Summary

In this thesis alterations in cellular function and structure were studied in heart failure with different underlying cause and phenotype to obtain insight into the cellular pathomechanisms underlying progressive deterioration of left ventricle pump function in human heart failure. The results obtained may be used to develop new-targeted therapeutic interventions for the treatment of heart failure in the future. Here, the main findings of the studies are presented and the implications of the findings for clinical practice and future research are discussed.

Chapter 1. In this chapter an introduction to the background and methods of the studies is given. The aim of this thesis is defined.

Chapter 2. In this chapter we focused on the functional role of individual sarcomeric protein isoforms and of post-translational protein modifications such as proteolysis and phosphorylation in diseased myocardium. In failing myocardium, sarcomeric dysfunction includes depressed maximum force development, increased calcium-sensitivity and increased passive stiffness. These changes could be largely explained by altered phosphorylation of sarcomeric proteins including troponin I, titin, myosin binding protein C and myosin light chain 2. Changes in phosphorylation are most likely caused by neurohumoral-induced alterations in the kinase-phosphatase balance inside the cardiomyocytes. A therapy which specifically targets phosphorylation sites within sarcomeric proteins or the kinases and phosphatases involved might be used to improve cardiac function in heart failure.

Chapter 3. Abnormalities in β-adrenergic receptor (βAR) signal transduction are not only involved in impairment of cardiac function, but they also play a role in the structural changes observed in heart failure. An increase in catecholamines resulted in down regulation and desensitization of the βAR. Moreover, it is evident that prolonged adrenergic stimulation causes alterations of the expression and activity of downstream components of the βAR signal transduction cascade.

In Chapter 3 a comparison was made between left ventricular tissue samples from patients with ischemic cardiomyopathy (ISHD) and idiopathic cardiomyopathy (IDCM) in order to investigate whether changes in the βAR signal transduction pathway translate into diverse functional and structural alterations. Donor hearts served as non-
failing controls. Diverse changes were found in cellular function and structure in failing human myocardium with different underlying cause. In both groups a marked reduction was found in the number of $\beta_1$AR and $\beta_2$AR. Different alterations in the expression level of G-coupled receptor kinase 5, G-inhibitory, protein phosphatase 1 and myosin light chain 2 phosphorylation were found between ISHD and IDCM. Furthermore, differences in sarcoplasmic reticulum calcium ATPase (SERCA2a) expression and phospholamban/SERCA2a ratio between ISHD and IDCM were found. Cardiomyocyte force measurements showed differences in calcium sensitivity, which could be explained by a difference in TnI phosphorylation. A lower collagen volume fraction in ISHD was found compared to IDCM using histological analyses.

Our results indicate that alterations in the $\beta$AR signaling pathway and in cardiomyocyte structure and function depend on underlying cause. These findings highlight the different alterations in ISHD and IDCM, which may modify heart failure risk, prognosis and response to treatment.

Chapter 4. Fabry disease is an inherited X-linked inborn lysosomal storage disorder characterized by intracellular glycosphingolipid depositions resulting from deficient $\alpha$-galactosidase A activity. To reveal if alterations in myofilament function and protein composition contribute to left ventricle myocardial dysfunction in Fabry disease patients, both were determined in left ventricle endomyocardial biopsies of patients with the cardiac variant of Fabry disease. In addition cardiomyocyte cross sectional area, area of glycosphingolipid vacuoles, myofibrillolysis and extent of fibrosis were determined. Our data showed high cardiomyocyte passive stiffness and low active tension which may both contribute to left ventricle myocardial dysfunction observed in Fabry disease patients. The high resting tension relates to the observed diastolic LV dysfunction and may be partly explained by decreased phosphorylation of myofibrillar and/or cytoskeletal proteins, while the low active tension relates to reduced tissue Doppler systolic shortening velocity and may be due to proteolysis of troponin I and desmin. As cardiomyocyte stiffness is an important determinant of left ventricle stiffness in heart failure, the augmented passive stiffness in Fabry disease patients may be detrimental for left ventricle diastolic function.

Chapter 5. The aims of $\beta$-blocker therapy are to improve survival and quality of life of patients. To investigate if $\beta$-blocker therapy induces disparate effects in heart failure with
normal ejection fraction (HFNEF) and heart failure with reduced ejection fraction (HFREF), this chapter compared myocardial structure, function and protein composition in HFNEF and HFREF patients without or with β-blocker therapy.

Changes in active tension, calcium sensitivity and troponin I phosphorylation are shared by HFNEF and HFREF and are all beneficial for left ventricle contractility. Changes in collagen volume fraction, cardiomyocyte diameter and passive stiffness are unique to HFNEF and affect diastolic left ventricle function. Lower G-inhibitory is unique to HFREF and may improve left ventricle contractility. β-blocker therapy induces myocardial effects unique to HFNEF or HFREF. This may explain the dissimilar outcome of β-blocker therapy in HFNEF and HFREF phenotype. The clinical benefits obtained with β-blocker therapy are multi-factorial, involving improvement of calcium handling, reversal of cardiac remodeling, improved cardiac efficiency. Our data showed diverse effects of β-blocker therapy on myofilament function. Myofilament contractile reserve to β-adrenergic stimulation (i.e. exogenous PKA) was blunted in HFNEF+β and enhanced in HFREF+β. Our data indicate that β-blocker therapy may improve systolic cardiac function in HFNEF patients by an increase in active cardiomyocyte force development and a reduction in cardiomyocyte hypertrophy. However, an increased passive stiffness was observed in cardiomyocytes from HFNEF patients receiving β-blocker therapy, which was no longer corrected to normal values upon PKA treatment. As it is generally believed that the dominant pathophysiological mechanism in HFNEF patients is abnormal diastolic function, our observations do not provide mechanistic evidence to support the use of β-blockers in HFNEF.

Chapter 6. Raised diastolic left ventricle stiffness importantly contributes to heart failure in diabetes mellitus (DM) and results from myocardial deposition of advanced glycation endproducts (AGEs) and interstitial fibrosis. In diabetic patients with heart failure a contribution to this high diastolic left ventricle stiffness of an elevated passive stiffness has so far not been assessed. This study was designed to compare myocardial fibrosis, AGEs deposition and passive stiffness of isolated cardiomyocytes between diabetic and non-diabetic HFNEF patients and between diabetic and non-diabetic HFREF patients. Increased myocardial collagen volume fraction was found only in diabetic patients with HFREF, while elevated cardiomyocyte passive stiffness was present only in diabetic patients suffering from HFNEF. Diabetes increased myocardial AGEs deposition in patients with HFREF and less so in HFNEF patients. The question remains if β-blocker
therapy reverses the changes observed in cardiomyocyte function and structure in heart failure patients with diabetes mellitus, especially the high passive stiffness observed in HFNEF patients. It is therefore very important to understand the cellular pathophysiology of the β-blocker therapy in these patients, to improve the clinical outcome of patients with diabetic cardiomyopathy.

Chapter 7. In this chapter, we investigated whether the antibodies directed against β-adrenergic receptors can be used to determine expression of β-adrenergic receptors (βAR) in human myocardium. Using western blotting we investigated the specificity of commercially available antibodies directed against β1AR and β2AR in human left ventricular tissue. The antibodies recognized several protein bands at different molecular weights in human myocardial samples and also multiple proteins in Chinese hamster ovary (CHO) cells expressing β1AR, β2AR and even β3AR, indicating that these antibodies are not specific and are not suited to study expression of β-adrenergic receptor in myocardium.

Chapter 8. In this review an overview is given of the perturbed balance between receptor-mediated kinases and phosphatases coordinating phosphorylation of regulatory proteins involved in cardiomyocyte contractility during heart failure. The imbalance between kinases and phosphatases is related to increased neurohumoral stimulation to enhance cardiac pump function in heart failure. The data from different animal and human studies underline the importance of careful biopsy procurement, and the need to investigate localization of kinases and phosphatases within the cardiomyocyte.
Conclusion

The research in this thesis was limited to the most important signal transduction pathway in the heart and the most frequent drug therapy in heart failure, in order to understand the alterations in the pathophysiology of heart failure patients. Despite the different studies performed, it is still unclear how we can improve the quality of life and prolong survival in patients with heart failure. Other options need to be taken into consideration for a better therapy of patients with heart failure. Altered maximum force development, calcium sensitivity and increased passive stiffness largely originate from changed sarcomeric proteins in diseased myocardium, caused by neurohumoral-induced alterations in the kinase-phosphatase balance inside the cardiomyocytes. However, alterations in β-adrenergic signaling pathway and cardiomyocyte function and structure depend on underlying cause of the cardiomyopathy. Therefore, these differences may explain in part the dissimilar outcome of β-blocker therapy in HFNEF and HFREF. Overall, the findings presented in this thesis indicate that cellular changes in heart failure depend on underlying cause and phenotype of the disease.