Chronic Treatment with Carvedilol Improves
Ca^{2+}-Dependent ATP-Consumption
in Triton X-Skinned Fiber Preparations of Human Myocardium


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Carvedilol reduces myofibrillar ATP-consumption

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Non-standard abbreviations

NF: non-failing

DCM: dilative cardiomyopathy

MET: metoprolol

CAR: carvedilol

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Abstract

Evidence is given that β-blocker treatment differentially influences gene expression and up-regulation of β1-adrenoceptors in human myocardium. Here, we investigate whether long-term treatment with carvedilol or metoprolol may functionally alter myofibrillar function in end-stage human heart failure. Investigations were performed in triton X (1%, 4°C, 20 hrs)-skinned fiber preparations of explanted hearts from patients undergoing heart transplantation due to idiopathic dilative cardiomyopathy. 5 patients were not on β-adrenoceptor blocker treatment (DCM_NBB), 5 patients received either carvedilol (DCM_CAR) or metoprolol (DCM_MET). Non-failing donor hearts (NF, n=5), which could not be transplanted due to technical reasons, were investigated for comparison. Ca²⁺-dependent tension development (DT) and acto-myosin ATPase activity (MYO) were measured and tension-dependent ATP consumption was calculated by the ratio of DT and MYO (‘tension cost’). In addition, we measured the phosphorylation of troponin I (TNI) by back phosphorylation. Maximal DT and TNI-phosphorylation were reduced, with myofibrillar Ca²⁺-sensitivity of DT and MYO as well as tension cost being increased in DCM_NBB compared to NF. Metoprolol treatment restored TNI-phosphorylation, decreased Ca²⁺ sensitivity of tension development and of myosin-ATPase activity but did not alter the tension-dependent ATP-consumption. Carvedilol-treatment improved maximal DT and significantly decreased tension-dependent ATP-consumption without altering myofibrillar Ca²⁺-sensitivity. TNI-dephosphorylation was increased in patients treated with carvedilol. In conclusion, chronic β-adrenoceptor blockade...
functionally alters myofibrillar function. The more economic cross-bridge cycling in patients under carvedilol treatment may provide an explanation for the efficacy of carvedilol in the treatment of chronic heart failure patients.
Introduction

The results of a recent clinical trial suggest that carvedilol may be superior to metoprolol in the treatment of heart failure patients (Poole-Wilson et al., 2003). This may be due to the fact that myocardial contractility is decreased less when carvedilol is used instead of metoprolol as shown by some clinical trials investigating smaller patient populations (Gilbert et al., 1996; Sanderson et al., 1999; Metra et al., 2000). The reasons for the minor cardiodepressant effects of carvedilol in comparison to metoprolol are unclear. It has been shown previously that treatment of heart failure patients with β-adrenoceptor blockers results in alterations of gene expression regarding myofibrillar proteins (Lowes et al., 2002). Thus, changes in the myofibrillar response to Ca^{2+} may be an explanation for the differences between the inotropic effects of β-adrenoceptor blockers. We have demonstrated previously that myofibrillar Ca^{2+} sensitivity of human myocardium is not altered acutely by carvedilol or metoprolol treatment under in vitro conditions (Bundkirchen et al., 2001), however, changes in myofibrillar Ca^{2+} responsiveness due to β-adrenoceptor blocker treatment may occur during chronic treatment. Therefore, we investigated Ca^{2+}-dependent tension (DT) and actomyosin-ATPase activity (MYO) in chemically skinned fiber preparations of left ventricular myocardium from patients who were on treatment with carvedilol (DCM_CAR) or metoprolol (DCM_MET). Left ventricular failing myocardium from patients that did not receive β-adrenoceptor blocker treatment (DCM_NBB) as well as non-failing left ventricular myocardium (NF) was studied in comparison.
Materials and Methods

Cardiac tissue

Failing left ventricular tissue was obtained during cardiac transplantation. Patients suffered from heart failure clinically classified as New York Heart Association class IV on the basis of clinical symptoms and signs as judged by the attending cardiologist shortly before operation. All patients gave written informed consent before surgery. Only male patients aged between 40-60 years were included for the present study. The treatment with carvedilol and metoprolol respectively had been administered for about 2 to 12 months. The patients’ characteristics are given in table 1. Drugs used for general anesthesia were flunitrazepam and pancuroniumbromide with isoflurane. Cardiac surgery was performed on cardiopulmonary bypass patients with cardioplegic arrest during hypothermia. The cardioplegic solution (a modified Bretschneider solution) contained (in mmol/L) NaCl 15, KCl 9, MgCl₂ 4, histidine 180, tryptophan 2, mannitol 30, and potassium dihydrogen oxoglutarate 1.

Nonfailing human myocardium was obtained from donor hearts, which were rejected for further transplantation due to technical reasons. The mean age of the donor group was 50.6±2.8 years. No cardiac catheterization had been performed in the organ donor group, but none of the donors had a history of heart disease and all had normal left ventricular function as measured by echocardiography. The study was approved by the local ethics committee.
Chemically skinned left ventricular fibers

Left ventricular muscle fibers were prepared with minor modifications as described previously (Brixius and Schwinger, 2000). Briefly, frozen cardiac tissue was slowly and carefully thawed and small fiber bundles (diameter <0.2 mm) were dissected and permeabilized at 4°C for 20 h in a solution containing 50% (v/v) glycerol, 1% Triton X, and (in mmol/L) Na3N 10, ATP 5, MgCl2 5, EGTA 4, 1,4-dithioerythritol 2, and imidazole 20 (pH 7.0). Afterwards, the fibers were stored in a similar solution but without Triton X at -20°C.

Immunocytochemistry and measurement of sarcomere length

Skinned fibers of human hearts, prepared as described above, were used for immunocytochemical labeling of the Z-lines by α-actinin staining. After three washes in 0.1 mM phosphate buffered saline buffer, the skinned fiber preparations were incubated in a 1:800 dilution of mouse anti rat α-actinin antibody for 1 h at room temperature, followed by treatment with a secondary biotinylated goat anti-mouse antibody (1:400) for 1 h and Cy3-labeled extravidin (1:600) for 1 h (Ji et al., 1999). Afterwards, the skinned fibers were washed with 0.1 M Tris-buffered saline and stored at -20°C until the sarcomeric length measurements.

The measurements of sarcomeric length were performed using a Zeiss Axiovert 135 fluorescence microscope (filter set 15 Zeiss; excitation BP 546/16, emission LP 590), a Sony three chip camera, and computer-assisted imaging software (Optimas 6.01). For investigation of the sarcomeric length, the skinned
fibers were fixed at slack position in relaxation solution. The distance of 10 to 15
actinin/Cy3-labeled Z-lines was measured at 10 different areas of each skinned
fiber using a 40 x Neofluar objective (Zeiss Oberkochen, Germany). The
sarcomeric length was calculated by dividing the measured distance by the
number of spaces between labeled Z-lines. The mean of sarcomeric length for
each skinned fiber was calculated from all investigated areas. The experiments
were performed as described previously (Brixius and Schwinger, 2000). Average
sarcomeric length was 1.95±0.04 µm.
Measurement of force and actomyosin ATPase activity

Force and actomyosin-ATPase activity were simultaneously measured as described before (Guth and Wojciechowski, 1986; Brixius and Schwinger, 2000; experimental setup, Scientific Instruments, Heidelberg, Germany). The actomyosin-ATPase activity was measured using a linked NADH-fluorescence assay. The relaxation solution contained (in mmol/L): imidazole 20, Na₂ATP 10, NaN₃ 5, EGTA 5, MgCl₂ 12.5, phospho(enol)-pyruvate 5, NADH 0.6, P₁,P₅-di(adenosine 5') pentaphosphate (myokinase inhibitor) 0.2, and cyclopiazonic acid 25, together with 100 units/ml pyruvate kinase and 125 units/ml lactate dehydrogenase. The contraction solution contained calcium EGTA (5 mmol/L) instead of EGTA. Both solutions were mixed by a gradient mixer so that Ca²⁺ was successively increased every 30s. Free Ca²⁺ concentration was determined by calculator programs designed for experiments in skinned muscle cells (Fabiato and Fabiato, 1979). Measurement of developed tension and myosin ATPase activity started 3 s after the solution was exchanged. Developed tension and myosin ATPase activity had reached a stable plateau at that time. By subtracting the basal ATPase activity obtained in the relaxation solution from the measured ATPase activity, the suprabasal ATP-splitting rate was obtained. The ratio of suprabasal ATPase activity and force in the steep part of the respective Ca²⁺-relation was assumed as a measure for the “tension cost”, since these parts reflect the Ca²⁺-dependent linear changes.
**Back phosphorylation**

Freeze-clamped skinned fibers (about 5-8 mg) were homogenized at 4°C with an Ultra Turrax T8 (Janke & Kunkel KG, IKA-Werke, Staufen i. Breisgau) for 3x20 sec, followed by 3 strokes of 30 sec with a glass-teflon-potter (B.Braun AG, Melsungen, Germany) in 3 times the volume of chilled preparation buffer containing (in mmol/L) histidine-HCl 5, dithiothreitol 0.2, NaF 25, EDTA 10, NaH₂PO₄ 50, and phenylmethylsulfonyl fluoride 0.1 (pH 7.4). The homogenates were stored at –80°C. For back phosphorylation 40 µg protein (finally verified by Bradford's assay (Bradford, 1976) were phosphorylated in a medium containing (in mmol/L) histidine-HCl 40, NaCl 100, MgCl₂ 10, NaF 15, EDTA 1, TritonX 1%, BSA100 µg, and 0.5 U/µl catalytic subunit of PKA in the presence of 50 µmol/l [γ-32P] ATP (pH 6.8). The reaction was stopped after 10 min with ice-cold stop solution containing 50 mmol/l H₃PO₄, 0.5 mmol/L ATP, and 15% trichloroacetic acid. After centrifugation (2000g, 20 min), the precipitate was directly processed for electrophoresis in sample buffer (50 mmol/L H₃PO₄, 5 mol/L EDTA, 1 % mercaptoethanol, 2% SDS, 10% glycerol, and a trace of bromphenol blue as tracking dye, pH 6.8 adjusted with Tris), boiled at 95 °C for 5 min and subjected to a 7.5 % urea/SDS-PAGE as previously described (Swank and Munkres, 1971). Gels were stained with Coomassie blue and destained with methanol/acetic acid/water (3:1:6; v/v). As molecular mass marker, the kaleidoscope stained marker (with 7 marker-proteins) from BioRad was used. The gels were exposed to x-ray films. Autoradiography using X-OMAT film (Kodak, New York, USA) and intensifying screens permitted the detection of 32P-
labelled proteins on the gels. Densitometric units of the signals were investigated by scanning the respective bands for troponin I of the whole autoradiogram. The band intensities were evaluated by densitometric scanning using a computerized imaging system. Previous measurements reveal evidence that the troponin I content is similar between non-failing and DCM (non-ischemic) failing hearts. Therefore, we assume that the protein content of our preparations reflects that of the myofibrillar proteins.

**Materials**

All chemicals were of analytical grade or the best grade commercially available. 30% acrylamide/bisacrylamide and kaleidoscope stained marker were from BioRad (Hercules, California, USA) and [γ-32P] ATP from ICN (Eschwege, Germany). All compounds were dissolved in twice distilled water. Applied agents did not change the pH of the medium.

**Statistics**

All values are means ± S.E.M. unless otherwise noted. One way ANOVA were used to test significance. $P$ -values of <0.05 were accepted as significant. pCa-force as well as pCa-actomyosin ATPase activity relationships were fitted by a modified Hill equation (Hill, 1910) as follows: $Y=Ca^{2+}H/([pCa_{50}]^H+[Ca^{2+}]^H)$, where $Y$ is the fractional force, or actomyosin-ATPase activity, $pCa_{50}$ is the $Ca^{2+}$-concentration giving half-maximal activation (inhibition), and $H$ is an index of cooperativity (Hill coefficient). The concentration needed for half-maximal $Ca^{2+}$
activation of tension development or myosin ATPase activity (EC₅₀ for Ca²⁺), all Hill-coefficients, and the tension cost (ratio of ATPase activity and tension development) were analyzed by GraphPad Prism (GraphPad, San Diego, CA).

Results

Non-failing versus failing myocardium in the absence of β-adrenoceptor blocker treatment

The present study investigated the influence of chronic β-adrenoceptor blocker treatment on myofibrillar function in human myocardium. Table 2 summarizes the results obtained in triton X-skinned fiber preparations by simultaneous measurements of Ca²⁺-dependent force and actomyosin-ATPase activity in human failing myocardium from patients who suffered from dilated cardiomyopathy and who had not been on β-adrenoceptor blocker treatment (DCM_NBB) in comparison to non-failing myocardium (NF).

In DCM_NBB, maximal Ca²⁺-dependent tension was significantly decreased as compared to NF. This was accompanied by a significant increase in Ca²⁺ sensitivity of tension and actomyosin ATPase activity in DCM_NBB compared to NF. There was no difference in Ca²⁺-activated actomyosin ATPase activity between the two groups.

Tension development after chronic treatment with carvedilol or metoprolol

Figure 1 summarizes the results obtained for Ca²⁺-dependent tension development in human non-failing and failing myocardium with and without β-
blocker treatment. The depression of maximal Ca^{2+}-dependent tension in DCM_NBB was not restored in patients chronically treated with metoprolol. However, metoprolol significantly shifted the Ca^{2+}-concentration/tension relationship to the right (EC_{50} Ca^{2+} tension DCM_MET: 0.60±0.03), i.e. metoprolol decreased the Ca^{2+}-sensitivity of human failing myocardium to values similar to that obtained in human non-failing hearts (tab. 2). In contrast to metoprolol, carvedilol significantly increased maximal Ca^{2+}-dependent tension (although the tension development was not fully restored as compared to human non-failing myocardium), without altering the Ca^{2+}-sensitivity of myofibrillar tension (EC_{50} Ca^{2+} tension DCM_CAR: 0.52±0.05). There were no significant alterations between the Hill-coefficients of the four groups (nHill tension NF: 2.24±0.06, DCM_NBB: 1.94±0.04, DCM_MET: 1.90±0.04, DCM_CAR: 2.06±0.04). This means that after chronic treatment with carvedilol, the Ca^{2+}-sensitivity of human failing myocardium is still significantly increased in failing as compared to non-failing hearts.

*Actomyosin ATPase activity*

In human heart failure, the ATP-turnover is a critical point for the pathophysiology of the disease, since the ATP-supply is hampered in the failing human myocardium. Both, carvedilol and metoprolol decreased the maximal Ca^{2+}-dependent actomyosin ATPase activity (fig. 2, left panel). In addition, metoprolol (EC_{50} Ca^{2+} ATPase DCM_MET: 0.54±0.03), but not carvedilol (EC_{50} Ca^{2+} tension DCM_CAR: 0.44±0.03) induced a rightward shift of the Ca^{2+}-sensitivity of the
actomyosin-ATPase activity (fig. 2, right panel, tab. 2). Hill-coefficients of the Ca²⁺-/actomyosin-ATPase activity were similar for all four groups (nHill ATPase NF: 1.33±0.06, DCM_NBB: 1.37±0.04, DCM_MET: 1.34±0.06, nHill:1.39±0.06, DCM_CAR: nHill: 1.40±0.04).

_Tension-dependent myofibrillar ATP-consumption and β-adrenoceptor blocker treatment_

The tension-dependent ATP-consumption was evaluated by the ratio of Ca²⁺-dependent tension and suprabasal actomyosin ATPase activity for the very steep part of the Ca²⁺-/tension, respectively actomyosin ATPase relationship. A linear line fit was done for all data points obtained in the different groups. Figure 3 shows the results. Metoprolol-treatment did not significantly alter the tension-dependent ATP-consumption in human failing myocardium. In contrast, carvedilol completely restored the increased tension-dependent ATP-consumption. The tension-dependent ATP consumption of Triton X-skinned fiber preparations obtained from human failing myocardium of patients who had undergone chronic treatment with carvedilol was similar to that of human nonfailing myocardium.

_Troponin I-phosphorylation_

It has been shown that the increased myofibrillar Ca²⁺-sensitivity of human failing myocardium may be due to alterations in the phosphorylation of contractile proteins, e.g. troponin I (van der Velden et al., 2003a; van der Velden et al., 2003b). Therefore, we investigated the phosphorylation status of troponin I using the back phosphorylation technique. Figure 4 presents the original blots as well
as the summarised data. Although the phosphorylation was varying within the different samples of one group, back-phosphorylation of troponin I was significantly increased in myocardium of heart failure patients who had not been treated with β-adrenoceptor blockers when compared to nonfailing myocardium, which indicates a higher phosphorylation status in nonfailing. While chronic treatment with metoprolol reversed this situation in DCM, the dephosphorylation of troponin I was further decreased in human failing myocardium in hearts of patients chronically treated with carvedilol.

**Discussion**

The present study investigated Ca\(^{2+}\)-dependent tension and myosin-ATPase activity in patients suffering from dilated cardiomyopathy who had been treated without a β-adrenoceptor blocker or who had received chronic treatment with carvedilol or metoprolol. Evidence is provided that β-adrenoceptor-treatment differentially influences myofibrillar function. Chronic treatment with metoprolol restores the increased Ca\(^{2+}\)-sensitivity of the myofilaments at least partly due to an increased phosphorylation of troponin I which may be the consequence of the β-adrenergic remodelling as has been described for metoprolol (Omerovic et al., 2003). Carvedilol did not alter myofibrillar Ca\(^{2+}\)-sensitivity but significantly increased maximal Ca\(^{2+}\)-dependent tension development by simultaneously decreasing the Ca\(^{2+}\)-dependent ATP-consumption. The more economic cross-bridge cycling in patients under carvedilol treatment may be one explanation for the efficacy of carvedilol in the treatment of chronic heart failure patients.
Alterations of myofibrillar function in human failing myocardium

In the present study it was shown that myofibrillar Ca$^{2+}$-sensitivity of myofibrillar function is significantly increased in heart failure patients without β-adrenoceptor blocker treatment. These findings are in agreement with previous results from our group (Brixius et al., 2002) and have also been confirmed in single cell preparations by different groups (Wolff et al., 1996; van der Velden et al., 2003b) indicating that the increased Ca$^{2+}$-sensitivity is a phenomenon which can be attributed to alterations on the cardiomyocyte itself and is not solely due to alterations of the extracellular matrix, which have been described in human heart failure (for review see Jane-Lise et al., 2000). However, the results of the present study are in contrast to a study on right ventricular myocardium of human failing hearts (Hajjar et al., 1992), indicating that regional differences exist in cardiac muscle regarding myofibrillar function. Only recently, regional differences have been described for human right atrial and left ventricular myocardium (Narolska et al., 2005). In addition, the present findings are in contrast to studies obtained in rat myocardium, in which a rightward shift of the Ca$^{2+}$-tension relation has been shown (Konhilas et al., 2002). However, the isoform composition of myosin differs between human and rat, especially under pathophysiological conditions. In rat myocardium, a reexpression of the alpha-myosin heavy chain may be the underlying reason for the rightward shift of the Ca$^{2+}$-tension relation (De Sousa et al., 1999).

Although the β-blockers metoprolol and carvedilol have been shown to be beneficial for heart failure patients and to significantly prolong the survival of
these patients to a similar extent (for review see Domanski et al., 2003), the pharmacodynamic profile of the two drugs is different. Thus, carvedilol but not metoprolol is a scavenger of radicals and thus unfolds antioxidant properties that may significantly contribute to its beneficial effects in heart failure (Arumanayagam et al., 2001; Flesch et al., 1999; Nakamura et al., 2002). As shown previously, chronic treatment with metoprolol reversed the hyperphosphorylation of the ryanodine receptor and restored the stoichiometry of the ryanodine receptor macromolecular complex (Reiken et al., 2003). The present study shows that metoprolol treatment also restores the functional integrity of the myofibrillar system, since metoprolol treatment improved the phosphorylation of troponin I and this was paralleled by a decrease in myofibrillar Ca$^{2+}$-sensitivity. Although these alterations may be advantages for the diastolic cardiac function and may prevent cardiac Ca$^{2+}$-overload, metoprolol did not alter the myofibrillar ATP-consumption and thus did not improve myofibrillar economy – at least under the in vitro system of skinned fiber preparations.

In contrast, carvedilol significantly reduced tension-dependent ATP-consumption. This may be of especial advantage in a situation of ATP-deprivation as has been described in failing myocardium (Hearse, 1979). In addition, carvedilol treatment increased maximal Ca$^{2+}$-dependent tension development. These effects of carvedilol may be the result of its antioxidant effects (Arumanayagam et al., 2001; Flesch et al., 1999; Nakamura et al., 2002). An alternative explanation may be an altered expression of myofibrillar proteins under chronic treatment with carvedilol. Thus, it has been shown in previous studies that carvedilol treatment
results in an up-regulation of the α-myosin heavy chain mRNA and a
downregulation of the β-myosin heavy chain mRNA (Lowes et al., 2002).
However, whether these alterations also occur on the protein level has to be
investigated in further studies. As shown in this study, carvedilol did not alter the
increased myofibrillar Ca²⁺-sensitivity and even increased the dephosphorylation
of the troponin I-protein. A very effective suppression of the β-adrenergic system
by carvedilol has been described previously and this β-adrenergic suppression
may be also present after the carvedilol treatment was stopped (Maack et al.,
2000).

Limitations of the present study
In contrast to our previous studies, we observed a significant decrease in
maximal Ca²⁺-activated force in human failing myocardium. One difference
between our present study and the previous studies is that for the present study
the myocardial samples had been frozen and were thawed for the preparation of
skinned fibers. The freezing and thawing treatment of the fibers may have altered
myofibrillar function. Nevertheless, functional data obtained in the present
samples are in the range of that obtained in fresh preparations (Schwinger et al.,
1994). In addition, by using this method, we were able to perform investigations
in a very clearly defined patient collective (all male, aged between 40-60 years).
The reason for the alterations in the maximal tension development by different
treatment of the fibers has to be investigated in further studies.
A further limitation of the study is the high variability inherent to studies in humans. However, to avoid this issue, we have selected patients from a group of over 200 heart transplantations with definite criteria. The groups, we investigated consisted only of male patients between the age of 40-60 years, who were on a definite pharmacological treatment (i.e. either no β-blocker, carvedilol or metoprolol). Thus, although the groups are very small they are very homogenous regarding the patients included.

In conclusion, a more detailed understanding on the molecular consequences of β-blockers may be of importance for a differential therapy of heart failure patients. Thus, in a situation of increased β-adrenergic suppression induced by carvedilol it may be contraindicated to treat the patients with Ca^{2+}-dependent positive inotropica, e.g. digitalis, because the risk for Ca^{2+}-overload of the cardiomyocytes may be increased. In addition, additional treatment with a Ca^{2+}-sensitizer like levosimendan may be unfavourable in addition to carvedilol, since the myofibrillar Ca^{2+}-sensitivity may be increased to such an extend that diastolic dysfunction may result. A recent clinical trial has demonstrated that levosimendan as well as carvedilol improve cardiac function. Thus, further studies are needed to more clearly define the pharmaco-molecular mechanisms underlying β-blocker treatment.
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Footnotes

The first 2 authors contributed equally to this work.

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Legends for Figures

Figure 1 left: Influence of DCM_NBB (n=15), DCM_MET (n=15) and DCM_CAR (n=15) on isometric tension development of skinned fiber preparations of human myocardium. *: p<0.05 vs. NF; # p<0.05 vs. DCM_NBB; right: Concentration-response curve for Ca$^{2+}$ on isometric tension development in skinned fiber preparations of human non-failing and failing myocardium with and without β-blocker treatment.

Figure 2 left: Influence of DCM_NBB (n=15), DCM_MET (n=15) and DCM_CAR (n=15) on maximum Ca$^{2+}$-dependent myosin ATPase activity of skinned fiber preparations of human myocardium. *: p<0.05 vs. NF; # p<0.05 vs. DCM_NBB; right: Concentration-response curve for Ca$^{2+}$ on the Ca$^{2+}$-sensitivity of the actomyosin-ATPase activity in skinned fiber preparations of human non-failing and failing myocardium with and without β-blocker treatment.

Figure 3: Influence of DCM_NBB, DCM_MET and DCM_CAR on tension cost of skinned fiber preparations of human myocardium at increasing Ca$^{2+}$ concentrations (0.01-32 µM free Ca$^{2+}$). The lines were obtained by a linear regression to all individual data points of each experimental group. Dashed lines to either side of the continuous lines indicate the confidence intervals.

Figure 4 left: Influence of NF, DCM_NBB, DCM_MET and DCM_CAR on troponin I phosphorylation status assessed via back-phosphorylation technique. Upper panel: Original gels from NF, DCM_NBB, DCM_MET and DCM_CAR. Lower panel: Densitometric analysis of the bands (*: p<0.05 vs. NF; # p<0.05 vs. DCM_NBB). Note that a low back phosphorylation signal indicates a high phosphorylation status.
### Table 1: Clinical characteristics of patients with DCM

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<td>11</td>
<td>CAR</td>
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<tr>
<td>12</td>
<td>CAR</td>
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</tr>
<tr>
<td>13</td>
<td>CAR</td>
<td>60</td>
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<td>25</td>
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<tr>
<td>14</td>
<td>CAR</td>
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<tr>
<td>15</td>
<td>CAR</td>
<td>53</td>
<td>19</td>
<td>18</td>
<td>2.6</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

EF: ejection fraction (%), LVDEP: left ventricular enddiastolic pressure (mmHg), CI: cardiac index (l/min · m⁻²), MET: metoprolol, CAR: carvedilol, NI: nitrates, Diu: diuretics, Gly: glycosides, ACE: angiotensin converting enzyme inhibitors, AT1: AT₁-antagonists
Table 2: Tension development, myosin-ATPase activity and tension cost in human non-failing and failing myocardium without β-blocker treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NF (n=5 men)</th>
<th>DCM_NBB (n=5 men)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tension</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum (mN/mm²)</td>
<td>21.4±1.9</td>
<td>13.7±1.5 *</td>
</tr>
<tr>
<td>EC₅₀ Ca²⁺ (µM)</td>
<td>0.71±0.07</td>
<td>0.43±0.04 *</td>
</tr>
<tr>
<td><strong>Actomyosin-ATPase activity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum (µM ADP/s)</td>
<td>40.2±2.2</td>
<td>45.4±1.6 *</td>
</tr>
<tr>
<td>EC₅₀ Ca²⁺ (µM)</td>
<td>0.64±0.04</td>
<td>0.38±0.02 *</td>
</tr>
<tr>
<td><strong>Tension-dep. ATP-consumption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α ((µM ADP<em>s⁻¹) / (mN</em>mm⁻²))</td>
<td>1.79±0.03</td>
<td>3.67±0.05 *</td>
</tr>
</tbody>
</table>

*: p<0.05 vs. NF

NF: non-failing, DCM_NBB: dilative cardiomyopathy patients without β-blocker treatment, α: slope of the myosin-ATPase/tension relationship, EC₅₀ Ca²⁺: Ca²⁺-concentration at which a 50% increase of tension or myosin-ATPase activity was achieved.
Fig. 1

HUMAN LEFT VENTRICULAR MYOCARDIUM
Skinned Fibers

Developed Tension

DT$_{\text{max}}$ (mN/mm$^2$)

DT$_{\text{max}}$(%)

NF  DCM NBB  DCM MET  DCM CAR

NF  DCM NBB  DCM MET  DCM CAR

pCa

8  7  6  5

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HUMAN LEFT VENTRICULAR MYOCARDIUM
Skinned Fibers

Myosin-ATPase Activity

maximum ATPase
(µmol/L ADP/s)

0 20 40 60 80
NF DCM NBB DCM MET DCM CAR

ATPase (% maximum)
0 20 40 60 80 100

pCa
8 7 6 5
NF DCM_NBB DCM_MET DCM_CAR

Fig. 2

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Fig. 3

Myosin-ATPase Activity
(µmol/L ADP/s)

Tension Cost

DT (mN/mm²)

- NF
- DCM_NBB
- DCM_MET
- DCM_CAR
**Fig. 4**

**HUMAN LEFT VENTRICULAR MYOCARDIUM**

*Troponin I-phosphorylation*

![Image of densitometry results showing phosphorylation levels for different conditions: NF, DCM_NBB, DCM_MET, DCM_CAR.](Image)

**Graph:**

- **X-axis:** NF, DCM_NBB, DCM_MET, DCM_CAR
- **Y-axis:** Densitometry units/µg Protein

- **Legend:**
  - *: Significant difference from NF
  - #: Significant difference from DCM_NBB

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