

Importance of Cardiac Reserve for Evaluation and  
Prediction of Cardiac Function and Morbidity  
assessed by low-dose dobutamine  
stress echocardiography

Margareta Scharin Täng



Department of Molecular and Clinical Medicine/Cardiology,  
Wallenberg Laboratory, Institute of Medicine,  
Sahlgrenska Academy at Göteborgs University

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

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*"Det är vägen som är målet."*

Inger Anckar-Svensson

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## Abstract

This thesis aimed to evaluate the cardiac reserves capacity to be used to predict treatment effects, sub clinical heart disease and to evaluate  $\beta_1$ -adrenoceptor (AR) gene polymorphism (Ser49Gly).

Studies were performed in patients with dilated cardiomyopathy, in rats (young, healthy, diabetic and hypertensive), in mice (immunized against the  $\beta_1$ AR) and in heart-transplanted patients.

The cardiac reserve was assessed by low-dose dobutamine stress echocardiography. In both patient studies by dobutamine infusion until an increased baseline heart rate with  $\sim 20$  bpm, in rats at doses of  $10 \mu\text{g}/\text{kg}/\text{min}$  and  $20 \mu\text{g}/\text{kg}/\text{min}$  dobutamine and in mice after an intraperitoneal injection of  $1 \mu\text{g}$  dobutamine/g of body weight.

Both global and regional cardiac reserve can be used to predict treatment effect of metoprolol in dilated cardiomyopathy patients. However, only cardiac reserve in the basal segments of the heart was independently associated with recovery. In heart transplanted patients a gene-polymorphism in the  $\beta_1$ AR in the graft affects cardiac reserve. Patients having the  $\beta_1$ AR Gly49 variants had a lower resting heart rate, a better stress endurance and chronotropic reserve than patients homozygous for Ser49. They also had better diastolic function shown as better lusitropic capacity. Cardiac reserve can also be used to investigate sub clinical heart disease in  $\beta_1$ AR immunized mice and to predict heart disease development in these animals. Furthermore, cardiac reserve decreases with age and is depressed both in hypertension and in diabetes rat model.

We conclude that cardiac reserve can predict left ventricular recovery during beta-blocker treatment and that  $\beta_1$ AR polymorphism affects cardiac reserve in humans. Cardiac reserve decreases with age and is impaired both in severe heart disease and during progression of myocardial dysfunction in rats. Furthermore, cardiac reserve can be used to predict cardiomyopathy development after  $\beta_1$ AR immunization in mice.

## *Svensk sammanfattning*

Hjärtats kardiella reserv är ett mått på skillnaden i dess pumpförmåga mellan vila och stress. Denna avhandling studerar möjligheten att använda den kardiella reserven för att prediktera subklinisk hjärtsjukdom och behandlingseffekt samt för att utvärdera den kliniska betydelsen av polymorfism (Ser49Gly) i genen för den  $\beta_1$ -adrenerga receptorn (AR).

Studierna utfördes på patienter med dilaterad kardiomyopati och hjärttransplanterade patienter samt i experimentella studier på råttor och möss. Den kardiella reserven studerades med lågdos dobutaminstress-ekokardiografi; på patienterna genom att öka vilopulsen med 20 slag/min, på råttor vid två doser dobutamin (10 $\mu$ g/kg/min; 20 $\mu$ g/kg/min) och på möss med en intraperitoneal dobutamininjektion (1  $\mu$ g/g kroppsvikt).

Kardiell reserv kan användas för att prediktera behandlingseffekt av metoprolol ( $\beta_1$ -selektiv receptorblockerare) hos patienter med dilaterad kardiomyopati. Den kardiella reserven i de basala delarna av hjärtat var oberoende knuten till förbättringen efter behandling. Hos de hjärttransplanterade patienterna sågs skillnader i kardiell reserv beroende på en gen-polymorfism i  $\beta_1$ AR. Framför allt syntes skillnader i hjärtats pulsreserv och relaxationsförmåga (lusitrop) samt i patienternas fysiska uthållighet. Den kardiella reserven sjunker med ålder och är nedsatt vid högt blodtryck och vid diabetes hos råttor precis som hos människor. Den kardiella reserven kan användas för att studera subklinisk och klinisk hjärtsjukdomsutveckling hos  $\beta_1$ AR immuniserade möss och prediktera hjärtsjukdomsutveckling hos dessa djur.

Slutsatserna av denna avhandling är att den kardiella reserven, utvärderad med lågdos dobutaminstress-ekokardiografi, kan användas för att prediktera behandlingsresultat av  $\beta$ -blockerare samt att den är påverkad av en genpolymorfism i  $\beta_1$ AR. Den kardiella reserven sjunker med åldern och kan användas för att utvärdera och prediktera klinisk och subklinisk hjärtsjukdom i experimentella studier. En nedsatt kardiell reserv kan vara det första tecknet på begynnande hjärtdysfunktion.

## List of papers

This PhD thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I. **The function of left ventricular basal segments is most important for long-term recovery.** *M Scharin Täng, F Waagstein, B Andersson.*  
Int J Cardiology 2007 doi:10.1016/j.ijcard.2006.11.014
  
- II. **Influence of age, hypertension, and diabetes on cardiac reserve in rat model.** *M Scharin Täng, E Haugen, A Isic, M Fu, B Andersson.*  
J Am Soc Echocardiography 2007 doi:10.1016/j.echo.2006.11.001
  
- III. **Antibodies against the  $\beta_1$ -adrenergic receptor induce progressive development of cardiomyopathy.** *L Buwall, M Scharin Täng, B Andersson, M Fu.* J Mol and Cell Cardiology 2007  
doi:10.1016/j.yjmcc.2007.02.007
  
- IV. **Cardiac reserve in the transplanted heart: effect of a graft polymorfism in the  $\beta_1$ -adrenoceptor.** *M Scharin Täng, E Lindberg, B Grüner Sveälv, Y Magnusson, B Andersson.* Submitted

## Abbreviations

|                               |   |
|-------------------------------|---|
| ANOVA                         | Analysis of variance  |
| ATP                           | Adenosintriphospate   |
| Av                            | Atrial myocardial velocity  |
| cAMP                          | Cyclic adenosin monophosphate   |
| BalbC                         | The Bagg albino mice  |
| bpm                           | Beats per minutes   |
| $\beta_1$ AR                  | $\beta_1$ -adrenoceptor   |
| $\beta_1$ AR EC <sub>II</sub> | the second extracellular loop of $\beta_1$ AR                         |
| CV                            | Coefficient of variation  |
| DCM                           | Dilated cardiomyopathy  |
| DSE                           | Dobutamine stress echocardiography                                    |
| DTI                           | Doppler tissue imaging  |
| E                             | Early transmitral flow velocity                                       |
| EF                            | Ejection fraction   |
| ERNA                          | Equilibrium radionuclide angiography                                  |
| Ev                            | Early myocardial velocity   |
| G-protein                     | Guanine nucleotide protein  |
| G <sub>i</sub> -protein       | Inhibitory G-protein  |
| G <sub>s</sub> -protein       | Stimulatory G-protein   |
| HR                            | Heart rate  |
| FS                            | Fractional shortening   |
| LV                            | Left ventricular  |
| LVEF                          | Left ventricular ejection fraction                                    |
| NYHA                          | New York Heart Association  |
| OR                            | Odd ratio   |
| PKA                           | Protein kinase A  |
| SHR                           | Spontaneously hypertensive rats                                       |
| Sv                            | Systolic myocardial velocity  |
| Vcf c                         | Velocity of circumferential fiber shortening corrected for heart rate |
| VTI                           | Velocity time integral  |
| WKY                           | Whistar Kyoto rats  |



## Introduction

### *The Heart*

The heart is unique compared to other organs in the body since it starts functioning before it is fully formed. After fertilization, the first indication of the development of a human heart is between day 16-19 and the heart starts to beat around the 22nd day. However, the circulation does not start until around the 28th day, at this point the heart is approximately 4 mm in length. This usually happens before the woman realizes she is pregnant and when the embryo is too small to be visualized in detail by ultrasound.

The heart beats about 100 000 times daily or about two and a half billion times over a 70 year lifetime. Heart disease is a major cause of death in the western world and about 43% of all deaths in Sweden during 2004 were caused by cardiovascular disease [Socialstyrelsen, 2007].

### **Left ventricular function**

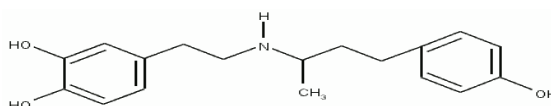
Left ventricular (LV) function is traditionally divided into systole and diastole, describing ventricular emptying and filling. Systolic function of the heart depends on end-diastolic volume (preload) and on myocardial activation (inotropy). Systolic function is most commonly measured as left ventricular ejection fraction (LVEF). Diastolic function depends on a series of events. The phase of isovolumic myocardial relaxation with a rapid LV pressure fall due to relaxation and elastic recoil is followed by the filling phases. The rapid early filling phase is related to the rate of myocardial relaxation and depends on the pressure gradient between the atrium and the left ventricle. In the study by Chemla *et al*, they came to the conclusion that the relaxation in the heart depends mainly on preload and inactivation (lusitropy) [Chemla, 2000]. The myocardial wall motion velocities both in systole and in diastole can be measure with Doppler tissue imaging (DTI). Mitral annulus velocity determined by DTI is a relatively preload independent variable and is superior to conventional mitral Doppler indexes [Farias, 1999] and some authors claim that diastolic early myocardial velocity can be a reliable marker for LV diastolic function [Galiuto, 1998, Sohn, 1997].

### *Cardiac reserve*

Cardiac reserve can be studied with different types of tests; exercise, pacing and by pharmacologic stress. In this thesis, the cardiac reserve has been studied by pharmacologic stress with dobutamine. Cardiac reserve refers to the heart's ability to adjust to the demands placed upon it. The traditional definition of cardiac reserve is the maximum percentage that the cardiac output can increase above the resting level. It is calculated as the cardiac output during stress minus cardiac output at rest. In the normal young adult the resting cardiac output is about 5-6 L/min and the cardiac reserve is nearly 30 L/min. For example, running to catch a tram would cause an increase in oxygen demand, which must be balanced by increased blood circulation. This increase in cardiac output is achieved by an increase in either heart rate or stroke volume or both. In the weak or elderly person, the cardiac reserve may be as low as 5-6 L/min. In severe heart failure the cardiac reserve can be markedly diminished or totally abolished [Guyton, 1991].

One of the components in cardiac reserve is stroke volume. Stroke volume is dependent on preload, afterload and contractility (inotropy). Where preload is the volume of blood in the ventricle at the end of diastole and afterload the resistance against which the ventricle must eject during systole. Dobutamine decreases preload and afterload [Leier, 1978] by decreasing pulmonary and systemic vascular resistance and thereby reduce the pulmonary capillary wedge pressure. Increased inflow causes the end diastolic volume to increase (increased myocardial fiber length). In response to this augmented inflow, the ventricles contract more forcefully resulting in an increased stroke volume. Changes in stroke volume can be accomplished by changes in ventricular inotropy (contractility), this ability to increase contractility decreases with development of heart failure. The LVEF is the fraction of the end-diastolic volume that is ejected with each beat (stroke volume divided by end-diastolic volume) and LVEF is the inotropic component of the cardiac reserve. Increasing inotropy leads to an increase in LVEF, while decreasing inotropy decreases LVEF. LVEF is therefore often used as a clinical index for evaluating the inotropic state of the heart during stress.

Heart rate and the heart rate reserve also referred to as chronotropic reserve are also components in cardiac reserve. It is generally acknowledged that fast resting heart rate is associated with increased cardiovascular mortality [Kannel, 1987]. In both men and women, independent of age, all-cause and cardiovascular mortality increased progressively with higher resting heart rates [Kannel, 1987]. Furthermore, heart rate reserve has shown to be a predictor of mortality and morbidity. Cheng *et al* have shown in 27 459 healthy men that heart rate reserve during exercise test was inversely associated with cardiovascular mortality [Cheng, 2002]. Especially low heart rate reserve in healthy young men (age 20-39) seemed to be associated with higher cardiovascular mortality. Savonen *et al* showed the same phenomena in healthy middle-age men [Savonen, 2006].



The chemical structure for dobutamine

## Dobutamine

Dobutrex<sup>®</sup> (Eli Lilly Sweden AB, Stockholm) were used in all studies. Dobutamine is a synthetic catecholamine, with strong agonistic activity at the  $\beta_1$ -adrenoceptor and mild agonistic activity at the  $\beta_2$ - and  $\alpha_1$ -adrenoceptors [Jewitt, 1974, Ruffolo, 1987]. Dobutamine can be used to assess lusitropic, inotropic and chronotropic reserve [Hees, 2006] and it is dose depending with a main increase in contractility and cardiac output at lower doses [De Wolf, 1999] and a dose-related increase in heart rate. Since dobutamine does not act on dopamine receptors to induce the release of norepinephrine (an  $\alpha_1$ -agonist), dobutamine is less prone to induce hypertension than dopamine. Dobutamine acts by increasing synthesis of cyclic adenosin monophosphate (cAMP) and is thereby dependent of both the adrenoceptors as well as of the G-protein (guanine nucleotide-binding protein) - adenylate cyclase - cascade.

### ***Dobutamine stress echocardiography***

Dobutamine stress echocardiography (DSE) is commonly used as a pharmacologic stress to determine the presence of significant coronary artery disease and low-dose DSE is a well-established method to investigate the cardiac reserve in humans with non-coronary heart disease [Kitaoka, 1999, Marwick, 2000, Naqvi, 1999, Paelinck, 1999, Scrutinio, 2000]. As previously shown by Kyriakides *et al* the cardiac reserve decreases with age [Kyriakides, 1986] and is also decreased in heart disease both of ischemic and non-ischemic etiology [Vigna, 1996]. DSE has been used in different studies to predict survival and clinical outcome in patients with cardiomyopathy [Paraskevaidis, 2001, Scrutinio, 2000]. Furthermore, studies have demonstrated that LV global contractile reserve can predict improvement in LVEF after beta-blocker treatment (carvedilol, bisindolol), in ischemic as well as non-ischemic cardiomyopathy patients [Eichhorn, 2003, Jourdain, 2002, Seghatol, 2004]. Cardiac reserve has shown to provide incremental prognostic information for the prediction of cardiac events in patients with right bundle branch block [Biagini, 2004]. In contrast, assessment of cardiac reserve does not give additional information for the early detection of cardiomyopathy induced by cytostatic treatment [Bountiukos, 2003].

High dose dobutamine stress is a surrogate for maximal exercise test but they are not interchangeable. High dose dobutamine test is frequently used to evaluate coronary artery disease and for determining prognosis in these patients [Armstrong, 2005, Rambaldi, 2005, Vigna, 1996]. During high dose dobutamine the contractility is substantially higher and afterload is lower than during exercise test. However, cardiac output is higher during physiological exercise due to higher heart rate and preload which leads to a higher cardiac reserve during maximal exercise test compared to stress test with high dose dobutamine [Cnota, 2003].

The technology of echocardiography is rapidly improving making it possible to perform more advanced studies on small animals with good precision and accuracy and therefore DSE has been widely used in several animal models. In both rats and mice, DSE has mainly been used to study ischemia and remodelling after myocardial infarctions [Cove, 1995, Dawson, 2005, Fiordaliso, 2005, Iwanaga, 2004, Salto-Tellez, 2004]. Studies on mice are now getting more

and more attention due to the unlimited resources of different gene manipulated mice (Knock-Out and Knock-In), elucidating differences in heart diseases and disease development.

### **Coronary flow reserve**

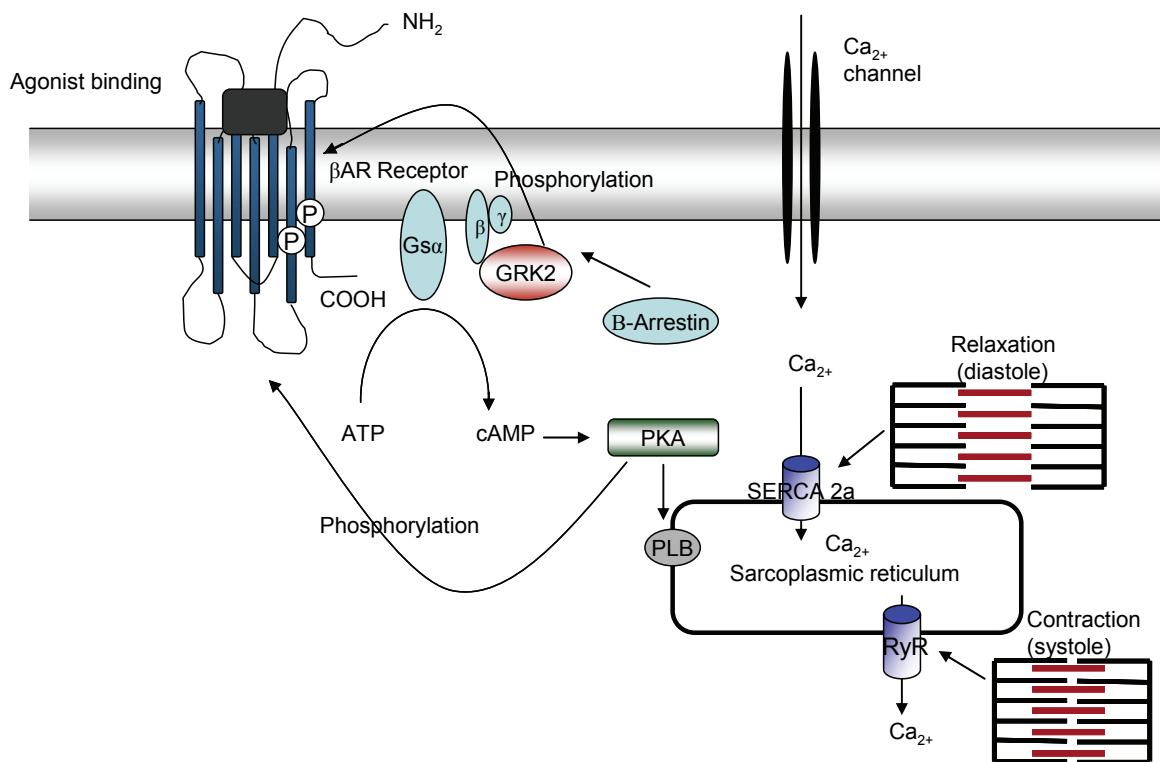
When exploring cardiac reserve, investigations of coronary flow reserve might be of interest. Studies have shown that coronary flow reserve is reduced in several diseases affecting the heart i.e. dilated and hypertrophic cardiomyopathy, coronary artery disease, hypertension and diabetes mellitus. Coronary flow reserve is considerably reduced even when the systolic function appears normal or only slightly decreased in patients with heart failure [Neglia, 1995]. Neglia *et al* also showed that myocardial blood flow at rest and during pacing correlated inversely with LV end-diastolic pressure and did not correlate with LVEF. Teragaki *et al* among others suggests that coronary flow reserve in patients with dilated cardiomyopathy correlates better with diastolic than systolic parameters [Teragaki, 2003]. However, detecting severely decreased coronary flow is a predictor of poor prognosis in patients with idiopathic LV dysfunction [Neglia, 2002].

### ***Adrenoceptors***

The adrenoceptors of most interest during DSE are  $\beta_1$ ,  $\beta_2$  and  $\alpha_1$ -adrenoceptors, since these receptors mainly regulate cardiac function. Ahlquist classified the adrenoceptor in 1948 into  $\alpha$  (excitatory) and  $\beta$  (inhibitory) for their function in blood vessels [Ahlquist, 1948]. The  $\beta$ -adrenoceptor consists of three intra- and three extra-cellular loops. The  $\beta$ -adrenoceptor signaling pathway plays an important role in regulating the contractility and heart rate, Figure 1. Both  $\beta_1$ - and  $\beta_2$ -receptors couples to Gs-protein (stimulatory G-protein) to activate adenylyl cyclase that dephosphorylates adenosintriphospate (ATP) to form cAMP and stimulation of this receptor subtype increases the synthesis of cAMP. Increased cAMP activates protein kinase A (PKA), which through phosphorylation of phospholamban and calcium channels increases myocardial contractility. The phospholamban is a regulatory phosphoprotein which modulates the active transport of  $Ca^{2+}$  into the lumen of the sarcoplasmic reticulum.

Cardiac  $\alpha_1$ -adrenoceptors couple also via G-protein [Bristow, 1988] but mediate only a weak positive inotropic effect, thus the density of the  $\alpha_1$ -adrenoceptor is only 10-15% of the  $\beta$ -adrenoceptor in the human heart. However, the  $\alpha_1$ -adrenoceptors in rat hearts, but not in mice have a five time higher density than in the human heart [Steinfath, 1992]. This could explain why rats generally have a greater increase in fraction shortening during dobutamine stimulation compared to that of humans and mice.

In the failing and the ageing heart the  $\alpha$ -adrenoceptor function is unchanged or only slightly decreased [Bristow, 1988, Brodde, 2004]. Whereas, the decreased  $\beta$ -adrenoceptor function both in failing and in ageing heart results in a reduction in cardiac reserve. The  $\beta$ -adrenoceptors downregulation with age appears already at a very young age (in 5 to 13 week old rats) in rats [Castellano, 1993] considering that the breeding age for Whistar Kyoto rats is 10-12 weeks.



**Figure 1. Signaling pathway of the  $\beta$ -adrenoceptor.**

G, G-protein; PKA, Protein kinase A; GRK2, G-protein-coupled receptor kinase 2; PLB, phospholamban; SERCA 2a, sarcoplasmic reticulum calcium ATPase; RyR2, Ryanodine receptor. Adapted from Lisa Buvalls thesis 2006.

## Polymorphisms of the $\beta_1$ -adrenoceptor

The  $\beta_1$ -adrenoceptor is encoded by an intron-less gene located on chromosome 10. There are at least two known polymorphisms in the  $\beta_1$ -adrenoceptor. The first polymorphism is located at nucleotide position 145 (Adenin→Guanin), resulting in an amino acid substitution of serine by glycine at codon 49, Ser49Gly [Borjesson, 2000]. The second polymorphism is located at nucleotide position 1165 (Guanin→Cytosin), resulting in an amino acid substitution of arginine by glycine at codon 389, Arg389Gly [Tesson, 1999], see Figure 2. Neither of the polymorphisms in the  $\beta_1$ -adrenoceptor can predict dilated cardiomyopathy [Magnusson, 2005] but dilated cardiomyopathy patients with the Gly49 variants had a improved long-term survival [Borjesson, 2000] and this polymorphism is also associated with resting heart rate. An additive model was presented, patients homozygous for Ser49 (74%) had the highest resting heart rate, patients homozygous for Gly49 (2.5%) had the lowest resting heart rate, and patients heterozygous for Ser49Gly (23.5%) were in between [Ranade, 2002].

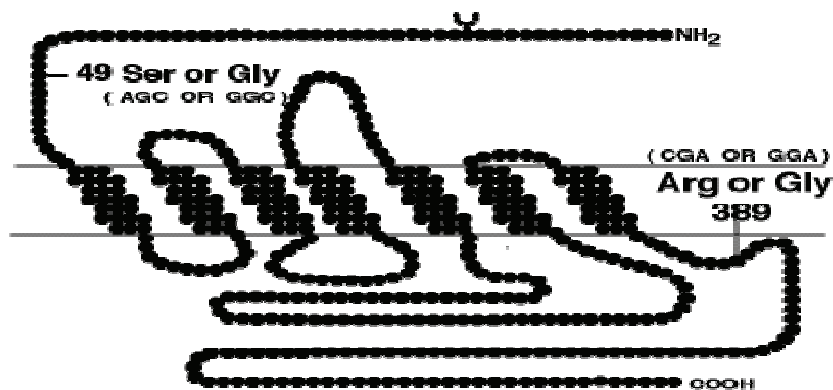


Figure 2. Human  $\beta_1$ -adrenergic receptor polymorphisms

## ***Cardiomyopathy***

The name cardiomyopathy comes from cardio = heart, myo = muscle and pathy = disease. In patients with cardiomyopathy, desensitization of the  $\beta$ -receptor pathway is observed due to the sustained increase in catecholamine stimulation. Catecholamine's induce positive inotropic and chronotropic response in the heart through the  $\beta$ -adrenoceptor. This sustained catecholamine stimulation results in a downregulation of the  $\beta$ -adrenoceptor density. In normal human myocardium the  $\beta_1$ :  $\beta_2$ -adrenoceptor ratio is approximately 75:25% and in rodents about the same. In failing myocardium, the percentage of  $\beta_1$ -adrenoceptors is reduced to about 60% and the  $\beta_2$ -adrenoceptors is proportionally increased to 40% [Bristow, 1986, Brodde, 2006].

The  $\alpha_1$ -adrenoceptor in the human myocardium is of relatively low density and the density is unchanged in the failing human heart [Bristow, 1988].

### **Idiopathic cardiomyopathy**

Idiopathic dilated cardiomyopathy is a heart disease of unknown origin and it is characterized by impaired systolic function and dilatation of one or both ventricles. The incidence is 5-8 cases per 100 000 per year. Idiopathic dilated cardiomyopathy is associated with high morbidity and poor prognosis and is the second most common reason for heart transplantation in Sweden.

About 30% of the idiopathic dilated cardiomyopathy patients have shown to have auto-antibodies against the  $\beta_1$ -adrenoceptor. The most immunogenic target on the  $\beta_1$ -adrenoceptor has been shown to be the second extracellular loop [Magnusson, 1994] and immunization with this part of the receptor has shown to induce dilated cardiomyopathy in rabbit [Matsui, 1999] and rat [Jahns, 2004].

### **Ischemic cardiomyopathy**

Ischemic heart disease is caused by arteriosclerosis in the coronary arteries which lead to imbalance between the myocardial blood flow and the metabolic demand of the myocardium. Ischemic cardiomyopathy is the most common type of cardiomyopathy, 50-75% of all heart failure is due to ischemia.



### ***Heart transplantation***

When the heart is transplanted it loses both its sympathetic and parasympathetic innervations, thus the transplanted denervated heart displays an exaggerated chronotropic response to exogenous catecholamines, probably due to the loss of the parasympathetic vagal tone [Gerber, 2001]. Consequently, they also display a higher resting heart rate. The total  $\beta$ -adrenoceptor density is unaltered in transplanted patients but some studies have shown that transplanted human heart exhibits an increase in the  $\beta_2$ -adrenoceptor density over time. With increasing time after transplantation Steinfath *et al* found that the  $\beta_1$ :  $\beta_2$ -adrenoceptor ratio was altered, with a decrease in  $\beta_1$ - and an increase in  $\beta_2$ -adrenoceptor densities from about 80:20 to 60:40 which is similar to that in the failing heart [Steinfath, 1992].

The intraventricular septum has an enhanced sympathetic innervation [Poston, 2004] and consequently septum is in greater jeopardy of damage during the autonomic cascade following brain death [Novitzky, 1986]. Further, this could be the reason that septum is especially liable to future injuries. Septal hypokinesis is the most common wall motion abnormality seen in donor hearts [Poston, 2004]. Compared with healthy subjects, patients with heart transplants have higher resting heart rate but reduced chronotropic reserve to endogenous sympathetic stimulation, causing a reduction of maximal exercise capacity [Ferretti, 2002, Hidalgo, 1989, Mandak, 1995]. Some reinnervation of the transplanted heart (anteriorly) has been shown to occur with time after transplantation. With no reinnervation seen at one year and about 20-25% reinnervation after five year [Bengel, 2001, Uberfuhr, 2000].

### ***Hypertension***

Hypertension results in  $\beta$ -adrenoceptor downregulation and Gi-protein (inhibitory G-protein) alterations, similarly to that in heart failure [Bohm, 1995, Castellano, 1997] and it is a well known cause of heart disease. Furthermore, in spontaneously hypertensive rats a relative decrease of  $\beta_1$ -adrenoceptor and an increase in  $\beta_2$ -adrenoceptor were observed [Girouard, 2003]. The risk of developing

heart failure in hypertensive compared with normotensive subjects has been shown to be 2-fold in men and 3-fold in women [Levy, 1996].

### *Diabetes*

There is a high frequency of heart failure accompanied by an increased mortality risk for patients with diabetes. Diabetic subjects have shown to have elevated levels of Gi-protein [Richardson, 2004] and an increased Gi-protein signalling is associated with heart failure [Neumann, 1988]. One experimental models of non-insulin depending diabetes is to inject a low dose of Streptozotocin after high fat diet [Zhang, 2003]. Streptozotocin-induced diabetic swine have an increase in the Gi:Gs-protein ratio [Roth, D. A., 1995] so although the  $\beta$ -adrenoceptor density is maintained, adenylyl cyclase is depressed in diabetics resulting in a depressed LV function as well as a depressed catecholamine responsiveness and cardiac performance.

## Aims

To study with low-dose DSE:

- ♥ if cardiac reserve could be used to predict, global and/or regional improvement after metoprolol treatment.
- ♥ cardiac reserve during concealed and progressing myocardial dysfunction and during ageing in rats.
- ♥ if cardiac reserve could predict future development of dilated cardiomyopathy in immunized ( $\beta_1$ AR EC<sub>II</sub>) mice.
- ♥ cardiac reserve in heart transplanted patients and if the  $\beta_1$ -adrenoceptor genotype has significance for the cardiac reserve.

## Material and methods

### *Ethic approval*

The human studies were carried out in accordance to the Declaration of Helsinki, with approval of the Ethic Committee at the Medical Faculty, Göteborg University, and informed written consent was obtained from all patients.

In all animal experiments the principle of laboratory animal care was followed and the studies have been carried out with approval of the regional Animal Ethic Committee at Göteborg University.

### *Patients*

#### **Study I.**

Twenty-nine patients, clinically stable, in NYHA class II-III and LVEF <50%, measured by equilibrium radionuclide angiography (ERNA), were randomised to metoprolol or placebo. During the first 6 months (the double-blind phase) 3 patients were withdrawn (ventricular fibrillation, skin reaction and patient request) and during open treatment 2 patients were withdrawn (increased heart failure and patient request) and 2 patients were missed due to technical problems. The remaining 22 patients (mean age 58 years, 5 females) were evaluated in this study and data from before and after 6 month treatment are shown. There were 16 patients with non-ischemic dilated cardiomyopathy and 6 patients with ischemic cardiomyopathy (post coronary bypass, 1; one-vessel disease, 2; two-vessel disease, 2; three-vessel disease, 1). The patients with ischemic cardiomyopathy were not suffering from angina pectoris, and had no sign of exercise-induced ST-segment depression.

#### **Study IV.**

The study was performed in 20 patients (mean age 48 years, 5 females). Maximal bicycle exercise test (ramp protocol) and echocardiography at rest and during low-dose DSE were performed 17 months (range 12 - 48 months) after heart transplantation. In 15 patients a central hemodynamic investigations was

performed within one week of the echocardiographic investigation. The origins of heart failure were; dilated cardiomyopathy in 12 patients, ischemic cardiomyopathy in 7 patients and hypertrophic cardiomyopathy in one patient. Exclusion criteria were coronary artery stenosis as confirmed by coronary angiography, other serious diseases potentially affecting the cardiac reserve and beta-blocker treatment. They were all on immunosuppressant treatment (9 tacrolimus, 11 cyclosporine).

The  $\beta_1$ -adrenoceptor polymorphism was determined by the allelic discrimination analysis and patients were divided into two groups: either homozygous for Ser49 (n=15) or with Gly49 in one or both alleles (Gly49; n=5).

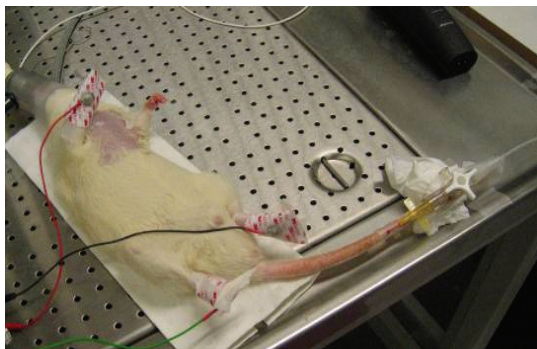
## *Rats*

### **Study II**

Forty-eight DSE examinations were performed in a total of 40 male rats (Charles River lab, Sulzfeld, Germany). Ten Whistar Kyoto (WKY) rats, age 16 weeks (WKY16); 10 WKY rats, age 26 weeks (WKY26); 10 WKY rats (26 weeks) with diabetes (WKY+D); and 10 spontaneously hypertensive (SHR) rats (26 weeks). For the study of repeatability, and inter- and intraobserver variability, 16 week old WKY rats were used (n=8).

The animals were acclimatized for at least 1 week and were housed under standard conditions. The 10 WKY rats, in which diabetes was induced, were first fed with a high saturated fat diet (MP Biomedicals Inc. Sweden, 4.39 kcal/g) for 8 weeks, followed by one intravenously (i.v) injection of 20 mg/kg streptozotocin (STZ) resulting in a blood glucose level of  $7.6 \pm 0.9$  mmol/L and became non-insulin depending animals.

All animals were weighed and lightly anesthetized with 1.6-2.7% isofluran (Abbot Scandinavia AB, Solna) [Kober, 2004, Roth, D. M., 2002] through a nose cone. The thorax was shaved, and the animal was placed in a slight left decubitus position on an electrical heating pad to maintain normothermia during the examination. A neoflon i.v cannula  $\varnothing$  0.7 mm was placed in the tail vein for the intravenous administration of dobutamine.



SHR originate from Okamoto in 1963 from outbred Wistar Kyoto rats. Bred from a male with mild hypertension, mated with a female with high blood pressure. Brother x sister mating with continued selection for high blood pressure. (Albino)

WKY originate from National Institutes of Health in 1971 from outbred Wistar stock from Kyoto School of Medicine. Inbred as a normotensive control strain for SHR. (Albino)

(An inbred strain is one that has been maintained by sibling (sister x brother) mating for 20 or more consecutive generations.)

## *Mice*

### Study III

Thirty-seven male BalbC mice (AgriSera, Sweden) were immunized with a synthetic peptide (H26R) corresponding to the human and mouse second extracellular loop on the  $\beta_1$ -adrenoceptor ( $\beta_1$ AR EC<sub>II</sub>). When the mice were 7 weeks old the immunization started by subcutaneous injection, of 0.2 mg H26R peptide, dissolved in 0.1M Na<sub>2</sub>CO<sub>3</sub>/1%  $\beta$ -mercaptoethanol and emulsified in Freund 's adjuvant, with a four week interval, for 14 or 25 weeks. Another 33 male BalbC mice were used as controls receiving vehicle in the same manner. Rest and DSE where performed in 12  $\beta_1$ AR EC<sub>II</sub> immunized (14 week old), 12 control (14 week old) and only rest echocardiography in 23  $\beta_1$ AR EC<sub>II</sub> immunized (25 week old) and 18 control mice (25 week old).

Thorax was shaved and the animal was placed in a slight left decubitus position on an electrical heating pad (38°C) to maintain normothermia. All

animals were anesthetized with 1.1% isofluran (Abbot Scandinavia AB, Solna, Sweden) via a nose cone during the echocardiographic examination.

BalbC mice originate from Dr MacDowell at the Carnegie Institution of Washington in Cold Spring Harbor, New York, who started inbreeding in 1920 with stock obtained from Dr Bagg at Memorial Hospital in New York who obtained albinos from a mouse dealer in Ohio in 1913.

## ***Methods***

### **Echocardiography**

*Study I.* The echocardiography investigations were performed on an Acuson XP 128 (Mountain View, CA, USA) and were recorded on VHS videotape. The evaluations were performed off-line on an Echo-Pac (Vingmed, Horten, Norway) system by one investigator who was blinded to patient data and time of investigation.

*Studies II - III* were performed on a HDI 5000 ultrasound system (ATL, Philip Medical System, Best). Using a high frequency 12-MHz phased array transducer (P12-5, Philip Medical System, Best) in the rat study, and a high frequency 15-MHz linear transducer (CL 15-7, Philip Medical System, Best) in the mice study. All animal data were evaluated off-line on an Echo-Pac (Vingmed, Horten, Norway) system by one investigator blinded to the animal's identity.

In *study IV*, an Acuson Sequoia 512 with a 3v2c phased array cardiac transducer (Mountain View, CA, USA) was used and the examinations were evaluated one-line on the Sequoia by one investigator blinded to patient  $\beta$ -receptor genotype.

All echocardiographic investigations were performed according to recommendations of the American Society of Echocardiography [Henry, 1980, Sahn, 1978, Schiller, 1991].

### **Dobutamine stress echocardiography**

In *study I* on dilated cardiomyopathy patients, dobutamine was infused i.v and the initial dose was 5  $\mu\text{g}/\text{kg}/\text{min}$  for 5 minutes, increased with 5  $\mu\text{g}/\text{kg}/\text{min}$  every 5

minute [Weissman, 1995] until the desired heart rate was achieved. The aim was to increase baseline heart rate by 20 bpm so that cardiac reserve could be studied without inducing myocardial ischemia.

*Study II* on rats, dobutamine was infused i.v via a neoflon in the tail vein at doses of 10 µg/kg/min and 20 µg/kg/min. Cardiac reserve was studied on each dose after at least 5 minutes dobutamine infusion.

In *study III* on mice, 1 µg dobutamine per gram of body weight was given intraperitoneally (i.p) and cardiac reserve was studied after 5 and 10 minutes.

*Study IV* on transplanted patients, an initial dose of 2.5 µg/kg/min was infused i.v with incrementing doses of 2.5 µg/kg/min until 7.5 µg/kg/min or until desired heart rate was achieved (same as in *study I*). Cardiac reserve was studied at each dose.

### **Doppler tissue imaging and estimated pulmonary capillary wedge pressure**

In *study IV*, myocardial velocities were measured by pulsed-wave Doppler tissue imaging (DTI) placing a 2.0 mm pulsed-wave sample volume at the junction of the mitral annulus in 4 basal sites: septum, lateral, anterior and inferior wall. Systolic velocities (Sv) together with early (Ev) and atrial (Av) diastolic velocities were measured from at least 3 cardiac cycles and averaged.

We compared the measured pulmonary capillary wedge pressure during heart catheterization with the estimated pulmonary capillary wedge pressure with Doppler tissue imaging, as proposed by Sundereswaran *et al*  $1.46 * (\text{early transmitral flow velocity (E)/ Ev lateral site}) + 2.6 = \text{estimated mean pulmonary capillary wedge pressure}$ , to investigate the effect of dobutamine in transplanted patient [Sundereswaran, 1998].

### **Validity and repeatability, intra- and intervariability**

Intra- and interobserver error (s) was calculated according to the formula  $s = \text{SD} / \sqrt{2}$ . The coefficient of variation (CV) describes the difference as a percentage of the pooled mean values ( $\bar{x}$ ) and was calculated according to the formula  $\text{CV}(\%) = s * 100 / \bar{x}$ .



In *study I* we found a good correlation ( $r=0.77$ ,  $p<0.001$ ) and acceptable agreement according to Bland-Altman between LVEF measured by ERNA and echocardiography, see Figure 3. In this study intraobserver variability for LVEF was shown and the coefficient of variation was 4.7% in these patients with a correlation of  $r=0.96$ ,  $p<0.001$ , see Figure 4.

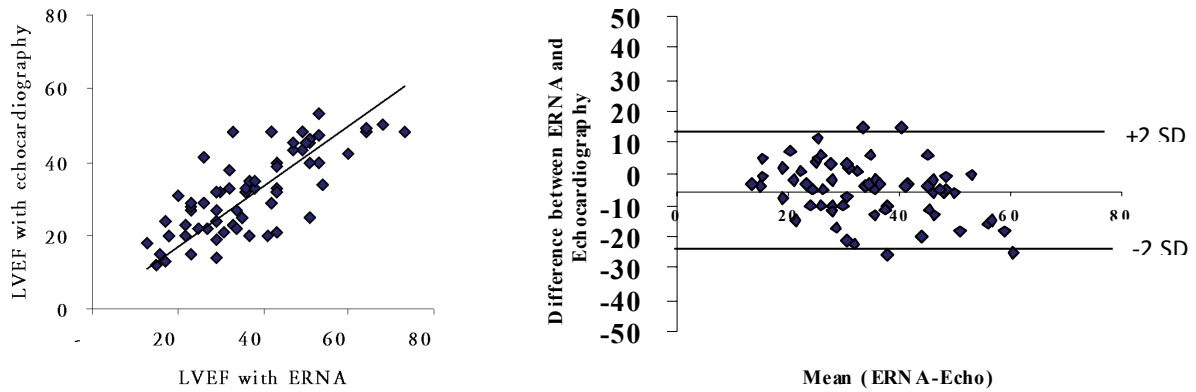


Figure 3. Correlation and agreement of left ventricular ejection fraction between radionuclear angiography (ERNA) and echocardiography.

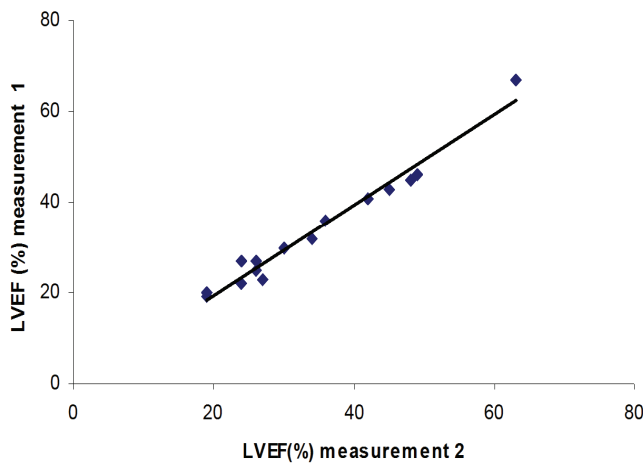


Figure 4. Intraobserver variation in left ventricular ejection fraction.

Data from the double-blind phase from the placebo group is not presented in *study I*. However, in the placebo group (n=9) the LVEF were not altered during the 6 month placebo period, rest echocardiography ( $26 \pm 6\%$  vs.  $26 \pm 11\%$ , ns) and DSE ( $32 \pm 9\%$  vs.  $37 \pm 9\%$ , ns).

In *study II*, we could show excellent intra- and interobserver variability, as well as repeatability data in rats (Table 1).

Table 1. Echocardiography data from rats

| Measurement                           | Repeatability             |                   | Intra       |                    | Inter       |                   |      |
|---------------------------------------|---------------------------|-------------------|-------------|--------------------|-------------|-------------------|------|
|                                       | 16w vs.17w                |                   | variability |                    | variability |                   |      |
|                                       | Difference                | CV (%)            | Difference  | CV (%)             | Difference  | CV (%)            |      |
| REST                                  | HR (bpm)                  | $3 \pm 21$        | 3.7         | $3 \pm 15$         | 1.4         | $4 \pm 15$        | 1.4  |
|                                       | PA VTI (cm <sup>2</sup> ) | $-0.08 \pm 0.66$  | 7.1         | $-0.07 \pm 0.91$   | 4.8         | $0.03 \pm 0.90$   | 4.8  |
|                                       | LVEDd (mm)                | $-0.09 \pm 0.16$  | 1.6         | $0.00 \pm 0.01$    | 0.7         | $0.01 \pm 0.01$   | 0.5  |
|                                       | LVESd (mm)                | $0.00 \pm 0.14$   | 3.4         | $0.01 \pm 0.03$    | 3.6         | $0.01 \pm 0.04$   | 5.1  |
|                                       | LVPWd (mm)                | $0.004 \pm 0.013$ | 6.9         | $-0.004 \pm 0.016$ | 4.1         | $0.005 \pm 0.013$ | 3.3  |
|                                       | FS (%)                    | $-0.6 \pm 2.6$    | 3.1         | $-0.4 \pm 4.5$     | 2.7         | $-1.2 \pm 5.9$    | 3.6  |
|                                       | Vcf c (circ/s)            | $0.13 \pm 0.28$   | 5.8         | $-0.17 \pm 0.38$   | 4.0         | $-0.18 \pm 0.49$  | 5.2  |
| 10 $\mu\text{g}/\text{kg}/\text{min}$ | HR (bpm)                  | $13 \pm 47$       | 8.7         | $1 \pm 5$          | 0.4         | $1 \pm 8$         | 0.8  |
|                                       | PA VTI (cm <sup>2</sup> ) | $0.22 \pm 0.57$   | 5.9         | $0.10 \pm 0.39$    | 2.1         | $0.01 \pm 0.35$   | 1.8  |
|                                       | LVEDd (mm)                | $-0.10 \pm 0.39$  | 4.1         | $0.00 \pm 0.03$    | 1.7         | $0.01 \pm 0.03$   | 1.7  |
|                                       | LVESd (mm)                | $-0.04 \pm 0.23$  | 7.0         | $-0.01 \pm 0.02$   | 2.5         | $-0.01 \pm 0.03$  | 3.8  |
|                                       | FS (%)                    | $-0.14 \pm 1.98$  | 2.1         | $0.85 \pm 3.05$    | 1.7         | $0.90 \pm 4.31$   | 2.3  |
|                                       | Vcf c (circ/s)            | $-0.38 \pm 0.32$  | 5.7         | $-0.05 \pm 0.35$   | 3.3         | $-0.02 \pm 0.33$  | 3.1  |
| 20 $\mu\text{g}/\text{kg}/\text{min}$ | HR (bpm)                  | $3 \pm 29$        | 4.9         | $-0 \pm 9$         | 0.8         | $-1 \pm 10$       | 0.8  |
|                                       | PA VTI (cm <sup>2</sup> ) | $-0.18 \pm 1.14$  | 10.4        | $0.03 \pm 0.55$    | 2.5         | $-0.15 \pm 0.67$  | 3.0  |
|                                       | LVEDd (mm)                | $-0.08 \pm 0.17$  | 1.8         | $-0.00 \pm 0.02$   | 1.1         | $0.01 \pm 0.02$   | 1.2  |
|                                       | LVESd (mm)                | $-0.09 \pm 0.40$  | 14.3        | $0.00 \pm 0.02$    | 15.8        | $-0.00 \pm 0.02$  | 12.0 |
|                                       | FS (%)                    | $1.4 \pm 1.3$     | 1.0         | $-0.48 \pm 3.10$   | 1.2         | $0.28 \pm 2.32$   | 0.9  |
|                                       | Vcf c (circ/s)            | $0.37 \pm 0.59$   | 7.0         | $-0.06 \pm 0.79$   | 4.9         | $-0.10 \pm 0.54$  | 3.3  |

Heart rate(HR), Pulmonary artery velocity time integral (PA VTI), Stroke volume (SV), Left ventricle end diastolic diameter (LVEDd), Left ventricle end systolic diameter (LVESd), Left ventricle posterior wall dimension (LVPWd), Fractional shortening (FS) and Velocity of the circumferential fiber shortening (Vcf c). Data is shown as differences  $\pm$  SD.

The repeatability data for heart failure patients, done in our laboratory are shown in Table 2, together with interobserver variability in a group of healthy controls, also shown in Table 2.

Table 2. Repeatability data from heart failure patients and variability data from healthy controls

| Measurement            | Repeatability in heart failure patients |       | Interobserver variability          |      |
|------------------------|---|-------|------------------------------------|------|
|                        | Difference                              | CV%   | Difference                         | CV%  |
|                        | mean days 35.5 ± 13.7<br>n=10           |       | in controls<br>n=6<br>(MST vs BGS) |      |
| LVEF (%)               | 0.44 ± 2.70                             | 4.22  | 1.34 ± 1.30                        | 1.47 |
| LVEDV (ml)             | 2.22 ± 16.51                            | 8.30  | 2.08 ± 8.35                        | 5.14 |
| LVESV (ml)             | 0.44 ± 11.31                            | 10.20 | 2.42 ± 3.45                        | 5.62 |
| E (m/sec)              | 0.00 ± 0.09                             | 10.52 | 0.00 ± 0.03                        | 2.60 |
| A (m/sec)              | -0.03 ± 0.10                            | 12.89 | -0.01 ± 0.02                       | 3.77 |
| VTI (cm <sup>2</sup> ) | -0.53 ± 2.64                            | 10.14 | 0.00 ± 0.01                        | 2.15 |
| Sv (cm/sec)            | 0.47 ± 1.07                             | 9.50  | 0.18 ± 0.20                        | 1.27 |
| Ev (cm/sec)            | 0.24 ± 1.08                             | 8.28  | 0.00 ± 0.39                        | 1.56 |
| Av (cm/sec)            | 0.61 ± 2.03                             | 14.66 | 0.24 ± 0.30                        | 2.57 |

Left ventricular ejection fraction (LVEF), Left ventricle end diastolic volume (LVEDV), Left ventricle end systolic volume (LVESV), Early filling velocity (E), Late filling velocity (A), Velocity time integral (VTI), Doppler tissue imagine: systolic (Sv) myocardial velocity, early (Ev) and atrial (Av) diastolic myocardial velocity. Margareta Scharin Täng (MST), Bente Grüner Sveälv (BGS) Data is shown as differences ± SD.

### Measurement precision

In the animal studies we have used a Phillips ATL HDI 5000 which has an axial resolution of approximately 0.05 mm (which means that two echoes closer than 0.05 mm cannot be separated). One image point (pixel) is approximately 0.05 mm. In each single measurement point, the maximal deviation from true value will be maximum half a pixel, 0.025 mm. However, measuring a distance between two echoes, a pair of points is marked. The maximal deviation from the true distance will therefore be 0.05 mm, and varies from the true value with a SD of

0.02 mm [Schmidt, 1999]. Phillips ATL HDI 5000 offers a good temporal resolution in M-mode with a frame rate of about 800 frames/s.

### *Statistic*

Data are presented as mean ( $\pm$  SD) in tables and text and as mean ( $\pm$  SEM) in figures. A p-value  $<0.05$  was considered statistically significant. Data were analyzed using SPSS 9-11 for Windows (Chicago, Ill, USA) and GrafPad 4 (San Diego, Ca, USA).

In the human studies (*I+IV*) the differences between groups were assessed by Mann-Whitney U test and in *study I* we also used Wilcoxon signed rank test, ANOVA (analysis of variance), Fischer's exact test and a multivariate logistic regression.

In the animal studies difference between groups and within groups was assessed by paired t-test and Student's t-test, or Mann-Whitney U test as appropriate. We have also used both one-way and two-way ANOVA.

For assessing correlation and agreement between two methods or repeatability (*Studies I, II and IV*), we used the approach suggested by Bland and Altman [Bland, 1986] and Spearman's rho for correlation in *study I+IV*.

## Results

### *Study I*

LVEF increased significantly after 6 months treatment with metoprolol both at rest ( $29 \pm 10\%$  vs.  $34 \pm 12\%$ ,  $p < .05$ ) and during DSE ( $33 \pm 11\%$  vs.  $42 \pm 12\%$ ,  $p < .01$ ). Cardiac reserve in the basal segments predicted improvement in global LV function best with sensitivity 89%, specificity 77%, and accuracy 82%,  $p < 0.01$  (Figure 5). Global and basal cardiac reserve was univariately predictive of LVEF improvement ( $p < 0.02$ ), only basal cardiac reserve was independently associated with recovery OR 1.07 [95% CI, 1.01-1.21],  $p = 0.02$  in a multivariate logistic regression analysis. Further, significant differences were observed in regional cardiac reserve between poor, moderate and good responders to metoprolol and the cardiac reserve of basal segments (Figure 6) were significantly better in good responders and negative in the poor responders.

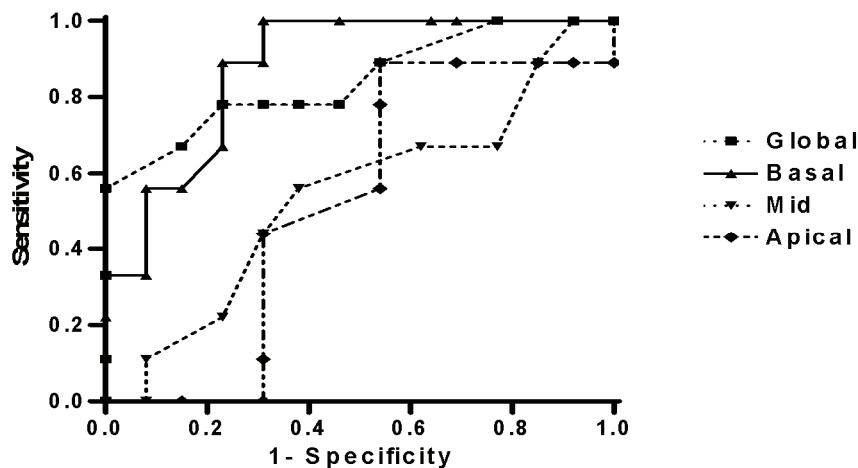


Figure 5. Different predictive models were compared by constructing receiver operating characteristic-area under curve (AUC). The capability to detect an improvement in global LVEF by  $>5\%$  during 6 months treatment with metoprolol is displayed. Assessment of cardiac reserve in basal segments (AUC 0.87) and global LV function (AUC 0.82) were best predictive of future improvement in LV function, the mid and apical segments displayed poorer values (AUC 0.54 and 0.52, respectively)

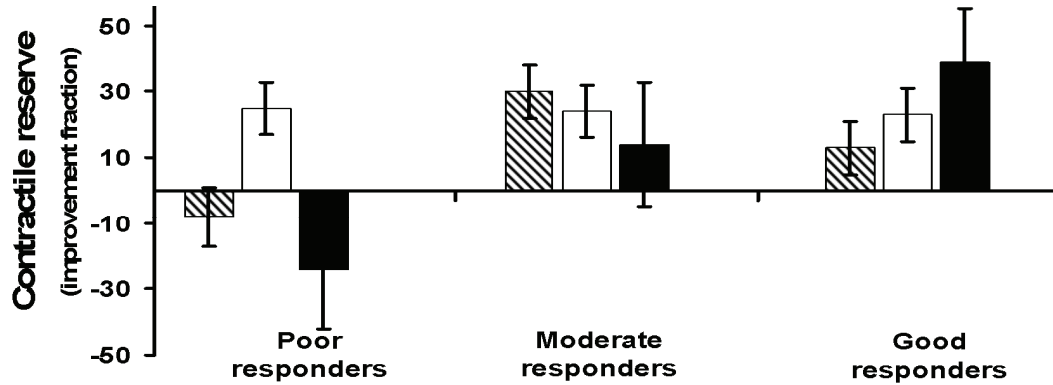
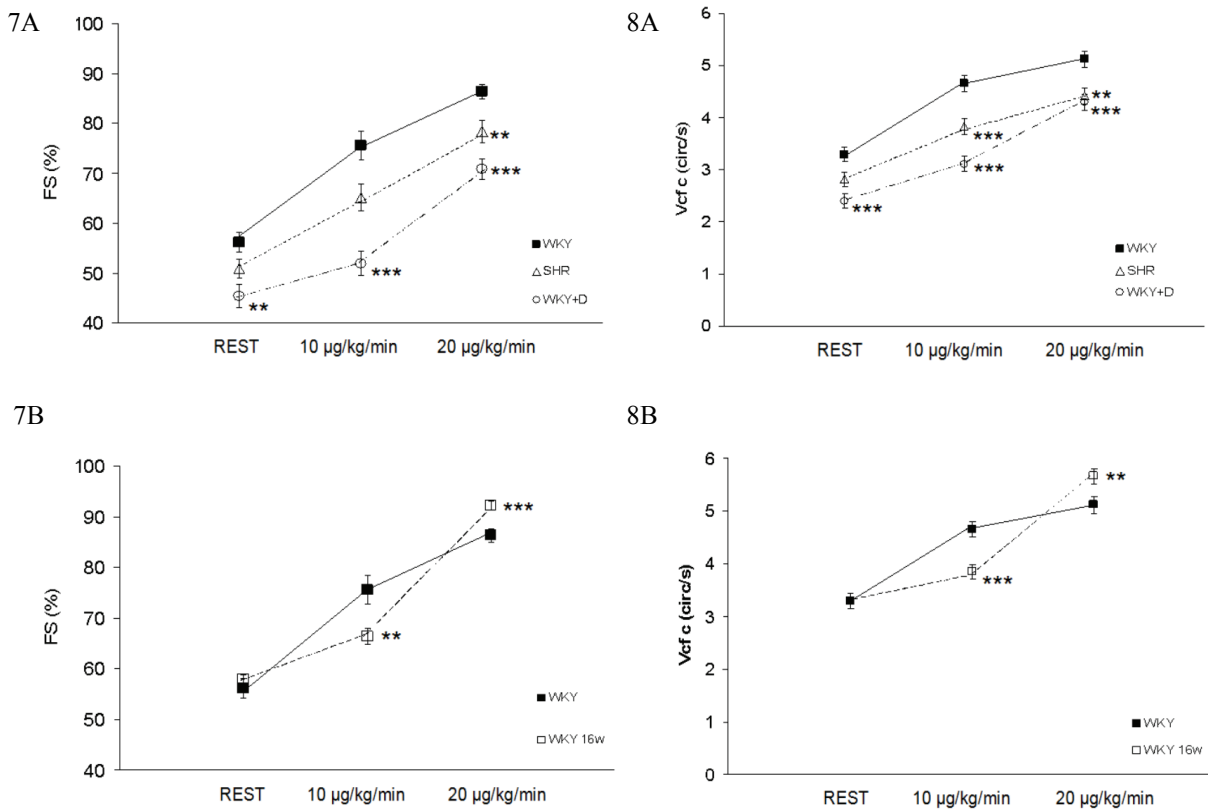


Figure 6. Cardiac reserve in different sections of the left ventricle at baseline, related to the response in global LVEF during long-term treatment. There was a significant difference among the three groups (poor, moderate and good responders) in contractile reserve of the basal segment ( $p < 0.05$ ), and in the apical segments ( $p < 0.01$ ). Data are given as fractional improvement of contractile reserve i.e. changes in amplitude/mean amplitude at baseline \* 100. Apical segments striped, mid segments white, and basal segments black.

## Study II

There were no differences in cardiac function at rest between younger and older WKY rats. At rest the hypertensive rats had lower velocity of circumferential fiber shortening ( $V_{cf\ c}$ ), compared to healthy age-matched controls (WKY26). All functional variables were impaired in diabetic rats, compared to WKY26.

Younger rats had significantly larger cardiac reserve during the second dose of DSE. Hypertensive rats showed decreased cardiac reserve and diabetic rats did not improve their cardiac reserve as much as age-matched WKY during the DSE (Figure 7 and 8).



**Figure 7 and 8.** Effect of dobutamine dose and disease (A) and age (B) on fraction shortening (7) and velocity of circumferential fiber shortening corrected for heart rate (8).

FS, Fractional shortening; SHR, spontaneously hypertensive rats;  $V_{cf\ c}$ , velocity of the circumferential fiber shortening corrected for HR; WKY16, Whistar Kyoto rats aged 16 weeks; WKY26, Whistar Kyoto rats aged 26 weeks; WKY+D, Whistar Kyoto rats with non-insulin depending diabetes. Values are mean  $\pm$  SEM

The repeatability study showed good agreement shown here with fractional shortening measurement (Figure 9) with good coefficients of variation at rest (<4%) and at both doses of dobutamine (<3%) in the WKY16 rats studied twice.

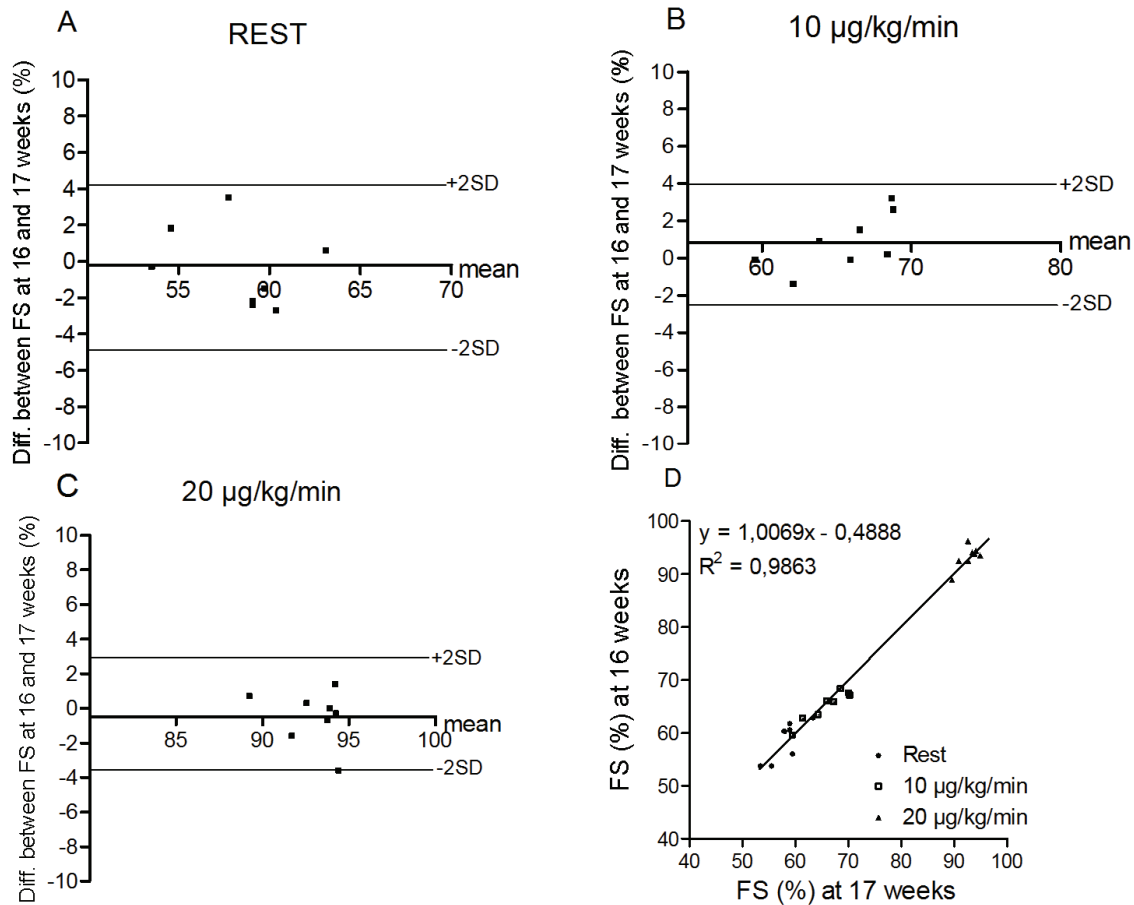


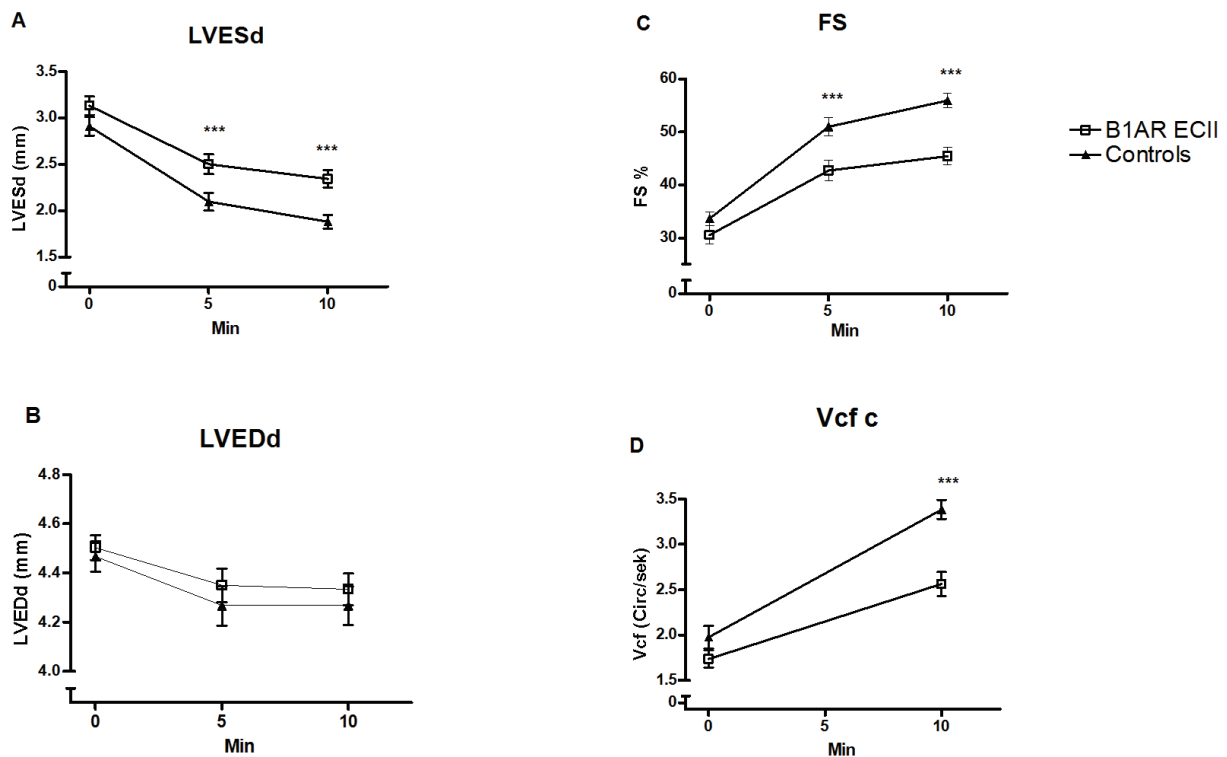
Figure 9. Repeatability of fractional shortening (FS) during DSE in rats at 16 and 17 weeks.

Bland-Altman plots at rest (A) and during 10  $\mu\text{g}/\text{kg}/\text{min}$  (B) and 20  $\mu\text{g}/\text{kg}/\text{min}$  (C) dobutamine. Correlation of fractional shortening at 16 weeks vs 17 weeks (D).



### Study III

When studying the mice at 14 weeks, no significant differences were seen between  $\beta_1$ AR EC<sub>II</sub> immunized mice and controls at rest. During DSE a significantly lower cardiac reserve was observed in the  $\beta_1$ AR EC<sub>II</sub> immunized mice at both time points studied (Figure 10). 25 weeks of immunization resulted in left ventricular dysfunction with a dilatation of the left ventricle, a decrease in fractional shortening and a thinner left ventricular posterior wall at rest in the  $\beta_1$ AR EC<sub>II</sub> immunized mice. The immunized animals also displayed increased levels of B-type natriuretic peptide ( $1.18 \pm 0.05$  vs.  $0.98 \pm 0.08$ ,  $p < 0.05$ ) and G-protein-coupled receptor kinase 2 ( $0.93 \pm 0.03$  vs.  $0.80 \pm 0.03$ ,  $p < 0.05$ ) in the heart tissue after 25 weeks of immunization.



**Figure 10. Cardiac reserve after 14 weeks of immunization**

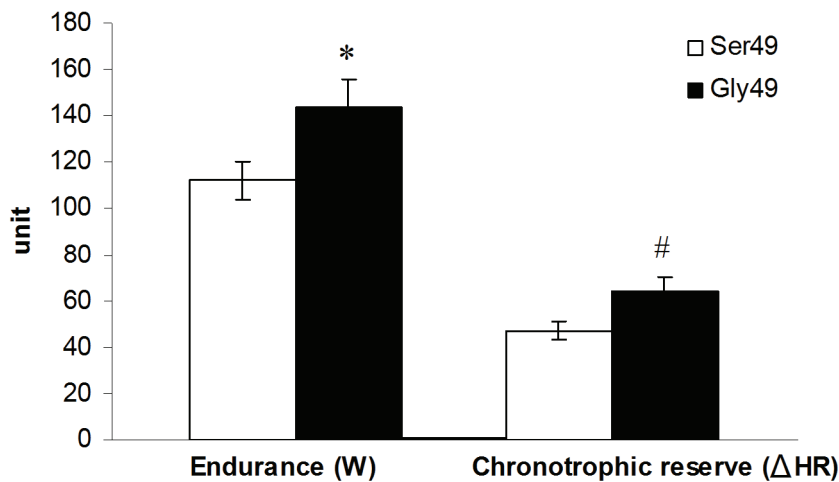
Left ventricular end systolic diameter (A). Left ventricular end diastolic diameter (B).

Fractional shortening (C). Velocity of circumferential fiber shortening(D).

$\beta_1$ AR EC<sub>II</sub> immunized mice vs. controls at baseline (0) and 5 and 10 min after dobutamine injection. Data is given as mean  $\pm$  SEM, \*\*\* $p < 0.001$  between groups.

### Study IV

Heart transplanted patients with grafts having the  $\beta_1$ -adrenoceptor Gly49 variants (n=5) had a lower resting heart rate ( $82 \pm 7$  vs.  $90 \pm 7$  bpm,  $p=0.04$ ), a better stress endurance and a trend towards a better chronotropic reserve than patients homozygous for Ser49 (n=15), see Figure 11. They also have better diastolic function shown as better lusitropic capacity in septum, see Table 3. There were no significant differences in LVEF between the two groups see Figure 12, with the exception of a decrease in cardiac reserve ( $\Delta$ LVEF) at the lowest dose of dobutamine in the patients with the Gly49 variants ( $-4.4 \pm 1.5$  vs.  $2.2 \pm 5.8$ ,  $p<0.05$ ).



**Figure 11. Stress bicycle endurance and chronotropic reserve**

*Gly49*, with Gly49 in one or both alleles; *Ser49*, homozygous for Ser49

Values are mean  $\pm$  SEM. \*  $p<0.05$ , #  $p<0.06$

Table 3. Pulsed wave Doppler tissue velocity at rest and during low-dose dobutamine infusion.

|                       | Septum   |          |      | Mean of 4 sites |          |      |
|-----------------------|----------|----------|------|-----------------|----------|------|
|                       | Gly49    | Ser49    | p    | Gly49           | Ser49    | p    |
| <b>Rest</b>           |          |          |      |                 |          |      |
| Sv (cm/s)             | 8.9±0.9  | 8.2±1.4  | ns   | 11.0±1.5        | 9.8±1.0  | 0.08 |
| Ev (cm/s)             | 14.5±3.2 | 10.4±2.0 | 0.03 | 16.4±4.1        | 13.0±1.8 | 0.07 |
| <b>2.5 µg/kg/min#</b> |          |          |      |                 |          |      |
| Sv (cm/s)             | 10.6±1.0 | 9.9±1.2  | ns   | 12.7±2.4        | 11.6±1.3 | ns   |
| Ev (cm/s)             | 15.0±3.7 | 10.9±2.5 | 0.04 | 17.6±4.7        | 13.5±2.6 | 0.08 |
| <b>ΔHR 20 bpm#</b>    |          |          |      |                 |          |      |
| Sv (cm/s)             | 11.4±2.9 | 12.8±3.1 | ns   | 15.1±2.3        | 14.4±2.3 | ns   |
| Ev (cm/s)             | 14.4±4.4 | 12.5±4.0 | ns   | 17.7±5.9        | 14.3±3.3 | ns   |

*Gly49*, with at least one Gly allele; *Ser49*, homozygous for Ser49

*bpm*, beats per minute; #, Dobutamine was infused at 2.5 µg/kg/min and at higher doses to induce an increase in heart rate of 20 bpm (ΔHR 20 bpm); *Ev*, early diastolic myocardial velocity; *Sv*, systolic myocardial velocity. Values are mean ± SD

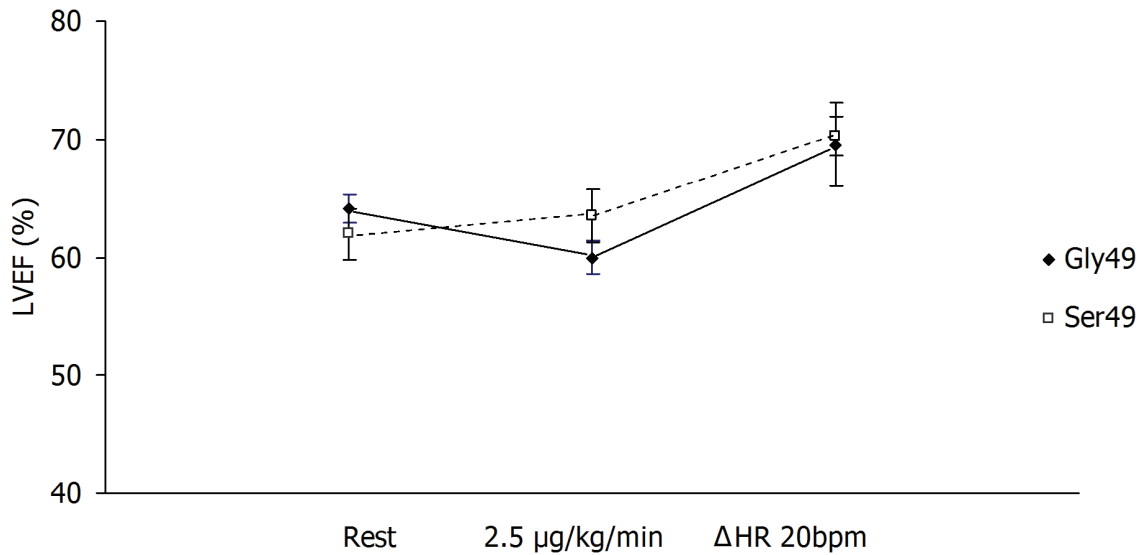


Figure 12. LVEF measured at rest and during low doses of dobutamine.

*Gly49*, with Gly49 in one or both alleles; *Ser49*, homozygous for Ser49; Dobutamine was infused at 2.5 µg/kg/min and at higher doses to induce an increase in heart rate of 20 bpm (ΔHR 20bpm). Values are mean ± SEM.

## Discussion

In this thesis we aimed to elucidate whether cardiac reserve, assessed by low-dose dobutamine stress echocardiography, can be used as a tool to evaluate and predict treatment effects, sub clinical heart disease and the influence of  $\beta_1$ -adrenoceptor gene polymorphism.

Investigation of cardiac function at rest cannot detect early and masked heart disease. The heart's capacity to adjust to the demands placed upon it can be the first and only sign of heart dysfunction. Therefore investigations under stress can be used to show these first alterations in heart function.

Heart failure is caused by diverse etiologies and is characterized by elevated levels of circulating catecholamines which affects the adrenoceptor-adenylyl cyclase - cascade in the heart. During the development of heart failure and hypertension there is an increased downregulation of the  $\beta_1$ -adrenoceptor density, while other diseases, like diabetes, mainly influence below the receptor level, by altering the Gi:Gs-protein ratio. All these alterations in the cascade result in an impaired signalling function and to a markedly blunted  $\beta_1$ -adrenoceptor-mediated contractile response.

Dobutamine acts by increasing synthesis of cAMP and thereby foremost depends on the  $\beta_1$ -adrenoceptor and the subsequent adenylyl cyclase - cascade. Function of this cascade mirrors the status of the heart. Low doses of dobutamine mainly increase the hearts contractility and dobutamine has some advantages compared with other drugs, like phosphodiesterase inhibitors i.e enoximone and milrinone that also affect the amount of cAMP in the myocytes. Clinically these drugs also mimic sympathetic stimulation and increase cardiac output but these drugs have the disadvantage of a long half-life, 2.5-10 hours, whereas dobutamine has a half-life of only 2 minutes. Furthermore, low doses of dobutamine rarely cause any undesirable side-effects and if any they are easily dissolved by stopping the infusion.

The cardiac reserve can be studied with different methods. However, we chose to study the cardiac reserve using low-dose DSE, as it has been shown to be a good predictor of prognosis and outcome in patients with heart failure [Paelinck,

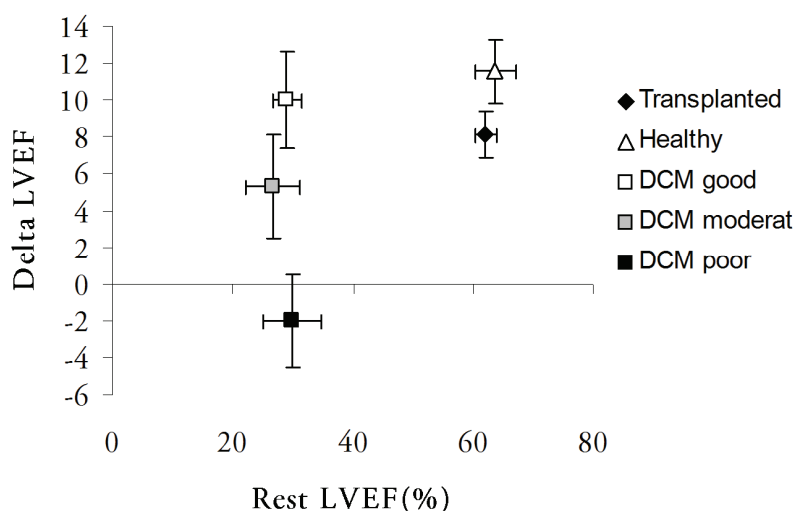
1999, *Scrutinio*, 2000]. *Study I* was a sub-study of a randomized, stratified, double-blind, placebo-controlled trial in mild to moderate heart failure [*Waagstein*, 2003].

Using radionuclide angiography *Waagstein et al* studied the cardiac reserve during steady state submaximal exercise. Treatment with metoprolol resulted in increased LVEF both at rest and during submaximal exercise, corresponding to what we reported studying the cardiac reserve with low-dose DSE, 7 units vs. 8 units compared to our 5 units vs. 9 units in  $\Delta$ LVEF.

Studying cardiac reserve with low-dose dobutamine using equilibrium radionuclide ventriculography can also be used in the same way as DSE to predict prognosis and outcome [*Ramahi*, 2001, *Ramahi*, 2001]. However, equilibrium radionuclide ventriculography is not a real-time examination, it requires average of the images over several minutes and the patient is exposed to nuclear radiation. Magnetic resonance imaging studies have shown to accurately determine segments with viability in the heart, in patients with ischemic cardiomyopathy [*Baer*, 1998, *Bree*, 2006]. Furthermore, low-dose dobutamine magnetic resonance imaging has shown to have higher sensitivity and specificity compared with echocardiography [*Saito*, 2000]. However, it is more time consuming, expensive and not as easily assessable when compared with echocardiography.

There is poor consensus in the literature of what a low-dose dobutamine test is and to find an easy and elucidative test would be of great importance. Cardiac function at rest provides modest or no information to detect early and silent myocardial dysfunction. A healthy individual has a good resting function and cardiac reserve. As disease develops, resting function is usually maintained, whereas cardiac reserve declines. During further disease development resting function also decreases. We chose to investigate the cardiac reserve in humans not at fixed doses of dobutamine but rather at the same level of “heart stress”,  $\Delta$ HR 20 bpm (see Figure 13) in all subjects studied. There might be limitations in the method we chose to use for studying the cardiac reserve, for example to use chronotropic response to study inotropic reserve assumes a parallel response to dobutamine both in the sinus node and in the myocyte, and this we cannot be certain of. However, to study the effect of low-dose dobutamine this way makes it possible to compare different types of patients and even compare the same patient before and after different therapies. So by using this unconventional low-dose stress test in patients with cardiomyopathy, we could show that these patients had

a cardiac reserve on beta-blocker treatment as well as before treatment. The cardiac reserve before beta-blocker treatment could also be used to discriminate between patients responding positively to treatment and patients not gaining any improvement in cardiac function with beta-blocker treatment. However, by using stress echocardiography we also observed that patients with poor response at baseline had a better cardiac reserve after metoprolol treatment.

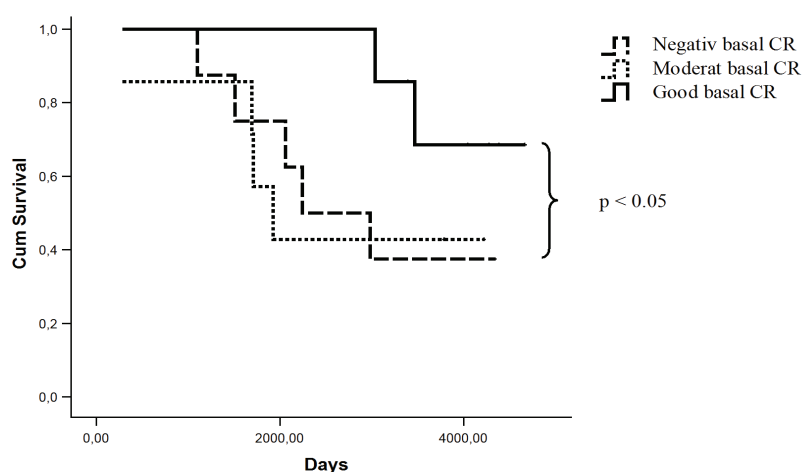


**Figure 13. Cardiac reserve studied at  $\Delta$ HR 20bpm during low-dose dobutamine infusion.**

Transplanted (n=20); Healthy controls (n=6); DCM, dilated cardiomyopathy patients with a good (n =8), moderat (n =7) and poor (n=7) response in global LVEF during long-term treatment with metoprolol.

The world has greatly benefited from different animal models of disease. Experimental studies have been performed allowing complex systems and processes to be investigated. DSE is used for a better understanding of myocardial behavior during stress and to clarify the mechanisms involved. In experimental studies, where the individual differences are small and the animals are genetically very similar, we used fixed doses of dobutamine to study the cardiac reserve. In rats, DSE has mainly been used to study ischemia and remodeling after infarction [Cove, 1995, Fiordaliso, 2005, Iwanaga, 2004]. However, our studies show that the cardiac reserve is easily studied with DSE. By using this non-invasive method the disease development can be studied repeatedly in the same animal.

Our results (*Study I*) suggest that both global cardiac reserve and cardiac reserve in basal segments can be used to predict improvement in global LV function. However, cardiac reserve in the basal segments seems to be of greater importance when patients with chronic heart failure are evaluated for drug treatment. Absence or negative cardiac reserve in basal segments was associated with negative or poor chances of improvement. The cardiac reserve is affected by a number of different factors like the percentage of fibrosis, the degree of apoptosis or necrosis and presence of auto-antibodies against the  $\beta_1$ -adrenoceptor in the heart. All these entities correlate with the prognosis of the patient and need to be further studied to determine if the cardiac reserve in the basal segments might be of importance for long-term outcome and survival. Well aware that this cohort (22 patients) is much too small to investigate long-term outcome, the ten-year survival in this cohort was significantly better in the patients with a good basal cardiac reserve compared with the patients that displayed a negative cardiac reserve in the basal segments of the heart, see Figure 14. I believe that low-dose dobutamine test has potential to be a good tool to find the patient group with the worst outcome. However, larger studies of cardiac reserve in the basal segments need to be performed. With improving echocardiography technology, for example with vector velocity imaging, the basal segments may be more easily accessible to study in the future.



**Figure 14.** Ten-year survival rate in patients, divided in relation to cardiac reserve in the basal segments before treatment with metoprolol. Negative basal cardiac reserve (n=7), moderate basal cardiac reserve (n=8) and good basal cardiac reserve (n=7).

The study in patients with heart transplants (*Study IV*) showed that patients with grafts with the Gly49 polymorphism on the  $\beta_1$ -adrenoceptor had lower heart rate, and better stress endurance and diastolic function compared with patients homozygous for Ser49. They also had a trend towards a better chronotropic reserve during the exercise test. Fast resting heart rate is associated with increased cardiovascular mortality [Kannel, 1987] so a polymorphism that contributes to a lower resting heart rate may be especially beneficial for heart transplanted patients, since heart transplanted patients display an elevated resting heart rate and a reduced chronotropic reserve compared with healthy controls [Mandak, 1995]. Studying the cardiac reserve in the heart transplanted patients revealed a decrease in cardiac reserve at the lowest dose of dobutamine in patients with grafts with the Gly49 variants. This might reflect that this  $\beta_1$ -adrenoceptor polymorphism has a greater responsiveness to circulating catecholamines. The results provide a possible explanation for differences in cardiac reserve among heart-transplanted patients.

Our experimental studies (*Studies II-III*) showed that low-dose DSE is a robust and reliable tool to investigate cardiac function in small animal models. In the study on  $\beta_1$ AR EC<sub>II</sub> immunized mice (*Study III*) we were able to demonstrate that early in the disease development no alterations were seen considering resting function and dimensions of the heart even though the cardiac reserve was decreased. Furthermore, with prolonged immunization the animals developed an eccentric dilated cardiomyopathy. In *study II* on rats, we could show that the cardiac reserve decreases with age and is impaired in hypertension and diabetes, diseases known to affect heart function. In addition, we have unpublished data showing a downregulation of the  $\beta_1$ -adrenoceptor density in the hypertensive rats which could be an explanation of the decreased cardiac reserve in these animals. However, the  $\beta_1$ -adrenoceptor density was not altered in the diabetic rat and this is in accordance with what others have reported [Roth, D. A., 1995]. In streptozotocin-induced diabetic swine, Roth *et al* found a maintained  $\beta$ -adrenoceptor density but an increase in the Gi:Gs-protein ratio which also leads to a decreased cAMP response to dobutamine stimulation.



### ***Clinical implications***

The response of the LV to low-dose dobutamine infusion adds valuable clinical prognostic information.

DSE has been shown to be an independent prognostic predictor of all-cause mortality and hard cardiac events in elderly patients [Biagini, 2005]. Furthermore, low-dose DSE has been shown to be able to predict clinical outcome both in idiopathic dilated cardiomyopathy and ischemic patients [Rambaldi, 2005, Scrutinio, 2000]. Low-dose DSE, not commonly used in infarct studies, has recently been shown to predict LV dilatation and provide prognostic information in patients with acute myocardial infarction [Norager, 2005] and a positive DSE is associated with improved clinical outcome and prognosis in patients with acute myocardial infarction treated with coronary angioplasty [Tomaszuk-Kazberuk, 2005].

Exercise electrocardiography is performed as a first-line non-invasive diagnostic stress test for evaluation of coronary artery disease. However, large numbers of patients referred for evaluation of chest pain are unable to perform adequate, exercise electrocardiographic testing. In these patients, DSE represents an exercise-independent stress alternative [Geleijnse, 1997]. Furthermore, considering the diagnostic problems of exercise electrocardiography and nuclear scintigraphy in women, DSE may be the stress test of choice because of its superior diagnostic specificity in women [Geleijnse, 2007].

### ***Conclusions***

We conclude that cardiac reserve can predict LV recovery in chronic heart failure patients during beta-blocker treatment and that  $\beta_1$ -adrenoceptor polymorphism (Ser49Gly) affects the cardiac reserve in humans. Furthermore, cardiac reserve can be used to evaluate and predict subclinical and clinical heart disease in experimental studies. A decreased cardiac reserve could be the first sign of heart disease development.

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## References

- Ahlquist R. A study of the adrenotropic receptors. *AM J Physiol* 1948; 143:586-600.
- Armstrong WF, Zoghbi WA. Stress echocardiography: current methodology and clinical applications. *J Am Coll Cardiol* 2005; 45:1739-1747.
- Baer FM, Theissen P, Schneider CA, Voth E, Sechtem U, Schicha H, Erdmann E. Dobutamine magnetic resonance imaging predicts contractile recovery of chronically dysfunctional myocardium after successful revascularization. *J Am Coll Cardiol* 1998; 31:1040-1048.
- Bengel FM, Ueberfuhr P, Schiepel N, Nekolla SG, Reichart B, Schwaiger M. Effect of sympathetic reinnervation on cardiac performance after heart transplantation. *N Engl J Med* 2001; 345:731-738.
- Biagini E, Elhendy A, Schinkel AF, Rizzello V, Bax JJ, Sozzi FB, Kertai MD, van Domburg RT, Krenning BJ, Branzi A, Rapezzi C, Simoons ML, Poldermans D. Long-term prediction of mortality in elderly persons by dobutamine stress echocardiography. *J Gerontol A Biol Sci Med Sci* 2005; 60:1333-1338.
- Biagini E, Schinkel AF, Rizzello V, van Domburg RT, Pedone C, Elhendy A, Krenning BJ, Bountiokos M, Vourvouri EC, Branzi A, Rapezzi C, Simoons ML, Bax JJ, Poldermans D. Prognostic stratification of patients with right bundle branch block using dobutamine stress echocardiography. *Am J Cardiol* 2004; 94:954-957.
- Bland M, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet* 1986; February 8:307-310.
- Bohm M, Kirchmayr R, Erdmann E. Myocardial Gi alpha-protein levels in patients with hypertensive cardiac hypertrophy, ischemic heart disease and cardiogenic shock. *Cardiovasc Res* 1995; 30:611-618.
- Borjesson M, Magnusson Y, Hjalmarsen A, Andersson B. A novel polymorphism in the gene coding for the beta(1)-adrenergic receptor associated with survival in patients with heart failure. *Eur Heart J* 2000; 21:1853-1858.
- Bountiokos M, Doorduijn JK, Roelandt JR, Vourvouri EC, Bax JJ, Schinkel AF, Kertai MD, Sonneveld P, Poldermans D. Repetitive dobutamine stress echocardiography for the prediction of anthracycline cardiotoxicity. *Eur J Echocardiogr* 2003; 4:300-305.
- Bree D, Wollmuth JR, Cupps BP, Krock MD, Howells A, Rogers J, Moazami N, Pasque MK. Low-dose dobutamine tissue-tagged magnetic resonance imaging with 3-dimensional strain analysis allows assessment of myocardial viability in patients with ischemic cardiomyopathy. *Circulation* 2006; 114:133-36.
- Bristow MR, Minobe W, Rasmussen R, Hershberger RE, Hoffman BB. Alpha-1 adrenergic receptors in the nonfailing and failing human heart. *J Pharmacol Exp Ther* 1988; 247:1039-1045.
- Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P, Jamieson S, et al. Beta 1- and beta 2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective beta 1-receptor down-regulation in heart failure. *Circ Res* 1986; 59:297-309.
- Brodde OE, Leineweber K. Autonomic receptor systems in the failing and aging human heart: similarities and differences. *Eur J Pharmacol* 2004; 500:167-176.
- Brodde OE, Bruck H, Leineweber K. Cardiac Adrenoceptors: Physiological and Pathophysiological Relevance. *J Pharmacol Sci* 2006.
- Castellano M, Bohm M. The cardiac beta-adrenoceptor-mediated signaling pathway and its alterations in hypertensive heart disease. *Hypertension* 1997; 29:715-722.

## References

- Castellano M, Beschi M, Rizzoni D, Paul M, Bohm M, Mantero G, Bettoni G, Porteri E, Albertini A, Agabiti-Rosei E. Gene expression of cardiac beta 1-adrenergic receptors during the development of hypertension in spontaneously hypertensive rats. *J Hypertens* 1993; 11:787-791.
- Chemla D, Coirault C, Hebert JL, Lecarpentier Y. Mechanics of Relaxation of the Human Heart. *News Physiol Sci* 2000; 15:78-83.
- Cheng YJ, Macera CA, Church TS, Blair SN. Heart rate reserve as a predictor of cardiovascular and all-cause mortality in men. *Med Sci Sports Exerc* 2002; 34:1873-1878.
- Cnota JF, Mays WA, Knecht SK, Kopser S, Michelfelder EC, Knilans TK, Claytor RP, Kimball TR. Cardiovascular physiology during supine cycle ergometry and dobutamine stress. *Med Sci Sports Exerc* 2003; 35:1503-1510.
- Cove CJ, Widman SC, Liang CS, Schenk EA, Hood WB, Jr. Dobutamine effects on systole and diastole in rats with myocardial infarction. *Am J Med Sci* 1995; 309:5-12.
- Dawson D, Lygate CA, Zhang MH, Hulbert K, Neubauer S, Casadei B. nNOS gene deletion exacerbates pathological left ventricular remodeling and functional deterioration after myocardial infarction. *Circulation* 2005; 112:3729-3737.
- De Wolf D, Foubert L, Van Belleghem Y, Mareels K, Matthys D, Verhaaren H, Van Nooten G. The influence of low afterload on the nature of the stress-velocity relationship. *J Am Coll Cardiol* 1999; 34:1219-1225.
- Eichhorn EJ, Grayburn PA, Mayer SA, St John Sutton M, Appleton C, Plehn J, Oh J, Greenberg B, DeMaria A, Frantz R, Krause-Steinrauf H. Myocardial contractile reserve by dobutamine stress echocardiography predicts improvement in ejection fraction with beta-blockade in patients with heart failure: the Beta-Blocker Evaluation of Survival Trial (BEST). *Circulation* 2003; 108:2336-2341.
- Elhendy A, Geleijnse ML, van Domburg RT, Nierop PR, Poldermans D, Bax JJ, TenCate FJ, Nosir YF, Ibrahim MM, Roelandt JR. Gender differences in the accuracy of dobutamine stress echocardiography for the diagnosis of coronary artery disease. *Am J Cardiol* 1997; 80:1414-1418.
- Farias CA, Rodriguez L, Garcia MJ, Sun JP, Klein AL, Thomas JD. Assessment of diastolic function by tissue Doppler echocardiography: comparison with standard transmitral and pulmonary venous flow. *J Am Soc Echocardiogr* 1999; 12:609-617.
- Ferretti G, Marconi C, Achilli G, Caspani E, Fiocchi R, Mamprin F, Gamba A, Ferrazzi P, Cerretelli P. The heart rate response to exercise and circulating catecholamines in heart transplant recipients. *Pflugers Arch* 2002; 443:370-376.
- Fiordaliso F, Chimenti S, Staszewsky L, Bai A, Carlo E, Cuccovillo I, Doni M, Mengozzi M, Tonelli R, Ghezzi P, Coleman T, Brines M, Cerami A, Latini R. A nonerythropoietic derivative of erythropoietin protects the myocardium from ischemia-reperfusion injury. *Proc Natl Acad Sci U S A* 2005; 102:2046-2051.
- Galiuto L, Ignone G, DeMaria AN. Contraction and relaxation velocities of the normal left ventricle using pulsed-wave tissue Doppler echocardiography. *Am J Cardiol* 1998; 81:609-614.
- Geleijnse ML, Fioretti PM, Roelandt JR. Methodology, feasibility, safety and diagnostic accuracy of dobutamine stress echocardiography. *J Am Coll Cardiol* 1997; 30:595-606.
- Geleijnse ML, Krenning BJ, Soliman OI, Nemes A, Galema TW, Ten Cate FJ. Dobutamine stress echocardiography for the detection of coronary artery disease in women. *Am J Cardiol* 2007; 99:714-717.
- Gerber BL, Bernard X, Melin JA, Delestinne T, Vanbutsele R, Goenen M, Vanoverschelde JL. Exaggerated chronotropic and energetic response to dobutamine after orthotopic cardiac transplantation. *J Heart Lung Transplant* 2001; 20:824-832.

## References

---

- Girouard H, Chulak C, LeJossec M, Lamontagne D, de Champlain J. Chronic antioxidant treatment improves sympathetic functions and beta-adrenergic pathway in the spontaneously hypertensive rats. *J Hypertens* 2003; 21:179-188.
- Guyton: Textbook of medical physiology, Eighth edn. Philadelphia: W.B. Saunders Company; 1991.
- Hees PS, Fleg JL, Mirza ZA, Ahmed S, Siu CO, Shapiro EP. Effects of normal aging on left ventricular lusitropic, inotropic, and chronotropic responses to dobutamine. *J Am Coll Cardiol* 2006; 47:1440-1447.
- Henry W, DeMaria A, Gramiak R. Report of the American Society of Echocardiography Committee on Nomenclature and Standards in Two-dimensional Echocardiography. *Circulation* 1980; 62:212-217.
- Hidalgo R, Alegria E, Castello R, Aparici M, Peteiro J, Saenz de Burugaga J, Martinez-Caro D. Stress testing in patients one year after orthotopic cardiac transplantation. *Angiology* 1989; 40:650-655.
- Iwanaga Y, Hoshijima M, Gu Y, Iwatate M, Dieterle T, Ikeda Y, Date MO, Chrast J, Matsuzaki M, Peterson KL, Chien KR, Ross J, Jr. Chronic phospholamban inhibition prevents progressive cardiac dysfunction and pathological remodeling after infarction in rats. *J Clin Invest* 2004; 113:727-736.
- Jahns R, Boivin V, Hein L, Triebel S, Angermann CE, Ertl G, Lohse MJ. Direct evidence for a beta 1-adrenergic receptor-directed autoimmune attack as a cause of idiopathic dilated cardiomyopathy. *J Clin Invest* 2004; 113:1419-1429.
- Jewitt D, Birkhead J, Mitchell A, Dollery C. Clinical cardiovascular pharmacology of dobutamine. A selective inotropic catecholamine. *Lancet* 1974; 2:363-367.
- Jourdain P, Funck F, Fulla Y, Hagege A, Bellorini M, Guillard N, Loiret J, Thebault B, Desnos M. Myocardial contractile reserve under low doses of dobutamine and improvement of left ventricular ejection fraction with treatment by carvedilol. *Eur J Heart Fail* 2002; 4:269-276.
- Kannel WB, Kannel C, Paffenbarger RS, Jr., Cupples LA. Heart rate and cardiovascular mortality: the Framingham Study. *Am Heart J* 1987; 113:1489-1494.
- Kitaoka H, Takata J, Yabe T, Hitomi N, Furuno T, Doi YL. Low dose dobutamine stress echocardiography predicts the improvement of left ventricular systolic function in dilated cardiomyopathy. *Heart* 1999; 81:523-527.
- Kober F, Iltis I, Cozzone PJ, Bernard M. Cine-MRI assessment of cardiac function in mice anesthetized with ketamine/xylazine and isoflurane. *Magma* 2004; 17:157-161.
- Kyriakides ZS, Kelesides K, Melanidis J, Kardaras F, Caralis DG, Voridis F. Systolic functional response of normal older and younger adult left ventricles to dobutamine. *Am J Cardiol* 1986; 58:816-819.
- Leier CV, Heban PT, Huss P, Bush CA, Lewis RP. Comparative systemic and regional hemodynamic effects of dopamine and dobutamine in patients with cardiomyopathic heart failure. *Circulation* 1978; 58:466-475.
- Levy D, Larson MG, Vasan RS, Kannel WB, Ho KK. The progression from hypertension to congestive heart failure. *Jama* 1996; 275:1557-1562.
- Magnusson Y, Wallukat G, Waagstein F, Hjalmarsen A, Hoebeke J. Autoimmunity in idiopathic dilated cardiomyopathy. Characterization of antibodies against the beta 1-adrenoceptor with positive chronotropic effect. *Circulation* 1994; 89:2760-2767.
- Magnusson Y, Levin MC, Eggertsen R, Nystrom E, Mobini R, Schaufelberger M, Andersson B. Ser49Gly of beta1-adrenergic receptor is associated with effective beta-blocker dose in dilated cardiomyopathy. *Clin Pharmacol Ther* 2005; 78:221-231.
- Mandak JS, Aaronson KD, Mancini DM. Serial assessment of exercise capacity after heart transplantation. *J Heart Lung Transplant* 1995; 14:468-478.

## References

---

- Marwick TH. Application of stress echocardiography to the evaluation of non-coronary heart disease. *Eur J Echocardiogr* 2000; 1:171-179.
- Matsui S, Fu ML, Hayase M, Katsuda S, Yamaguchi N, Teraoka K, Kurihara T, Takekoshi N. Active immunization of combined beta1-adrenoceptor and M2-muscarinic receptor peptides induces cardiac hypertrophy in rabbits. *J Card Fail* 1999; 5:246-254.
- Naqvi TZ, Goel RK, Forrester JS, Siegel RJ. Myocardial contractile reserve on dobutamine echocardiography predicts late spontaneous improvement in cardiac function in patients with recent onset idiopathic dilated cardiomyopathy. *J Am Coll Cardiol* 1999; 34:1537-1544.
- Neglia D, Parodi O, Gallopin M, Sambuceti G, Giorgetti A, Pratali L, Salvadori P, Michelassi C, Lunardi M, Pelosi G, et al. Myocardial blood flow response to pacing tachycardia and to dipyridamole infusion in patients with dilated cardiomyopathy without overt heart failure. A quantitative assessment by positron emission tomography. *Circulation* 1995; 92:796-804.
- Neglia D, Michelassi C, Trivieri MG, Sambuceti G, Giorgetti A, Pratali L, Gallopin M, Salvadori P, Sorace O, Carpeggiani C, Poddighe R, L'Abbate A, Parodi O. Prognostic role of myocardial blood flow impairment in idiopathic left ventricular dysfunction. *Circulation* 2002; 105:186-193.
- Neumann J, Schmitz W, Scholz H, von Meyerinck L, Doring V, Kalmar P. Increase in myocardial Gi-proteins in heart failure. *Lancet* 1988; 2:936-937.
- Norager B, Husic M, Moller JE, Pellikka PA, Appleton CP, Egstrup K. The Doppler myocardial performance index during low-dose dobutamine echocardiography predicts mortality and left ventricular dilation after a first acute myocardial infarction. *Am Heart J* 2005; 150:522-529.
- Novitzky D, Wicomb WN, Cooper DK, Rose AG, Reichart B. Prevention of myocardial injury during brain death by total cardiac sympathectomy in the Chacma baboon. *Ann Thorac Surg* 1986; 41:520-524.
- Paelinck B, Vermeersch P, Stockman D, Convens C, Vaerenberg M. Usefulness of low-dose dobutamine stress echocardiography in predicting recovery of poor left ventricular function in atrial fibrillation dilated cardiomyopathy. *Am J Cardiol* 1999; 83:1668-1671, A1667.
- Paraskevaidis IA, Adamopoulos S, Kremastinos DT. Dobutamine echocardiographic study in patients with nonischemic dilated cardiomyopathy and prognostically borderline values of peak exercise oxygen consumption: 18-month follow-up study. *J Am Coll Cardiol* 2001; 37:1685-1691.
- Poston RS, Griffith BP. Heart transplantation. *J Intensive Care Med* 2004; 19:3-12.
- Ramahi TM, Longo MD, Cadariu AR, Rohlf K, Slade M, Carolan S, Vallejo E, Wackers FJ. Dobutamine-induced augmentation of left ventricular ejection fraction predicts survival of heart failure patients with severe non-ischaemic cardiomyopathy. *Eur Heart J* 2001; 22:849-856.
- Ramahi TM, Longo MD, Cadariu AR, Rohlf K, Carolan SA, Engle KM, Samady H, Wackers FJ. Left ventricular inotropic reserve and right ventricular function predict increase of left ventricular ejection fraction after beta-blocker therapy in nonischemic cardiomyopathy. *J Am Coll Cardiol* 2001; 37:818-824.
- Rambaldi R, Bax JJ, Rizzello V, Biagini E, Valkema R, Roelandt JR, Poldermans D. Post-systolic shortening during dobutamine stress echocardiography predicts cardiac survival in patients with severe left ventricular dysfunction. *Coron Artery Dis* 2005; 16:141-145.
- Ranade K, Jorgenson E, Sheu WH, Pei D, Hsiung CA, Chiang FT, Chen YD, Pratt R, Olshen RA, Curb D, Cox DR, Botstein D, Risch N. A polymorphism in the beta1 adrenergic receptor is associated with resting heart rate. *Am J Hum Genet* 2002; 70:935-942.
- Richardson MD, Kilts JD, Kwatra MM. Increased expression of Gi-coupled muscarinic acetylcholine receptor and Gi in atrium of elderly diabetic subjects. *Diabetes* 2004; 53:2392-2396.



## References

- Roth DA, White CD, Hamilton CD, Hall JL, Stanley WC. Adrenergic desensitization in left ventricle from streptozotocin diabetic swine. *J Mol Cell Cardiol* 1995; 27:2315-2325.
- Roth DM, Swaney JS, Dalton ND, Gilpin EA, Ross J, Jr. Impact of anesthesia on cardiac function during echocardiography in mice. *Am J Physiol Heart Circ Physiol* 2002; 282:H2134-2140.
- Ruffolo RR, Jr. The pharmacology of dobutamine. *Am J Med Sci* 1987; 294:244-248.
- Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 1978; 58:1072-1083.
- Saito I, Watanabe S, Masuda Y. Detection of viable myocardium by dobutamine stress tagging magnetic resonance imaging with three-dimensional analysis by automatic trace method. *Jpn Circ J* 2000; 64:487-494.
- Salto-Tellez M, Yung Lim S, El-Oakley RM, Tang TP, ZA AL, Lim SK. Myocardial infarction in the C57BL/6J mouse: a quantifiable and highly reproducible experimental model. *Cardiovasc Pathol* 2004; 13:91-97.
- Savonen KP, Lakka TA, Laukkanen JA, Halonen PM, Rauramaa TH, Salonen JT, Rauramaa R. Heart rate response during exercise test and cardiovascular mortality in middle-aged men. *Eur Heart J* 2006; 27:582-588.
- Schiller NB. Two-Dimensional Echocardiographic Determination of Left Ventricular Volume, Systolic Function, and Mass. *Circulation* 1991; 84:I-280-I-287.
- Schmidt C, Wendelhag I. How can the variability in ultrasound measurement of intima-media thickness be reduced? Studies of interobserver variability in carotid and femoral arteries. *Clin Physiol* 1999; 19:45-55.
- Scrutinio D, Napoli V, Passantino A, Ricci A, Lagioia R, Rizzon P. Low-dose dobutamine responsiveness in idiopathic dilated cardiomyopathy: relation to exercise capacity and clinical outcome. *Eur Heart J* 2000; 21:927-934.
- Seghatol FF, Shah DJ, Diluzio S, Bello D, Johnson MR, Cotts WG, O'Donohue JA, Bonow RO, Gheorghiade M, Rigolin VH. Relation between contractile reserve and improvement in left ventricular function with beta-blocker therapy in patients with heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol* 2004; 93:854-859.
- Socialstyrelsen: 2007:*The national board of health and welfare*. Available from: [www.socialstyrelsen.se](http://www.socialstyrelsen.se)
- Sohn DW, Chai IH, Lee DJ, Kim HC, Kim HS, Oh BH, Lee MM, Park YB, Choi YS, Seo JD, Lee YW. Assessment of mitral annulus velocity by Doppler tissue imaging in the evaluation of left ventricular diastolic function. *J Am Coll Cardiol* 1997; 30:474-480.
- Steinfath M, von der Leyen H, Hecht A, Neumann KH, Schmitz W, Scholz H, Haverich A, Heublein B. Decrease in beta 1- and increase in beta 2-adrenoceptors in long-term follow-up after orthotopic cardiac transplantation. *J Mol Cell Cardiol* 1992; 24:1189-1198.
- Steinfath M, Chen YY, Lavicky J, Magnussen O, Nose M, Rosswag S, Schmitz W, Scholz H. Cardiac alpha 1-adrenoceptor densities in different mammalian species. *Br J Pharmacol* 1992; 107:185-188.
- Sundereswaran L, Nagueh SF, Vardan S, Middleton KJ, Zoghbi WA, Quinones MA, Torre-Amione G. Estimation of left and right ventricular filling pressures after heart transplantation by tissue Doppler imaging. *Am J Cardiol* 1998; 82:352-357.
- Teragaki M, Yanagi S, Toda I, Sakamoto K, Hirota K, Takeuchi K, Yoshikawa J. Coronary flow reserve correlates left ventricular diastolic dysfunction in patients with dilated cardiomyopathy. *Catheter Cardiovasc Interv* 2003; 58:43-50.
- Tesson F, Charron P, Peuchmaurd M, Nicaud V, Cambien F, Tiret L, Poirier O, Desnos M, Jullieres Y, Amouyel P, Roizes G, Dorent R, Schwartz K, Komajda M. Characterization of a unique genetic variant in the beta1-adrenoceptor gene and evaluation of its role in idiopathic dilated cardiomyopathy. CARDIGENE Group. *J Mol Cell Cardiol* 1999; 31:1025-1032.

## References

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- Tomaszuk-Kazberuk A, Musial WJ, Dobrzycki S, Korecki J. The prognostic significance of early dobutamine echocardiography in patients with acute myocardial infarction treated with primary coronary angioplasty. *Kardiol Pol* 2005; 63:613-619; discussion 620-611.
- Uberfuhr P, Ziegler S, Schwaiblmair M, Reichart B, Schwaiger M. Incomplete sympathetic reinnervation of the orthotopically transplanted human heart: observation up to 13 years after heart transplantation. *Eur J Cardiothorac Surg* 2000; 17:161-168.
- Waagstein F, Stromblad O, Andersson B, Bohm M, Darius M, Delius W, Goss F, Osterziel KJ, Sigmund M, Trenkwalder SP, Wahlqvist I. Increased exercise ejection fraction and reversed remodeling after long-term treatment with metoprolol in congestive heart failure: a randomized, stratified, double-blind, placebo-controlled trial in mild to moderate heart failure due to ischemic or idiopathic dilated cardiomyopathy. *Eur J Heart Fail* 2003; 5:679-691.
- Weissman NJ, Nidorf SM, Guerrero JL, Weyman AE, Picard MH. Optimal stage duration in dobutamine stress echocardiography. *J Am Coll Cardiol* 1995; 25:605-609.
- Vigna C, Russo A, De Rito V, Perna GP, Testa M, Lombardo A, Lanna P, Langialonga T, Salvatori MP, Fanelli R, Loperfido F. Regional wall motion analysis by dobutamine stress echocardiography to distinguish between ischemic and nonischemic dilated cardiomyopathy. *Am Heart J* 1996; 131:537-543.
- Zhang F, Ye C, Li G, Ding W, Zhou W, Zhu H, Chen G, Luo T, Guang M, Liu Y, Zhang D, Zheng S, Yang J, Gu Y, Xie X, Luo M. The rat model of type 2 diabetic mellitus and its glycometabolism characters. *Exp Anim* 2003; 52:401-407.

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*I am still confused, but on a higher level.*  
Enrico Fermi, Nobel Prize Laureate in Physics 1938

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