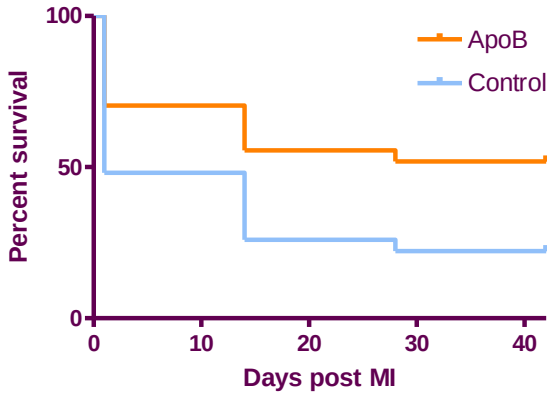


Figure 8 *Survival in apoB and wild-type mice after myocardial infarction*

An important finding of this study was a difference in myocardial response in rats compared to mice. This suggests a species specific apoB response to myocardial injury. 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. Furthermore, DOX-induced heart failure in rats, resulted in a ~70% reduction of myocardial apoB content compared to controls.

Echocardiographic examinations of the heart 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. But 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. Tthe apoB transgenic mice showed a two-fold better survival at 6 weeks post MI compared to wild type control mice (Figure 8). The largest part of the mortality occurred acute post MI i.e. within the first 24 hours post-infarction.

Paper V

The aim of this paper was to study the effects of complete heart block (CHB) on cardiac function, morphology, and energy metabolism in a rat model. At first we tried to induce CHB by ethanol injections, but despite increasing amounts of ethanol and repeated injections, the success rate for establishment of permanent CHF was as low as 5%. We changed method and started using electrocautery to induce CHB instead. Using this method we were able to increase the success rate of permanent CHB induction to 54%. The animals with permanent CHB recovered and appeared healthy throughout the course of the experiment. Both short-term and long-term effects of CHB were evaluated using echocardiography examinations at 1, 3 and 12 weeks post CHB induction. The CHB animals had significantly lower ventricular rates both at the early and the late time points compared to the controls, but the atrial rates were similar between the groups throughout the experiment. LV dimensions were increased in the CHB animals compared with the controls and relative LV wall thickness was decreased, which suggests development of eccentric hypertrophy. The LV hypertrophy and LV dilation was detected as early as 1 week post CHB induction and was sustained until 12 weeks after. As a compensation for the lower HR the CHB animals had a 2.5 fold increase in their stroke volume (SV), which was sufficient to maintain the CO at similar values as the control animals. The myocardial contractility, seen as FS, was however decreased at 3 and 12 weeks, but this was not seen as early as 1 week. The animals were also examined using invasive hemodynamics at12 weeks after the CHB induction. There were no differences between the groups in left-ventricular and right-ventricular end-diastolic pressures suggesting that CHF had not developed in these animals. There were also no signs of disturbed myocardial energy metabolism. The myocardial content of creatine and the high-energy phosphometabolites did not differ between the groups.

Discussion

Paper I

Scientists and clinicians need to better understand the pathophysiology and molecular mechanism of the disease processes involved in development of MI and CHF to define the targets for future interventions in order to e.g. decrease MI size, prevent or suppress malignant arrhythmias, prevent or attenuate pathologic LV remodeling, improve function and morphology of the failing heart etc. To achieve these goals, we will continue to depend on relevant animal models. One such valuable model is experimental MI in rats.
Induction of MI and development of CHF that follows in rats is a widely used experimental model for studies of various aspects of MI and CHF. Complete occlusion of the left coronary artery results in MI of variable sizes with occurrence of overt heart failure in a subset of animals with large MI[78, 90-92]. The impairment of LV function is related to the loss of functional myocardium. Development of CHF is associated with LV dilatation, reduced systolic function and increased filling pressures as well as with neurohormonal activation similar to that seen in patients[90, 93-95]. Besides a large body of knowledge derived from this model in terms of basic science and clinical aspects of MI and CHF the model is also associated with lower costs for experimental procedures and animal handling as compared to the equivalent large animal models. On the other hand, the major disadvantage is high mortality particularly in the setting of a large MI which presence is necessary to commence the process of LV remodeling. In this study we have demonstrated that malignant ventricular arrhythmias (ventricular tachycardia and ventricular fibrillation) and progressive HF are the major causes of death. Ventricular arrhythmias were responsible for ~ 80 % of all deaths while progressive HF-cardiogenic shock developed in the minority (~ 20%) of the rats. Other possible causes of death (usually accidental) include bleeding, infection, excessive inadvertent overdosage of anesthesia and drugs. Acute mortality (i.e. within first 24 hours) is responsible for ~ 95 % of all deaths that will occur within first 3 months after induction of MI. When performing animal experiments one is obliged to follow the laws regulating the use of animals for experimental purposes based on the rules stipulated by national and international ethical comities. We believe that the use of animals in medical research is not only valuable scientifically but is also an ethical imperative prior to clinical trials. However we also support the argument that we must improve and develop experimental models which will lead to a decrease in unnecessary loss of animal life[96, 97].
In conclusion, the study has shown that the use of pre-treatment with the antiarrhythmic drug amiodarone, respiratory support, isoflurane gas anaesthesia and aggressive treatment of sustained MVA with electrical cardioversion are simple and effective measures which reduce mortality in rats with acute MI. Improving survival rates increases ethical acceptance and cost-efficiency of this important experimental model.

Paper II

The most important results of this study showed that myocardial Cr depletion results in disturbed LV function, pathologic LV remodeling, and altered energy metabolism. Induction of acute MI in the setting of creatine depletion is associated with high acute mortality caused by ventricular arrhythmias and worsening heart failure. Our results support the hypothesis

that intact myocardial energy metabolism is necessary for maintenance of normal structure and function of the heart.

The rat model of myocardial creatine depletion induced by BGP feeding has been described perviously[55]. Here, however, osmotic minipumps were used for BGP delivery because we found it to be more convenient and much less expensive than the food enrichment protocols used in previous studies[56, 61, 98].

We found that the LV function was deteriorated under both basal and stress conditions. This differs from the result in a previous study[55] in which Cr depletion resulted in LV hypertrophy and dysfunction during stress. The reason for this discrepancy between the studies is not clear, but could be due to the use of different methods and possibly to a more severe Cr depletion using subcutaneous BGP administration.

Even though it is known that the failing heart is characterized by disturbed energy metbolism[20], it is not yet known to which extent the supply and demand mismatch in chemical energy, contributes to the development of CHF. Our study provides in vivo evidence, that Cr depletion may be a part in the pathogenesis of LV dysfunction, pathologic remodeling, and disturbed energy metabolism, as seen in CHF patients.

Others have reported increased LV mass and volume after prolonged BGP feeding[56]. In our study we saw evidence of pathologic remodeling already after 4 weeks of BGP treatment, using noninvasive *in vivo* echocardiographic measurements. This confirms the previous findings. From the present data we have not been able to determine a cause for the LV dilatation, but there are a number of possible explanations. The heart could be trying to preserve cardiac output by compensating for the disturbed function, by employing the Frank-Starling mechanism. Others have implicated Cr in regulation of protein synthesis[99], if so, maybe the LV dilatation could be a direct consequence of the Cr depletion.

The ^{31}P MRS examinations showed a decreased PCr + P-BGP/ATP ratio in the BGP treated animals. This is the first *in vivo* evidence of disturbed energy metabolism in the BGP induced Cr depletion.

A major cause of CHF is ischemic heart disease[2]. A large portion of CHF patients, suffer sudden death mostly caused by malignant ventricular arrhythmias[100]. The more advanced the CHF is, the higher the mortality rate, and with that the rate of sudden death[101]. Trying to decrease this fatal clinical outcome the search for novel therapies and pharmacologic interventions is of great importance. Based on the evidence found in this study, one way to achieve this goal may be to prevent Cr depletion and improve the energy metabolism of the heart. In a similar model, others have reported that rats suffering from Cr depletion die within 24 hours post MI induction[56], however they did not determine the cause of these deaths. Our data shows that the major causes of death are ventricular arrhythmias, worsening HF and cardiogenic shock. This demonstrates that electrophysiological instability is an important consequence of disturbed myocardial energy metabolism. An intact Cr metabolism appears to be essential for survival during the acute phase of MI. Even though our data in this study does not explain the molecular mechanisms behind the increased susceptibility for arrhythmias in the energetically altered heart, there is other evidence that support this coupling[102, 103]. But other mechanisms may also be involved in the development of ventricular arrhythmias in the Cr-depleted heart. There is a possibility that the left ventricular hypertrophy of the Cr-depleted heart may have contributed to the increased incidence of ventricular arrhythmias. Evidence suggest that there is a link between left ventricular hypertrophy and increased

incidence of ventricular arrhythmias[104]. However, the sole presence of left ventricular hypertrophy does not always lead to an increased incidence of arrhythmias[105]. A possible direct pro-arrhythmic effect of BGP itself cannot be excluded.

The absence of data concerning MI size is an important limitation of this study, because it is not known how a decrease in myocardial Cr affects MI size. Bothe the severity of left ventricular dysfunction and the incidence of ventricular arrhythmias may be dependent on MI size. This should be examined in future research.

In a recent study it was shown also supranormal levels of Cr in the heart may result in left ventricular hypertrophy and dysfunction[48]. This provides a strong indication that it is very important for the heart to maintain the narrow physiologic Cr concentrations in order to uphold normal structure, function and energy metabolism. Further studies should address the question of whether disturbances in left ventricular morphology and function caused by CR depletion, may be a reversible process.

Paper III

In this study the aim was to use the same Cr depletion model established in paper II, but in a mice model instead, and see if the disturbances in LV function, morphology and creatine metabolism was reversible after normalization of the Cr levels. We also wanted to investigate if Cr depletion leads to alterations in myocardial lipid metabolism.

The most important findings of this study is 1) that Cr depletion in mice results in compromised cardiac function, development of pathologic LV remodeling and accumulation of intracellular triglycerides, and 2) that normalization of the myocardial Cr levels results in a complete reversal of these cardiac abnormalities.

In paper II we found evidence of disturbances in LV function, morphology and energy metabolism caused by Cr depletion in a rat model. Those findings are similar to those found in this mice model of Cr depletion, in regards to compromised LV function, geometry and disturbed energy metabolism.

In this study we also wanted to investigate, the simple but highly important question whether the functional, morphological and metabolic consequences of the Cr depletion were reversible. This important question has not been previously addressed. Cr depletion and disturbances in the myocardial energy metabolism are generally regarded as a characteristic part of the pathological remodeling in CHF. However there are some researchers that argue that Cr depletion may be a compensatory biochemical mechanism activated to preserve the normal value of ΔG of ATP hydrolysis, and that any measures to increase Cr in the failing heart may be deleterious [16, 20, 50]. 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. These findings support the theory that Cr metabolism could be an important pharmacologic target for future CHF therapies.

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[74]. It is believed that lipotoxicity may significantly contribute to the development and progression of CHF. 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[21, 106]. 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. [23]. Future studies should look into the mechanisms behind the coupling between Cr depletion and triglyceride accumulation.

Transgenic models of both the depletion of myocardial creatine[49] and the excess of creatine[48] 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 (paper II) 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.

Paper IV

The most important findings in this study are the biphasic pattern of the myocardial apoB response, the attenuated/reduced response in DOX-induced acute HF and the marked decrease in mortality following MI in mice with apoB overexpression.

The apoB system may play an important role in the cardioprotection by counteracting excessive intracellular lipid accumulation and thereby prevent/attenuate development of lipotoxic heart disease[107, 108]. This hypothesis is based on the results from transgenic models of myocardial lipotoxicity and development of cardiomyopathic HF. When these transgenic mice were crossed with mice that overexpressed apoB in the heart, the result was improved cardiac function and survival[107].

Our aim was to investigate how myocardial apoB expression affects survival, normal cardiac function and structure, and ventricular arrhythmias, in a model of acute MI and HF. This has not been studied previously.

Increased microsomal transfer protein (MTP) expression was found in the hypoxic areas of the LV of patients with ischemic heart disease undergoing cardiac surgery, and the MTP expression was negatively correlated with TG content[108]. Our results are in agreement with these data from the clinical study. We show that ischemia induce a rapid and sustained response in apoB expression 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[109]. 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[110, 111].

An unexpected finding was that apoB dramatically decreased in the hearts of animals with chronic post-MI HF. The mechanisms for this time-dependent difference in the apoB response is not clear. We have not measured the apoB expression between five days and eight weeks, but it is plausible to assume that the initial increase in the apoB response reaches a plateau a few days postinfarction, and then steadily decreases to the subnormal levels found eight weeks post-MI. This suggests a development of exhaustion in the apoB response. These findings are in agreement with the functional and mortality data from our substudy of survival postinfarction in apoB transgenic mice. The excessive mortality in the control group was most notable within the first 24h post-MI. This is the phase that shows upregulation of apoB ~2 times the normal level. This early postinfarction phase is also characterized by development of acute HF due to a large MI. The reasons and mechanisms behind the improved survival of the apoB overexpressing mice may not be determined by the present data and needs further investigation. However, we speculate that myocardial overexpression of apoB results in a better and more prompt activation of a compensatory mechanism to maintain contractile function. Furthermore, the overexpression of apoB may give rise to mechanisms that protects the heart against arrhythmias, by isolating and exporting pro-arrhythmic lipids during and after an ischemia-reperfusion period[112, 113]. Although intracellular lipid accumulation has been shown to exert pronounced pro-arrhythmic effects[114, 115] we did not observe any difference in arrhythmia incidence between the groups within 45 min post-MI. But we believe that this finding should be interpreted cautiously since mice has a low incidence of arrhythmias in the setting of MI, compared to other animal models[116, 117]. There are also differences in regards to arrhythmias between different mouse strains[118]. Future studies using other models should address the question whether apoB could provide an anti-arrhythmic protection. The fact that the incidence of arrhythmias was no different between the groups and that the systolic function early post-MI was better in the mice overexpressing apoB, strongly suggests that the reason for the increased survival of the apoB mice is related to a better ability to cope with the hemodynamic consequences of acute HF. One explanation could be that apoB overexpressing hearts are remodeled in terms of function, structure and biochemistry, and that these alterations together provide a better ability to adapt during life-threatening conditions such as large MI and acute CHF. Further studies are needed to find the mechanisms for these and/or other possible differences in the cardiac phenotype of apoB overexpression.

There was no apparent effect of apoB overexpression on infarct size measured six weeks post-MI. That the infarct size was similar, but the heart weight was lower indicates an anti-remodeling effect of apoB. Previous studies have shown that there is a close relationship between myocardial lipid metabolism and hypertrophy[119-121]. In the late post-MI phase there was no longer any improvement in systolic function between the groups, but the survival benefit remained however. One could speculate that this may be related to the biphasic time-dependent apoB response. Exhaustion of apoB maybe a part of a pathologic biochemical remodeling, that contributes to the phenomenon of transition from compensated LV dysfunction to overt HF.

The regulation of apoB synthesis in the liver is relatively well studied[122], but the regulation of endogenous apoB in the heart is more unknown. It is possible that the mechanisms in the heart are similar to those in liver. Given the fact that neurohormonal, mechanical and metabolic processes are of great importance for the cardiac function and structure after injury, it will be important to explore how these processes affect the apoB regulation in the heart post-MI and in HF. It should also be explored how myocardial apoB regulation is affected by pharmacological agents used for treating MI and HF.

The finding that doxorubicin-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. Doxorubicin -induced cardiotoxicity is a well described model of experimental CHF[123, 124].This agent induces myocardial injury through several mechanisms including severe mitochondrial dysfunction, disturbed energy metabolism, lipid accumulation and DNA damage (for review see [125, 126]. We should explore the possibility that doxorubicin - due to the DNA damage - may disrupt the genetic message involved in the synthesis of MTP and apoB molecules. Indeed, doxorubicin although being one of the most effective cytostatic agents for treatment of different malignancies, may cause a special form of toxic cardiomyopathy and CHF[127, 128]. This leads to the question whether doxorubicin -induced cardiomyopathy at least in part may be mediated by its ability to suppress the apoB response.

Paper V

The most important results in this study was that induction of complete heart block (CHB) in rats resulted in early and sustained cardiac remodeling. The animals with CHB developed eccentric LV hypertrophy but have preserved normal systolic function. No evidence of disturbed myocardial Cr metabolism was found.

Animal models of CHB can provide valuable information about the hemodynamic, electrophysiological, morphological and metabolic consequences of ventricular bradycardia and loss of synchrony between the atrial and ventricular activation. There are several experimental models both in large[129, 130] and small animals[131, 132]. In a rat model recently described the CHB was induced by injection of ethanol into the AV-node area[131]. We were unsuccessful in our attempt to reproduce this previous method for induction of permanent CHB. Most of the animals developed CHB rapidly upon the injections but the state was transient for the most part, only ~5% of the animals developed permanent CHB. We then tried direct electrocautery instead and found that to be a much more reliable model for induction of CHB.

The use of echocardiography for non-invasive and longitudinal evaluation of the CHB rat model has not been done previously. This investigation revealed important information about the LV remodeling and LV function in the rat suffering from CHB. After 1 week post CHB, there was already a marked decrease in LV diameters and SV - compensatory mechanisms to maintain a normal cardiac output. The animals had developed eccentric hypertrophy, but showed no signs of disturbed LV function. The remodeling continued and the eccentric hypertrophy increased further during the following weeks. At 3 and 12 weeks the LV contractile function, measured as FS, was decreased but no evidence of increasing RV and LV filling pressures were found. Decreased FS has not been reported previously in the

experimental model of CHB. We speculate that it may be explained by the "Bodwitch-phenomenon". This is a force-frequency relationship that could be explained shortly as; the myocardial contractility decreases when HR decreases and vice versa[133, 134]. This hypothesis is supported by human data from elite athletes in whom a decreased FS has been demonstrated when compared to healthy controls[135, 136].

Intracellular depletion of Cr and alterations in the high energy phosphometabolites are important indicators of disturbed energy metabolism in the heart. Disturbances in the myocardial energy metabolism in general and Cr depletion in particular, have been associated with pathologic biochemical remodeling in different types of LV hypertrophy and CHF[28, 47]. However, we were unable to detect any signs of disturbances in this system after 12 weeks of chronic volume-overload due to complete AV-block, even though the animals now had developed pronounced hypertrophy. Other studies have shown a decreased PCr/ATP ratio in the failing and hypertrophied heart[20, 50, 51]. Cr depletion is regarded as the major cause for decreased PCr content in the myocardium, since 70% of all Cr exists in its phosphorylated form, in the setting of chronic CHF and advanced hypertrophy. Therefore it is likely to assume that if Cr content is unaltered, the PCr/ATP ratio is also normal. The reason that our data differ from previous reports where hypertrophy is associated with disturbed energy metabolism[137, 138], could be that the eccentric LV hypertrophy associated with chronic CHB is more adaptive and physiologic than hypertrophy associated with pure volume-overload.

Cardiac remodeling is 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[9]. The process is influenced by hemodynamic load, neurohormonal activation, extent and location of myocardial damage and probably many other factors which are currently the focus of clinical and experimental research[139-141]. Cardiac remodeling ultimately leads to development of progressive myocardial dysfunction during time. It is generally accepted that early remodeling in response to pathologic stimuli (e.g., abnormal wall stresses) is an adaptive and useful response in the short term but it is the continuation of the process that is regarded as a maladaptive response. This small-animal model of post – CHB remodeling offers the possibility to study neurohormonal, metabolic, cellular, subcellular and other processes involved in development of adaptive and more physiological LV remodeling in contrast to the pathologic remodeling. Insights into molecular mechanisms behind beneficial cardiac remodeling may be useful for development of pharmacological and other interventions to attenuate and prevent progression of pathologic cardiac remodeling that leads to development of CHF. Our findings on LV remodeling and functional adaptation in this model are in agreement with those described in the canine model[129, 142]. One important limitation of this small animal model of CHB at the present time is a difficulty to implement different pacing strategies. This type of studies is more appropriate in large-animal models although the future advances in pace-maker technology with development of smaller devices may allow pacing studies even in small- animal models.

Conclusions

Application of measures of intensive cardiac care effectively decreases mortality in the rat model of myocardial infarction and acute heart failure.

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. The biochemically remodeled heart is extremely prone to malignant ventricular arrhythmias and to rapid progression of acute heart failure when subjected to acute myocardial infarction.

The myocardial apoB could be an important cardioprotective system mobilized during pathophysiological conditions such as ischemia, pathologic remodeling and heart failure which may protect the heart from pathologic consequences of intracellular lipotoxicity. Myocardial apoB system may be involved in the development of doxorubicin-induced cardiomyopathy.

Long-term bradycardia due to complete heart block leads to left ventricular remodeling with development of pronounced eccentric hypertrophy but with preserved energy metabolism and no signs of heart failure. This model may be useful in future studies of mechanisms behind the beneficial cardiac remodeling.

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