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Release characteristics of cardiac proteins after reversible or irreversible myocardial damage

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Chapter 1

General introduction

Cardiovascular disease (CVD) is the most important cause of mortality and morbidity in the Western world. CVD is a complex syndrome with a heterogeneous etiology, but atherosclerosis and thrombosis underlie the occurrence of most cardiovascular events such as stroke, angina pectoris, acute coronary syndromes, heart failure and dysrhythmias. Atherosclerosis of the coronary arteries is characterized by the presence of atherosclerotic plaques that result in narrowing of the arteries and a restricted blood flow causing ischemia¹. In the majority of patients, plaques are partially obstructive or only transiently obstructive. Ischemic episodes are commonly associated with reversible cell damage. Thrombus formation due to plaque rupture may completely occlude one of the coronary arteries, causing severe ischemia and irreversible cell damage (acute myocardial infarction). Figure 1 presents a schematic representation of the consequences of coronary atherosclerosis.

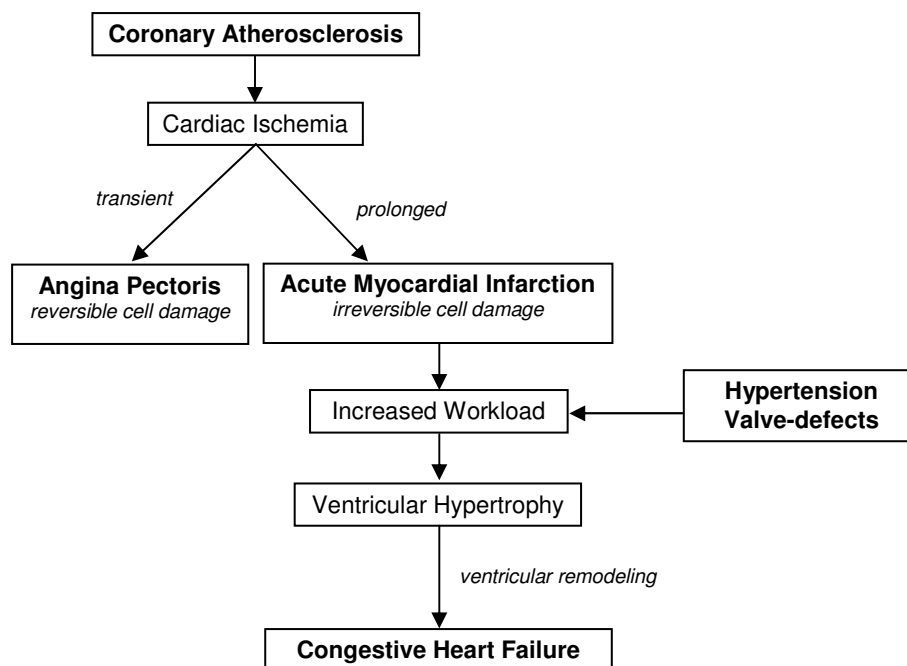


Figure 1. Schematic representation of the consequences of coronary atherosclerosis.

Acute myocardial infarction

Acute myocardial infarction (AMI) occurs when the coronary flow is severely reduced causing necrotic cell death in the myocardium distal from the occlusion. The exact mechanisms leading to necrosis during infarction are still controversial but many events are involved, including (1) poor oxygen delivery and poor washout of metabolites, (2) decreased production of adenosine 5'-triphosphate (ATP), (3) accumulation of fatty acid metabolites, (4) accumulation of lactate and protons, (5) calcium overload, and (6) inhibition of ion pumps leading to K^+ loss, Na^+ accumulation and water retention². Necrotic cell death occurs following severe cell damage and is characterized by cellular swelling and membrane rupture. This membrane damage allows cellular proteins to leak into the myocardial interstitium and finally into the circulation. Plasma concentrations of "cardiac" proteins are serving as biomarkers of necrotic cardiomyocyte death.

Dead cells act as a stimulus to inflammation with macrophage infiltration, fibroblast activation and ultimately scar formation³. Scar tissue replacing the infarcted area is characterized by stiffer mechanical properties compared to healthy myocardium, and may contribute to an impaired cardiac function and increased cell stretch at the border zone of the infarcted tissue. Postinfarction remodeling that refers to an adverse process occurring in the surviving, noninfarcted myocardium, may progress to congestive heart failure.

Congestive Heart Failure

The term congestive heart failure (CHF) describes the clinical syndrome arising when delivery of oxygen to the metabolizing tissues is impaired because of defective function of the heart as a pump. Heart failure has many causes and clinical manifestations but ischemic heart disease and idiopathic dilated cardiomyopathy are the two disease entities most frequently underlying heart failure. Any structural, mechanical or electrical abnormality of the heart may lead to the development of heart failure, either acutely, in a short time (over days or weeks), or over a long period (months or years). Increased workload of the heart due to myocardial infarction, hypertension, or valve defects, causes myocardial hypertrophy, a compensatory mechanism of cardiac tissue to adapt

to increased workload. Depending on the degree or duration of increased workload, ventricular hypertrophy may progress from a compensatory state to impaired systolic and/or diastolic function and heart failure. Systolic failure is often associated with ventricular remodeling, that is characterized by alterations in myocardial structure, composition, and function.

Ventricular remodeling

The underlying mechanisms of myocardial remodeling are complex, but identification of translational or post-translational modifications of cardiac proteins during the development of heart failure may provide insight into the mechanisms and may represent novel avenues toward the development of therapeutic approaches to treat or prevent CHF. Ventricular remodeling has been attributed to (1) intrinsic changes in cardiomyocytes, (2) changes in the linkage of the extracellular matrix (ECM) and cardiomyocyte, and (3) alterations in the composition of the ECM^{4;5}.

(1) Intrinsic changes in cardiomyocytes. Contractile activity of the heart is generated within the sarcomere of cardiomyocytes, by the interaction between thick and thin filaments (Fig. 2). The thick filament proteins include myosin heavy chain (MHC), myosin light chain (MLC), and myosin-binding protein C. The thin filament proteins include actin, tropomyosin (Tm), troponin I (TnI), troponin C (TnC), and troponin T (TnT).

Depressed cardiomyocyte contractility is an important determinant of a reduced pump function observed in heart failure⁶, and is associated with translational or post-translational modifications of myofilament proteins that are responsible for a reduced rate of myosin cross-bridge cycling, and a reduced power generation^{7;8}. As to translational modifications of myofilament proteins, several “fetal” proteins are becoming expressed, such as MHC- β ^{9;10}, α -skeletal-actin¹¹, atrial MLC-2a, and β -tropomyosin¹². Idiopathic dilated cardiomyopathy often has an inherited etiology. Over 300 dominant mutations in genes encoding sarcomere proteins have been identified in human heart failure¹³. As to post-translational modifications of myofilament proteins, phosphorylation¹⁴, degradation¹⁵, and an altered Ca^{2+} sensitivity of myofilament

proteins¹⁶, such as troponin I, may be responsible for a diminished contractility in heart failure⁷.

(2) *Cell-ECM linkage*. For efficient function, the heart requires a mechanical linkage that (i) provides the transmission of contraction forces from sarcomeres to the ECM, and (ii) prevents slippage of cardiomyocytes during contraction^{17;18}. Cardiomyocytes adhere to the ECM at transmembrane adhesion complexes, called costameres. These costamere complexes form a mechanical linkage, extending from the sarcomeres, through the plasma membrane, to the ECM, and are composed of vinculin, talin, integrin, laminin and several other extra- and intracellular components¹⁷⁻²⁰ (Fig. 2).

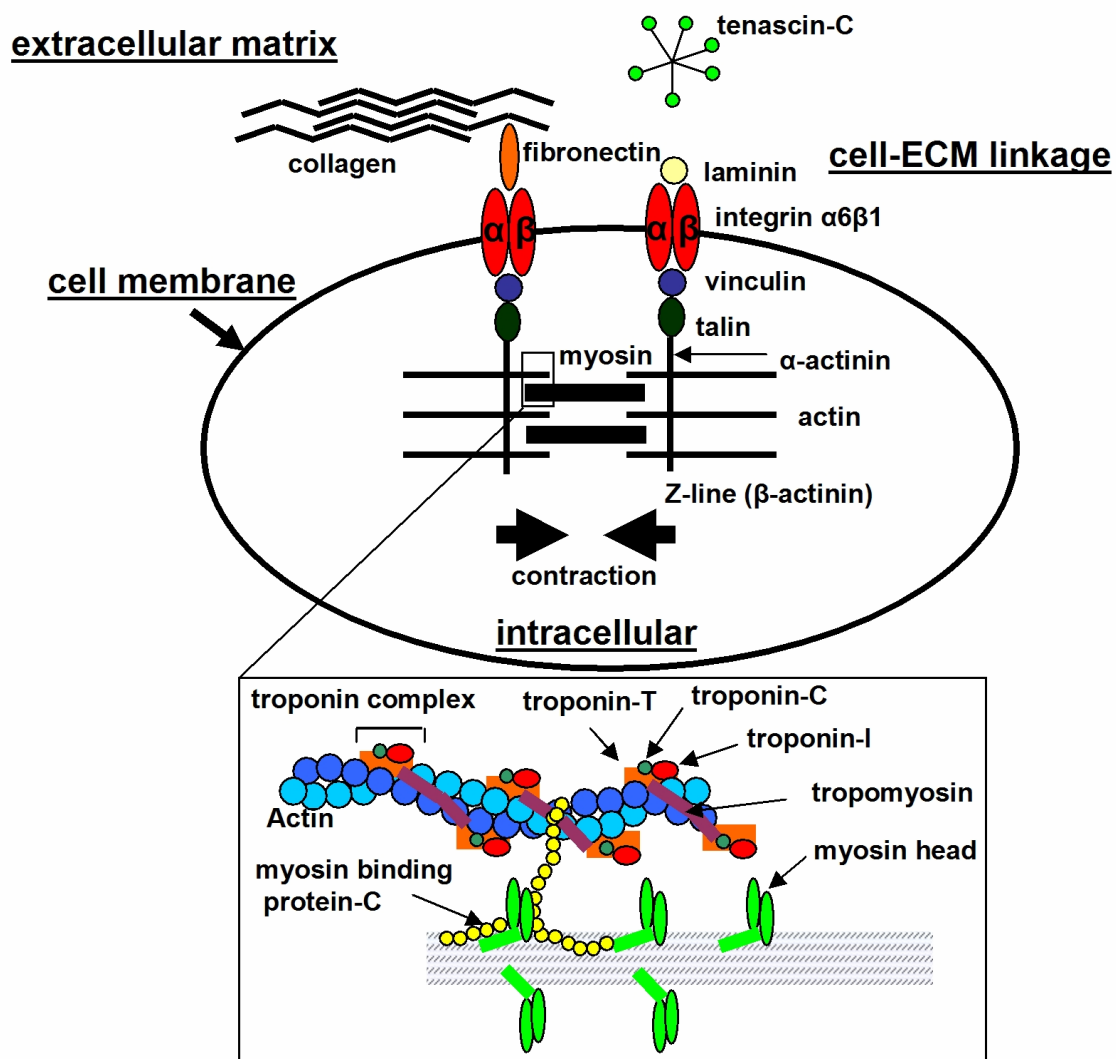


Figure 2. Cardiomyocyte sarcomere and the linkage to the extracellular matrix.

Integrins comprise a large family of heterodimeric cell-surface receptors, each being composed of an α (120-180 kD) and a β (90-110 kD) subunit. Several α - and β -subunits exist, forming multiple integrins of $\alpha\beta$ -dimers, which have varying specificity for cells and ligands. The expression of integrins is closely coordinated with ECM expression. The extracellular domain of integrins binds to ECM proteins or adhesion molecules on other cells, whereas the intracellular domain binds to cytoskeletal proteins and intracellular signaling molecules, including α -actinin and focal adhesion kinases^{21;22}. Engagement of integrins with ECM proteins results in clustering and activation of integrins on the cell surface and the formation of focal adhesion sites^{21;22}. Integrins function as mechanotransducers^{23;24} but also orchestrate bi-directional intracellular signaling^{21;24-26}. In cardiac hypertrophy, dilated cardiomyopathy, and myocardial infarction, the protein composition of ECM component, and the α - and β -subunit composition of integrins are altered^{24;27}. During ventricular remodeling, cardiomyocytes loosen their attachment with the ECM leading to structural changes because of cardiomyocyte slippage. A key molecule that influences the linkage to the ECM during ventricular remodeling is tenascin-C (TNC). TNC is a multimeric ECM glycoprotein that is synthesized by interstitial fibroblasts. TNC is specifically expressed during the embryonic development or early stages of tissue remodeling during inflammation, wound healing or cancer progression²⁸⁻³¹. In the heart, TNC appears during cardiogenesis but it is barely detected in the normal adult heart. However, TNC reappears in the adult heart under various pathological conditions, such as AMI^{31;32}, myocarditis^{33;34}, and dilated cardiomyopathy³⁵. An important factor responsible for the re-expression of TNC is mechanical stretch³⁶⁻³⁹. Several studies have suggested that TNC participates in ventricular remodeling by modulating the attachment of cardiomyocytes to ECM components⁴⁰. TNC is able to bind to ECM components such as fibronectin⁴¹ and collagen⁴², as well as to cellular integrins²⁹ and may also cause inhibition of the formation of costameric adhesion complexes³¹. De-adhesive properties of TNC may lead to cardiomyocyte slippage and ventricular dilatation. Nevertheless, the effects of TNC on myocardial structure and function during myocardial repair and ventricular remodeling remain to be elucidated.

(3) *The extracellular matrix.* The specific arrangement of individual components of the ECM, particularly the interstitial collagens, and their interaction with the cell surface and cytoskeleton of cardiomyocytes play a significant role in structure and function of the heart⁴³. The ECM is defined as a network surrounding and supporting cardiomyocytes and capillaries. The main components of the ECM include structural proteins (collagen type I and III, elastin), adhesive proteins (laminin, fibronectin, collagen IV and VI), de-adhesive proteins (tenascin-C, thrombospondin, osteopontin) and proteoglycans²³. Contractile activity of the heart, generated within the sarcomere of cardiomyocytes, is transmitted via cytoskeleton and integrins to ECM to allow contraction of the chamber. Postinfarction remodeling is associated with proliferation and differentiation of cardiac fibroblasts that cause excessive ECM protein production⁴⁴. Differentiated fibroblasts are the primary mediators of interstitial fibrosis, leading to a loss of myocardial compliance and diastolic failure⁴⁵. Elevated myocardial collagen levels (collagen type I, II, III and VI) have also been demonstrated in patients with hypertension^{46;47}.

On the other hand, alterations in ECM composition may lead to ventricular dilatation, wall thinning and systolic failure. Responsible for ECM degradation during ventricular remodeling are the matrix metalloproteinases (MMPs), a family of zinc-containing enzymes^{5;48-50}. The general classification of MMPs is based on substrate binding and to date, more than 20 MMPs have been described⁵¹. MMPs are synthesized by a number of cell types including fibroblasts, smooth muscle cells, endothelial cells and cardiomyocytes⁵². After synthesis, MMPs are secreted in the ECM as a latent proform that is activated by either proteolytic cleavage or by conformational changes induced by cytokines, reactive oxygen species (ROS), peroxynitrite, or other MMPs⁵³⁻⁵⁵. Active MMPs are inhibited by the tissue inhibitors of MMPs (TIMPs). MMP activity within the myocardium is strictly regulated at three levels (i) transcription, (ii) activation and (iii) inhibition/deactivation, indicating a complex and dynamic system. MMPs, particularly MMP2 and MMP9, have been implicated in the pathogenesis of several cardiovascular diseases, such as myocardial infarction, ischemia-reperfusion injury, and heart failure⁵. Myocardial MMP mRNA levels can be influenced by a variety of neurohormones and cytokines and enhanced MMP expression and activation has been identified in both animals and patients with LV dilatation and CHF^{56;57}. In addition, Spinale *et al.*⁵⁸ have

demonstrated that MMP activity directly contributes to ECM degradation and ventricular remodeling in CHF, because inhibition of MMP activity during the development of CHF resulted in limited LV dilatation and less wall strain.

Biochemical markers of cardiovascular diseases

Reversible and irreversible myocardial damage are accompanied by translational and/or post-translational modifications of cardiac proteins. Characterization of the release kinetics of these altered cardiac proteins, from the myocardium into the circulation, may lead to new prognostic and diagnostic biomarkers of myocardial injury. The release kinetics of various biochemical markers depend partly on their original location in the cell, molecular weight, and the route by which they are cleared from the circulation⁵⁹.

Biochemical markers of acute myocardial infarction

Myocardial necrosis during myocardial infarction is characterized by lethal disruptions of the sarcolemma, allowing structural proteins and other intracellular macromolecules to leak into the myocardial interstitium and finally into the circulation. Plasma concentrations of cardiac proteins serve as biomarkers of necrotic cell death. Nowadays, clinical information including history, anamnesis and ECG is integrated with data of biomarkers. The curve describing plasma concentrations of biomarkers of myocardial necrosis in time can be used to calculate the extent of myocardial injury⁶⁰. Biomarkers of myocardial necrosis after AMI include lactate dehydrogenase (LDH), α -hydroxybutyrate dehydrogenase (α -HBDH), myoglobin, creatine kinase (CK), CK-MB, troponin I and T (Table 1). These biochemical markers differ in sensitivity and specificity and differ in release kinetics^{61;62}.

Table 1. Biomarkers of myocardial necrosis

Biochemical marker	Molecular weight (kDa)	Cardiac specificity	Rise after onset of AMI (h)	Peak level (h)	Duration of elevation
LDH	134	-	4-6	24-36	> 72 h
α -HBDH	134	+	4-6	24-36	> 72 h
Myoglobin	18	-	1-3	6-8	12-24 h
CK-MB _{activity}	85	++	3-6	12-24	2-3 days
CK-MB _{mass}	85	+++	3-6	12-24	2-3 days
cTnI	29	++++	4-6	16-18	4-7 days
cTnT	39	++++	4-6	16-18	10-14 days

Adapted from Morrow *et al.*⁶³

Lactate dehydrogenase (LDH)

LDH is a cytoplasmic enzyme that plays a key role in determining whether pyruvate enters the tricarboxylic acid cycle, or whether pyruvate is converted to lactate. Under aerobic conditions, LDH converts lactate to pyruvate, which, after conversion to acetyl coenzyme A by pyruvate dehydrogenase, is oxidized in the tricarboxylic acid cycle. A large supply of free energy is generated when NADH is oxidized by the respiratory chain in mitochondria. Under anaerobic conditions, LDH converts pyruvate in lactate, which provides a small supply of NAD⁺ during glycolysis. LDH is a tetramer composed of two subunits and the five possible combinations of these subunits results in five isoenzymes. LDH1 is the most prominent form in heart tissue, and LDH5 is the most prominent form in skeletal muscle.

When the plasma membrane is damaged, LDH is released from irreversibly damaged cardiomyocytes. Serum LDH activity rises within 4-6 h after the onset of myocardial infarction, and peaks at 24-36 h. Differences in substrate specificity for LDH isoenzymes were used to develop assays for serum *α-hydroxybutyrate dehydrogenase* (*α*-HBDH), which showed an increased specificity for the detection of myocardial damage⁶⁴. Measurement of serum *α*-HBDH activity has provided a convenient mean for detection of a relative increase in LDH isoenzymes LDH1 and LDH2⁶⁵. However, due to the lack of cardiac specificity, both LDH and *α*-HBDH have been replaced by other biomarkers.

Myoglobin

Myoglobin is a small heme-containing protein responsible for the oxygen transport in muscle tissue. Myoglobin is known as a marker of irreversible cell damage for more than three decades. After membrane damage, it rapidly migrates from the myocardial interstitium into the circulation. Plasma myoglobin levels begin to rise as early as 1-3 h after onset of myocyte damage, peak after 6-8 h and are normalized within 12-24 h⁶⁶. Despite the early detection after AMI, myoglobin is not a very specific biomarker for myocardial injury as it is also present in skeletal muscles⁶⁷. Nowadays, myoglobin is used as an early biomarker of AMI, if combined with other biomarkers⁶⁸.

Creatine kinase (CK) and isoenzymes (CK-MB)

CK is a cytosolic enzyme that regulates the phosphorylation of creatine by ATP. CK is a dimeric protein that consists of two subunits B (brain) and M (Muscle), resulting in three isoforms being MM, MB and BB. Skeletal muscle contains predominantly CK-MM, whereas heart tissue contains the largest fraction of CK-MB (15%). A mitochondrial CK, mCK is also present in the heart but is not released during heart cell necrosis. Although total CK activity in plasma is a sensitive marker of myocardial damage, it has poor specificity due to its high concentrations in skeletal muscles. The MB isoenzyme of CK offers an improvement in sensitivity and specificity compared with total CK^{69;70}. CK-MB levels are elevated within 3-6 h after AMI, peak at 12-24 h, and remain elevated for 2-3 days. Nevertheless, CK-MB represents 1-3% of CK in skeletal muscle, and therefore has limited specificity for irreversible cardiac damage.

Cardiac troponin I and T

The troponin complex, located in the thin filaments of sarcomeres, plays an important role in the regulation of contraction and relaxation. The troponin complex consists of three different subunits troponin C, troponin I, and troponin T. In contrast to CK-MB, both TnI and TnT have cardiac isoforms that are unique to cardiomyocytes; cardiac TnI (cTnI, 29 kDa) and cardiac TnT (cTnT, 39 kDa). Within 4-6 h after onset of AMI troponins are released from necrotic cardiomyocytes into the circulation. In addition to CK-MB, troponin does not permit detection of myocardial necrosis very early (1-3 h) and does not support maximal sensitivity during the first 6 h after the onset of AMI⁷¹. Typically, troponin levels peak at 16-18 h, and remain elevated for \approx 10 days after AMI, long after most other markers have normalized⁷²⁻⁷⁵. Troponins are assayed by immunoassays, allowing a highly sensitive and specific detection of myocardial injury. Troponins are released as intact protein and as degradation products. Several degradation products are detected in serum of patients with AMI, and the release pattern of these degradation products changes in the days following AMI⁷⁶⁻⁷⁹.

Nowadays, troponins are the most frequently used serum markers to diagnose myocardial infarction. In 2000 the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction published

a consensus document about the redefinition of myocardial infarction⁸⁰. In this document the presence of a serum troponin concentration exceeding 95th or 99th percentile of the reference distribution is considered to confirm the diagnosis "myocardial infarction", even in case of microscopically small zones of myocardial necrosis. Moreover, it is likely that future generations of cardiac troponin assays will push this limit even lower.

Although cardiac troponins are nowadays the most frequently used biomarkers of irreversible cell damage after AMI, cardiac troponin levels are frequently exceeding the reference range in patients without acute coronary syndromes in whom myocardial necrosis is not a prominent aspect⁸¹⁻⁸⁵. Renal failure has been demonstrated to cause an increase of serum cTnT levels due to an impaired renal clearance of cTnT and cTnT degradation products, in the absence of any cardiac pathology⁸⁶. Elevated serum troponin concentrations also have frequently been reported in pulmonary embolism^{84;87} and conditions like congestive heart failure⁸², idiopathic dilated cardiomyopathy⁸⁸, myocarditis⁸⁹, unstable angina pectoris⁹⁰, as well as in athletes after ultra-endurance exercise⁹¹. The exact mechanism underlying troponin release from viable cardiomyocytes in the absence of necrosis and thus without lethal disruptions of the cardiomyocyte sarcolemma remains to be elucidated. Several studies have postulated that viable cardiomyocytes may release cardiac troponins by a stretch-related mechanism^{84;92}, but whether troponins are released as intact protein or as degradation products is still unknown.

Biomarkers of ventricular remodeling and congestive heart failure

Heart failure is a complex clinical syndrome and a single biomarker may not reflect all aspects of the syndrome. However, combined measurements of biomarkers may prove valuable in heart failure. Biomarkers of congestive heart failure, which are indicative for cardiac overload and/or ventricular remodeling, are brain natriuretic peptide (BNP) and matrix metalloproteinases (MMP).

Brain natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP)

BNP is a peptide hormone, which is mainly synthesized by cardiomyocytes, as a 134-amino acid prepropeptide that is cleaved into a signal peptide and proBNP (a.a. 27-134).

In the circulation, proBNP splits into BNP and the N-terminal part of proBNP (NT-proBNP). BNP plays an important role in maintaining the cardiorenal homeostasis under physiological and pathological conditions.

ProBNP is released from cardiomyocytes in response to increased ventricular wall stress⁹³. The effects of BNP (vasodilatation, natriuresis, and diuresis) lead to some improvement of the loading conditions of the heart. However, the role of BNP in ventricular remodeling remains undefined. Tsuruda *et al.*⁹⁴ reported that BNP is also synthesized by cardiac fibroblasts (myofibroblasts) *in vitro*. Cardiac fibroblasts play a crucial role in ECM metabolism by synthesizing collagen and other matrix proteins as well as promoting ECM degradation by secreting MMPs. Increased secretion of BNP by cardiac fibroblasts has been demonstrated to participate in ECM degradation by decreasing collagen synthesis and increasing MMP production^{94;95}.

Circulating levels of BNP and NT-proBNP are strongly increased during the early phase of acute myocardial infarction⁹⁶ and both plasma markers are predictors of adverse ventricular remodeling after myocardial infarction⁹⁷⁻⁹⁹. In addition, elevated plasma levels of BNP and NT-proBNP have also been demonstrated in patients with congestive heart failure¹⁰⁰, and both levels correlate with the functional classification of patients according to the New York Heart Association (NYHA)¹⁰¹. Unloading of the left ventricle in patients with CHF by a left ventricular assist device has been demonstrated to result in a decrease of BNP mRNA and protein expression in the heart¹⁰² and also results in reduced serum levels of both BNP and NT-proBNP^{102;103}. These studies suggest that serum levels of BNP and NT-proBNP may also be useful as biomarkers of reverse ventricular remodeling.

Matrix metalloproteinases (MMPs)

In both animals and patients with LV dilatation and CHF, enhanced expression and increased activation of MMPs have been identified^{56;57}. Spinale *et al.*⁵⁸ have demonstrated that MMP activity within the myocardium directly contributes to ventricular remodeling in CHF, because inhibition of MMP activity during the development of CHF resulted in limited LV dilatation and less wall strain. Circulating levels of MMP2 and MMP9 are elevated in patients with CHF and several studies have demonstrated that

plasma MMPs levels in patients with CHF correlated positively with LV volumes and negatively with LV ejection fraction¹⁰⁴⁻¹⁰⁶. These findings indicate that circulating MMP levels in patients with CHF reflect the actions of MMP within the myocardium, and that circulating MMP levels are useful biomarkers of ventricular remodeling.

In addition to MMPs, several MMP-like enzymes have been discovered, such as ADAMs, A Disintegrin And Metalloproteinase, and EMMPRIN, Extracellular Matrix MetalloPRoteinase Inducer, that may play a role in cardiac remodeling. ADAMs can down-modulate cell surface receptors, causing a switch-off of signals from a cellular receptor whose ectodomain is cleaved and “shed” by the ADAM. Goldsmith *et al.*¹⁰⁷ and Ding *et al.*¹⁰⁸ have shown that integrins are shed from the myocyte surface during the evolution of hypertrophy transitioning to heart failure. Shedding of integrins could rapidly “disconnect” cells from the ECM, leading to cardiomyocyte slippage and ventricular dilatation. EMMPRIN, also called basigin or CD147, is a 58 kDa transmembrane glycoprotein belonging to the Ig superfamily. EMMPRIN stimulates several cell types, including fibroblasts, to produce MMP1, MMP2, MMP3, MT1-MMP, and MT2-MMP. In human hearts with aortic stenosis, MMP2 and EMMPRIN were found to be upregulated at mRNA and protein level, and MMP1, MMP3 and MMP9 were downregulated at protein level¹⁰⁹. As TIMP4 protein levels were found to be upregulated markedly, these authors conclude that the balance between MMP and TIMP is shifted towards MMP inhibition in human aortic stenosis, which may contribute to collagen accumulation. However, ADAMs and EMMPRIN play an important role in tumor biology but little is known about the role of ADAMs and EMMPRIN in the (patho)physiology of cardiomyocytes.

Thesis Outline

Identification of translational and/or post-translational modifications of cardiac proteins after AMI or during the progression to CHF is highly relevant to gain insight into the mechanisms underlying these syndromes. In addition, characterization of the release kinetics of cardiac proteins from the injured myocardium into the circulation may provide information about their value as diagnostic biomarkers. The main objective of the first part of this thesis was to characterize the release kinetics of cardiac troponins from irreversibly versus reversibly damaged cardiomyocytes. Although troponins are nowadays the most frequently used biomarkers of myocardial infarction⁸⁰, controversy continues about whether the initial release of troponins from the infarcted myocardium occurs later than that of cytoplasmatic enzymes used previously, like LDH and CK-MB^{71;110-112}. In **Chapter 2**, release kinetics of intact cTnI from cultured neonatal rat cardiomyocytes undergoing rapid necrosis were compared with the release kinetics of LDH. In **Chapter 3** we investigated the release kinetics of cTnI and cTnT including their degradation products from neonatal rat cardiomyocytes undergoing a slowly developing necrosis process.

Elevated troponin levels have also been observed in patients without acute coronary syndromes with normal CK-MB levels⁸¹⁻⁸⁵. Several studies have suggested that troponins may also be released from viable cardiomyocytes by a stretch-related process^{84;92}. In **Chapter 4** we investigated the release of cTnI from viable cardiomyocytes *in vitro* by stimulation of stretch-responsive integrins. In **Chapter 5** we studied whether the presence of cTnT in serum of patients with CHF is caused by myocardial necrosis or by a stretch-dependent process initiated by severe cardiac overload.

In heart failure, myocardial expression patterns of many proteins undergo marked changes. The main objective of the second part of this thesis was to identify modifications of cardiac proteins in myocardium and in serum during ventricular remodeling and the progression to heart failure, and to investigate the relevance of these proteins as biomarker of CHF. A model frequently used to investigate functional, structural, and molecular changes associated with ventricular remodeling and heart failure is the monocrotaline(MCT)-treated rat. MCT induces pulmonary hypertension that

is associated with the development of compensated right ventricular (RV) hypertrophy and the progression to RV failure, within several weeks depending on the doses of MCT. In **chapter 6**, we characterized RV function in relation to structural changes after MCT-induced pulmonary hypertension in the intact rat.

The specific expression pattern of the ECM protein, TNC, in response to myocardial stretch suggests that TNC might be a relevant biomarker of ventricular remodeling. However, the effects of TNC on myocardial structure and function are still unknown. In **chapter 7** we investigated whether MCT-induced RV dilatation is associated with re-expression of myocardial TNC and with elevated TNC plasma levels and whether TNC can be used as biomarker of ventricular remodeling.

In heart failure patients, cardiac resynchronization therapy (CRT) leads to *reverse* ventricular remodeling. However the molecular and cellular mechanisms underlying *reverse* ventricular remodeling following CRT are not completely understood. In **chapter 8** we evaluated whether CRT induces changes in levels of circulating biomarkers, such as TNC, MMP2, MMP9 and NT-proBNP, in patients who showed a beneficial response to CRT and whether these proteins can be used as biomarkers of *reverse* ventricular remodeling. **Chapter 9** is a general discussion of these studies and presents future research, and **chapter 10** summarizes the major findings of the studies presented in this thesis.

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