Reverse remodeling following insertion of left ventricular assist devices (LVAD): A review of the morphological and molecular changes

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Abstract

Left ventricular assist devices (LVAD) are used to “bridge” patients with end-stage heart failure until transplantation of a donor heart can be performed (“bridge to transplantation”). However, in a subset of patients, support by LVAD sporadically results in improved cardiac function, with heart transplantation no longer necessary even after removal of the LVAD (“bridge to recovery”). Also, LVAD appears to be an optional treatment alternative to heart transplantation in patients with contraindications for organ replacement (“destination therapy”). The processes resulting in these effects have descriptively been termed “reverse remodeling”. Although the molecular mechanisms are incompletely understood at present, there are several aspects of the reverse remodeling process that have been identified in the past. Alterations of many molecular pathways are involved in the development of chronic heart failure. Some of these appear to be reversible and have been shown to be regulated by LVAD treatment.

LVAD lead to lowered cardiac pressure and volume overload in the myocardium followed by decreased ventricular wall tension, reduced cardiomyocyte hypertrophy, improved coronary perfusion and decreased chronic ischemia. Improved coronary flow and myocardial perfusion as well as decreased ventricular wall tension may possibly alter the molecular systems involved in the development of chronic cardiac insufficiency.

Aside from describing the morphological changes, this review focuses on the roles of signal transduction, transcriptional regulation, apoptosis, stress proteins, matrix remodeling, and neurohormonal signaling in the failing human heart before and after mechanical circulatory support.

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1. Introduction

Morbidity and mortality due to end-stage myocardial failure constitute a major medical problem and pose considerable socio-economic problems in industrialized nations. Besides medical treatment, at present cardiac transplantation is the only curative way to treat terminal heart failure. Almost 63,000 heart transplantsations have been performed worldwide, but the number of new transplants is declining, wherein the main limitation is the shortage of donor organs [1]. The long waiting period until transplantation, the need for intervention in cases of acute cardiac failure and the treatment of patients not amenable to cardiac transplantation have led to the development of left...
ventricular assist devices (LVAD). Improved survival rates of patients treated with LVAD until transplantation compared to conservative medical approaches could be demonstrated. Consequently, LVAD were approved as treatment for “bridging” patients until transplantation [2].

LVAD consist of electrically powered pumps, which can be installed either extracorporally or intrathoracically. These pumps or turbines are switched in parallel to the normal circulation, i.e. blood is transported from the left ventricle to the ascending aorta.

Today, LVAD are used to “bridge” patients until transplantation (“bridge to transplantation”) or to restore basic cardiac function without subsequent transplantation (“bridge to recovery”). It is optionally used for patients with contraindications for transplantation (“destination therapy”) [3].

The perioperative mortality and morbidity of LVAD insertion remain high, with patient survival rates ranging from 60% to 75% until transplantation or device removal [4]. Patient-related risk factors for poor survival include age, sepsis and renal/hepatic dysfunction. Device-related problems encompass production of HLA antibodies, infection and device failure [4].

A case of hypersensitivity myocarditis which resolved due to LVAD support was reported [5].

LVAD no longer increase the risk of transplantation itself, according to the ISHLT database [1] and other studies [4]. LVAD support does not influence the outcome of transplantation with regard to survival rate and acute rejection episodes [6].

Patients treated with LVAD have a significant improvement in quality of life (QOL) and functional status compared to their medical counterparts [3].

LVAD provide profound volume and pressure unloading of the left ventricle and restore systemic blood pressure and flow to near normal levels. This leads to a normalization of the neurohormonal and local cytokine milieu contributing to myocardial recovery [7]. Several reports suggest a recovery of the native ventricular function after long-term mechanical support with reversal of chronic ventricular dilation [2,8].

Thompson and co-workers found that brain natriuretic peptide (BNP) and endothelin-1 (ET-1) are associated with improved ejection fraction and cardiac output as well as decreased end-diastolic diameter following LVAD [9].

The molecular processes of improved cardiac performance after LVAD have been descriptively termed “reverse remodeling”. In a subset of patients, LVAD support reverses the complex processes of chronic left ventricular remodeling to the point where these patients could be weaned from the device with restored basic cardiac function. The proportion of these patients has been reported to be approximately 5% [10].

In the following, the latest results on morphological changes and important molecular mechanisms of “reverse remodeling” due to mechanical support are reviewed, including results from our group.

2. Chronic heart failure: “remodeling” versus “reverse remodeling”

The pathogenesis of chronic heart failure (CHF) is a complex step-wise process involving morphological and molecular alterations of the myocardium (“remodeling”). At the onset of cardiac insufficiency, a given factor, as diverse as myocardial infarction, myocarditis or genetic defect, is believed to have initiated this process. This event is combined with the compensatory attempt of the body to counteract by pathobiological mechanisms.

After LVAD, native cardiac function can recover in single patients and removal of the device is possible without subsequent transplantation (“reverse remodeling”).

2.1. Morphological changes of the myocardium in CHF

Hearts with CHF very often show organ enlargement (dilation) and increased ventricular wall thickness. Dilation is followed by increased ventricular wall stress resulting in decreased coronary blood flow and impaired pump function reflected by diminished cardiac output [11].

Histologically, compensatory enlargement (hypertrophy) of cardiomyocytes consisting of increased cell volume, length, diameter and thickness is observed. Furthermore, changes in the amount of interstitial connective tissue (fibrosis) is noted [12].

2.2. Morphological changes of the myocardium in CHF after LVAD support

Echocardiographic investigations of hearts after LVAD showed decreased left ventricular diameter and increased left ventricular wall thickness [13]. Reduction of organ size was noted radiologically [14].

Among others [15,16], we have shown a significant decrease of cardiomyocyte diameter by morphometric examination after LVAD (Fig. 1A and C). This difference, however, was only significant in subendocardial myocytes (Fig. 1B) [17].

Zafeiridis and co-workers compared samples from patients with CHF to controls by measuring cell volume, length and diameter and described an increase of 50%, 48% and 20% of these parameters, respectively. LVAD treatment (average duration 75±5 days) resulted in a significant decrease of 28% of the cell volume, 20% of length and diameter and 36% of the profile area of cardiomyocytes. The major changes occur in cardiomyocyte length, suggesting that LVAD support has its greatest impact on the cell dimension that is most distorted in CHF [15].

Whether there is reduction of interstitial fibrosis is a matter of intense debate. Müller and co-workers report a striking reduction of fibrosis to near-normal levels [18].

Bruckner and co-workers found a significant reduction of total collagen content in 9 out of 18 patients with improvement of ejection fraction. Patients who had less fibrosis and
myocyte hypertrophy before LVAD insertion showed the biggest improvement of ejection fraction. The authors propose that tissue profiling with regard to fibrosis and myocyte size can predict myocardial improvement during LVAD support [19].

Another study demonstrated a reduction of total collagen content and significantly decreased TNFα levels after LVAD support [20].

In contrast, Taketani and co-workers found increased interstitial fibrosis after LVAD support [21]. Another study reports a significant increase in fibrosis and slight increase in myocyte diameter [22].

Our own data did not show a significant reduction of either interstitial or perivascular fibrosis after LVAD support [23].

It is important to note that, in the studies cited, the amount of fibrosis was determined by computerized semi-quantitative analysis of Sirius red stained sections yielding density data such as sectional profile areas per unit area or 3D quantities per unit volume.

Caution is usually advised in order to derive meaningful biological conclusions from relative values or ratios. It should be kept in mind that, when using ratios, no significant changes of the structures of interest can be detected when both the latter and the reference structure show equivalent changes, i.e. when changes of both structures are correlated. This problem, called “reference trap”, is discussed in detail elsewhere [24].

The ratio of the volume of the structure of interest to the volume of the reference structure (organ) can mask differences which are seen when using absolute values. For a suitable normalization procedure, an independent reference variable has therefore to be introduced. However, in the present case, normalization to an unchanged reference volume is not feasible, as the only reference volume (i.e. heart) changes. Thus, one approach to circumvent the reference trap would be to estimate the volume of the heart based on MRI images and extrapolate the volume fraction of the fibrotic tissue (estimated on histological sections) to the absolute volume of fibrosis, assuming that the extent of fibrosis is homogenous throughout the heart.

Importantly, in many studies a significant reduction of heart volume and cardiac hypertrophy following mechanical support was demonstrated by both imaging and histomorphometric investigations, indicative of a smaller reference (total) volume.

Fig. 1. A: Effects of LVAD support concerning the cardiomyocyte diameter. Data are expressed in box plots. Circles indicate outliers [17]. B: Effects of LVAD support concerning the cardiomyocyte diameter with regard to the distribution in different regions. Data are expressed in box plots. Circles indicate outliers [17]. C: Histomorphology of cardiomyocytes before (left) and following LVAD (right). Note the decrease in diameter (magnification ×400; PAS-stain).
Thus, it can be concluded that, while the ratio or volume fraction of fibrotic tissue is unchanged between the two groups with a decreased reference volume, the absolute volume of fibrosis is also reduced. It is of note that the “reference trap” does not always fully explain all the discrepancies between the studies cited.

3. Molecular mechanisms involved in CHF and “reverse remodeling”

A multitude of molecular changes is noted, which can be partly considered as causal and partly as corollary of proceeding CHF. Some are regarded to be compensatory mechanisms at the beginning but eventually become deleterious and contribute to the progression of the disease in the long run. They are associated with apoptosis as well as with the regulation of genes and their encoded proteins related to cellular stress and extracellular matrix turnover.

3.1. Extracellular matrix changes in CHF

The extracellular matrix provides the support necessary for the alignment of myocytes in the myocardium and myofibrils within the myocyte. CHF is associated with profound changes in extracellular matrix turnover mediated by matrixmetalloproteinases (MMP). [25–27]

Thomas and co-workers analysed different MMP sub-species in chronic dilated cardiomyopathy (DCM). Specifically, interstitial MMP-1 was reduced, while stromelysin MMP-3 and MMP-9 were increased, whereas MMP-2 remained unchanged. Moreover, tissue inhibitors of matrix-metalloproteinases 1 and 2 (TIMP) were selectively downregulated in ischemic cardiomyopathy (IHC) at the protein level suggesting posttranscriptional inhibition.

Several studies have demonstrated increased myocardial MMP activity in dilated cardiomyopathy (DCM) [29]. MMP-3 is important because of its ability to degrade a wide range of ECM components and to activate latent MMP [30].

Li and co-workers investigated the expression of TIMP at the mRNA and protein levels. In CHF, TIMP-1 and -3 are downregulated regardless of the underlying disease. TIMP-4 was selectively downregulated in ischemic cardiomyopathy (IHC) at the protein level suggesting posttranscriptional inhibition.

The downregulation of TIMP and upregulation of MMP favour matrix degradation during cardiac remodeling [27]. These findings suggest that, upon activation during CHF, MMP degrade the interstitial connective tissue, therefore destabilizing the mechanical scaffold of the heart reflected by ventricular dilation.

Li and co-workers found that MMP-9 and -1 were significantly reduced after LVAD insertion. MMP-2 gelatinolytic activity remained unaltered. MMP-3 was expressed at low levels prior to mechanical support and was not influenced by LVAD. TIMP-1 and -3 protein levels were significantly upregulated, whereas TIMP-2 and -4 were unchanged. The decrease of MMP and increase of TIMP lead to a reduction of interstitial connective tissue turnover resulting in less pronounced ventricular remodeling, but total collagen content remains unchanged [25].

MMP can be induced by mechanical stretch. LVAD reduce ventricular wall tension and can therefore sustain a balanced MMP/TIMP ratio [25].

3.2. Changes in cardiomyocyte biology in CHF and after LVAD

3.2.1. Alterations of β-adrenergic signal transduction

In CHF, a number of abnormalities have been identified in the β-adrenergic signal transduction which include: (1) downregulation of β1 adrenergic receptors, (2) upregulation of the inhibitory G protein (Gi), (3) increase in the β-adrenergic receptor kinase, (4) decrease in cyclic AMP (cAMP), (5) decrease in sarcoplasmic reticulum ATPase activity, resulting in elevated resting intracellular calcium and an abnormal calcium transient, (6) decrease in myofibrillar ATPase activity, (7) decrease in creatine kinase activity, and (8) decrease in myofibrillar protein content [31].

Regulation of inotropy, chronotropy, dromotropy, and lusitropo depends on signaling by β-adrenergic receptors and the subsequent activation of the G protein–adenylylcy clase signal transduction cascade. β-adrenergic receptors are coupled to G proteins and serve as targets of protein kinase A and C as well as β-adrenergic receptor kinases (β-ARK) [31].

β-Adrenergic receptor signaling can be desensitized by the following mechanisms: (1) receptor downregulation due to decreased synthesis or to increased sequestration (requiring hours) and (2) receptor dysfunction as a result of uncoupling from the signal transducing G protein (Gs) (requiring seconds) and induced by receptor phosphorylation by protein kinases A and C and β-ARK [31].

Isolated muscles and cardiomyocytes from failing human hearts show a decreased inotropic response to β-adrenergic agonists while β-adrenergic receptor density is markedly reduced in the myocardium of patients with CHF due to DCM or ischemic heart disease [32].

β2-Receptors are not downregulated but functionally uncoupled [33].

Ogletree-Hughes and co-workers investigated baseline contractile parameters and inotropic response to β-adrenergic stimuli as well as β-adrenergic receptor density in isolated trabecular muscles of failing hearts with and without LVAD compared to controls. They demonstrated that unloading leads to a restoration of β-adrenergic receptor density in cardiomyocytes. Muscles from LVAD hearts produced an inotropic response to isoproterenol similar to non-failing hearts and were significantly better than that observed in muscles from failing hearts without LVAD. Importantly, the duration of the mechanical support did not
completely predict either the response to inotropic stimulation or the density of β-adrenergic receptors. Receptor density alone does not predict the magnitude of the inotropic response.

The mechanisms underlying the increase of receptor density and improved adrenergic responsiveness may include normalization of plasma neurohormones and decrease in both plasma and cardiac cytokine levels following LVAD [34].

3.2.2. Alterations of Ca\(^{2+}\) homeostasis

Myocardial contractile dysfunction in heart failure of either etiology has been linked to alterations of Ca\(^{2+}\) cycling [35]. Downregulated gene expression of sarcoplasmic endoreticular Ca\(^{2+}\) ATPase subtype 2a (SERCA2a), of the sarcoplasmic reticular (SR) ryanodine-sensitive Ca\(^{2+}\) release channel (ryanodine receptor [RyR]), and altered regulation of the sarcolemmal Na\(^{+}/Ca^{2+}\) exchanger have been reported and appear to correlate with contractile dysfunction [36].

Studies of isolated cardiomyocytes have demonstrated LVAD-induced recovery of contractile strength in unloaded cells, coinciding with normalization of magnitude and time course of the intracellular Ca\(^{2+}\) transient [37].

Heerdt and co-workers found enhanced force generation in myocardium following LVAD support (duration from 24 to 142 days) by determining the force–frequency relationship (FFR) of isolated trabecular muscles. This improved force generation is accompanied by an increased gene expression and protein level of SERCA 2a [38].

Additionally, there was an increased expression of the gene encoding for RyR after LVAD, while there were only little changes of the protein. Hyperphosphorylation of RyR in failing hearts disrupts the normal coupled gating of neighbouring receptors, resulting in abnormal ensemble gating patterns, less coordinated SR Ca\(^{2+}\) release during excitation and Ca\(^{2+}\) leak during diastole. After LVAD, these abnormalities are reversed, possibly in response to normalization of the β-adrenergic signaling pathway [37].

3.2.3. Altered expression of natriuretic peptides and receptors

Cardiac atrial (ANP) and B-type natriuretic peptides (BNP) play a crucial role in maintaining arterial pressure and volume homeostasis. They exert antihypertrophic (ANP) and antifibrotic (BNP) effects via guanylyl cyclase A (GC-A) [39].

Patients with cardiac hypertrophy and/or CHF display elevated cardiac and plasma levels of ANP and BNP, with these peptide levels being highly related to disease severity. However, there is a markedly decreased diuresis/natriuresis, vasodilatation and vascular synthesis of cGMP in response to exogenous ANP and BNP, suggesting a downregulation or impaired receptor or postreceptor responsiveness of GC-A in peripheral tissues from CHF patients. Increased peripheral expression of NPR-C receptors mediating enhanced metabolic clearance of NPs may also be involved [40].

We investigated changes of ANP, BNP and NPR-C by quantitative RT-PCR and determined the activity of the ANP/GC-A system. Increased cardiac ventricular ANP and BNP expression in CHF patients is accompanied by increased cardiac expression of the NP-metabolising NPR-C receptor, decreased ratios of GC-A to NPR-C receptors and blunted responsiveness of GC-A to ANP by reduced cGMP synthesis. Reverse remodeling of the heart by mechanical support reverses these changes and re-establishes the local responsiveness of GC-A to ANP.

Interestingly, there was a clear positive correlation between cardiomyocyte diameters and cardiac ANP, BNP as well as NPR-C mRNA levels. However, whereas cardiac hypertrophy does not fully reverse during LVAD support, the changes of ANP, BNP and receptors were completely reversible, suggesting that they are partly independent from cardiac hypertrophy but dependent on other local factors such as myocyte stretch [41].

3.2.4. Apoptosis

Apoptosis contributes to cardiomyocyte loss and progressive decline in left ventricular function in cardiomyopathy [42]. Several different apoptotic mechanisms are involved in the course of CHF.

3.2.4.1. (Extrinsic) death receptor pathway. “Death receptors” belong to the tumor necrosis factor (TNF) superfamily of cell membrane receptors. Upon ligand binding, they initiate apoptosis by ultimately activating caspase 8. TNF-R1 also activates the transcription factor NF-κB, which may induce the expression of survival genes and counteracts the apoptotic pathway [43].

Fas/Fas-ligand pathway. There is evidence that the Fas/FasL system participates in various types of stress-induced apoptosis in the heart, where stress may sensitize cardiomyocytes to Fas in vitro [44].

Soluble Fas-ligand (FasL) is elevated in patients with advanced CHF and Fas mRNA is expressed in failing human myocardium [45].

The myocardial Fas system (APO-1/CD 95) appears to respond very sensitively to changes in pressure and volume overload, as observed in CHF [46]. Mechanical support leads to normalized expression of the anti-apoptotic protein FasExo6Del, suggesting a decreased cardiomyocyte susceptibility to apoptosis [47].

TNF-receptor pathway. TNF-α is increased in CHF. There seems to exist a direct relationship between disease severity and circulating levels of TNF-α [48]. Cardiomyocytes can undergo apoptosis after stimulation with TNF-α in vitro.

A significant decrease of the pro-apoptotic cytokine TNF-α in the myocardium of LVAD-supported hearts was demonstrated [49].
In contrast, a number of studies suggest a beneficial role for TNF-α in the heart. TNF-α pre-treatment was protective in a rat model of ischemia/reperfusion [50]. It is unknown whether the net effect of TNF-α is pro- or antiapoptotic.

3.2.4.2. (Intrinsic) mitochondrial pathway. The mitochondrial pathway plays an important role in CHF. Narula and co-workers found release of cytochrome c from the mitochondria and activation of caspase 3 in patients with end-stage cardiomyopathy [42]. Currently, there are no data dealing with this apoptotic pathway in response to LVAD.

3.2.4.3. Bcl-2 family. These proteins exert either antiapoptotic (Bcl-2 and Bcl-xL) or pro-apoptotic properties (Bax, Bad, Bid, and Bnip3) primarily at the level of the mitochondria [51].

Among other conditions as chronic hypoxia and mechanical stretch, upregulation of both pro- and antiapoptotic Bcl-2 proteins were observed in end-stage heart failure [52].

3.2.4.4. Apoptosis and left ventricular mechanical unloading. The expression of genes involved in the regulation of apoptosis of cardiomyocytes is significantly altered upon long-term LVAD treatment.

In CHF, bcl-2 was shown to be downregulated, resulting in a decrease in its anti-apoptotic properties. Conversely, the expression of the pro-apoptotic molecule Bax remained unaltered [45].

The expression of the anti-apoptotic protein Bcl-xL was shown to be upregulated after LVAD, suggesting that cardiomyocyte susceptibility to apoptosis is abated by activation of anti-apoptotic Bcl-xL [47].

Our own data also showed significantly reduced apoptosis after LVAD [53].

3.3. Signal transduction

3.3.1. Transduction pathways

Three signal transduction pathways have been implicated in the development of cardiac hypertrophy: (1) the mitogen-activated protein kinases (MAPK) with the extracellular signal-related kinases (Erks), c-Jun N-terminal protein kinases (JNKs), and p38 MAPK subfamilies [54]; (2) the Ca2+/calmodulin activated protein kinase (CaM kinase) and phosphatase (calcineurin) [55], and, (3) the protein kinase B/Akt and its downstream target glycogen synthase kinase 3β (GSK3β) [56].

All three signal transduction pathways become activated during CHF in humans. While the Erks are powerfully activated by hypertrophic stimuli such as phenylephrine, angiotensin II and endothelin-1 via G-Protein coupled receptors [54], the JNK and p38 MAPK are activated by potentially cytotoxic cellular stresses and seem to play a role in cardiomyocyte apoptosis [57].

Akt exerts powerful anti-apoptotic properties via inhibitory phosphorylation of GSK3β, which is an essential negative regulator of cardiac hypertrophy [58].

The phosphatidylinositol-3-OH kinase (PI3K) is a second signaling pathway that acts through Akt and p70S6 kinase (p70S6K), the latter being a key factor in angiotensin II receptor type 2-mediated cardiac hypertrophy [59].

We investigated the activity of mitogen-activated protein kinases (MEKs), Erks, Akt, GSK3β, p70S6K, JNKs and p38 in end-stage heart failure before and after mechanical support.

The phosphorylation status of these kinases was determined and revealed a dramatic decrease in dually phosphorylated (Thr 202/Tyr 204) active forms of Erk-1 and Erk-2 after LVAD support, as determined by Western blot analysis. The erk-activating kinases (MEK-1/2) also showed a significant decrease in phosphorylation after LVAD.

Akt and GSK 3β phosphorylation was significantly decreased after LVAD support. There was a significant correlation between Akt and GSK 3β changes, suggesting an inactivation of Akt and an activation of GSK 3β, respectively, under LVAD support [53].

In the same study, the apoptotic index was determined and was found to be significantly decreased after LVAD support. However, there was no significant correlation between the apoptotic index and the decrease of Akt, Erk or GSK 3β phosphorylation [53].

Besides Akt, another kinase involved in the PI3K signal transduction pathway, P70S6K, showed a dramatic decrease in its phosphorylation in a subset of patients. P70S6K and its isoform p85S6 are associated with cardiac hypertrophy mediated by angiotensin II receptor 2 [59]. However, there was no correlation between cardiomyocyte diameter reduction and phosphorylation of p70S6K.

In contrast, neither the JNK nor the p38-mediated signaling cascades were altered under LVAD support in our study, suggesting specific regulation of kinase signaling after mechanical support in human hearts in vivo.

Recently, another study reported an inactivation of Erks as well as lack of JNK phosphorylation in line with our findings. In contrast to our data, these authors found an activation of p38 and JNK, which was most probably due to different durations of LVAD support or to different medications [60].

In summary, the decrease in MEK activity correlated with that of the Erks, while the decrease in Akt activity correlated with an increase in GSK 3β activation. Our findings underline the emerging evidence of MEK/Erks and Akt/GSK 3β in the development and regulation of cardiac hypertrophy and cardiomyopathy in vivo (Fig. 2A and B) [61]. The inactivation of MEK/Erks and the activation of GSK 3β after LVAD support are in line with the opposing effects of the two signaling pathways with regard to cardiac reverse remodeling.
Activation of some of the signal transduction pathways mentioned above can be explained by “classical” G-Protein coupled receptor (GPCR) signaling. There is evidence of a GPCR-mediated “transactivation” of the epidermal growth factor receptor (EGFR) lacking intrinsic tyrosine kinase activity. These studies propose a pathway starting from AT1-receptor transactivation of EGFR on cardiomyocytes that may involve membrane-bound metalloproteinases, which cleave EGFR ligands from a plasma membrane-associated precursor. Subsequent activation of the EGFR leads to mobilisation of signaling pathways including ERK 1/2, PI3K, Akt and mTOR/S6 kinase [62].

### 3.3.2. Transcription factor NF-κB

NF-κB plays a pivotal role in the regulation and expression of genes involved in the cellular response to stressful stimuli [63]. It is a crucial regulator of genes involved in anti-apoptosis [63] and activation of NF-κB has been observed in the myocardium of patients with CHF [64].

NF-κB regulates several of the factors involved in the pathogenesis of CHF including interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), Bcl-xL, and heme oxygenase-1.

We investigated the expression as well as the DNA-binding activity of NF-κB before and after LVAD. Immunohistochemical analyses revealed positivity for the active p65 subunit of NF-κB predominantly in cardiomyocytes. At the time of LVAD insertion, the median percentage of NF-κB positive nuclei was 77.7 and significantly decreased about twofold following LVAD (Fig. 3A and B).

To test whether the immunoreactivity for active NF-κB correlates with its DNA-binding activity, gel-shift assays were performed with nuclear extracts from left ventricular myocardial tissue acquired before and after LVAD support. In all four patients examined, the DNA-binding activity of NF-κB was significantly decreased after LVAD support in line with the immunohistochemical results.

In summary, we demonstrated abundant activity of NF-κB in cardiomyocytes in the failing heart that was drastically reduced by LVAD support. Therefore, NF-κB appears to be a specific and reverse functional response to molecular signals induced by overload [65].

Thus, we were able to identify NF-κB as the first transcription factor that is negatively regulated under mechanical unloading. As NF-κB transcriptionally regulates genes encoding TNFα, IL-6 and heme oxygenase, the specific changes in their expression in failing and supported human hearts may result from changes in the activity of NF-κB. In this scenario, NF-κB may regulate a subset of genes associated with “reverse remodeling”.

The stimuli leading to increased NF-κB activity in the failing heart and its downregulation after LVAD support still remain unknown. However, we have observed a gradient in the NF-κB activity in failing myocardium being more prominent in the subendocardium than in the subepicardium. This phenomenon disappears after LVAD support. The subendocardium is the least well perfused region of the failing myocardium and the most vulnerable area to relative ischemia due to abnormally high wall stress in the presence of overload. The resulting tissue hypoxia may contribute to the high NF-κB activity in the subendocardium since hypoxia has been shown to increase NF-κB activity [66].

This may explain the similar transmural gradient observed for ANP/BNP [67], COX-2 [64], metallothionein [17] and heme oxygenase [68]. As LVAD can substantially improve oxygen delivery, this may explain the subsequent decline of NF-κB activity.

### 3.4. Cell stress associated factors

#### 3.4.1. Heme oxygenase-1

Heme oxygenase-1 (HO-1, HSP 32) belongs to the family of heat shock proteins, which can be induced by ischemia, local hypoxia, oxidative stress and other stressful stimuli [69].

HO-1 has been shown to have cell-protective and anti-apoptotic properties [69]. Hence, increased expression of HO-1 indicates perturbed cellular homeostasis.

We investigated the distribution of HO-1 in tissue samples from patients with end-stage CHF of different etiology. Immunohistochemically, especially cardiomyocytes and to a lesser extent arterial wall smooth muscle...
cells, endothelial cells and infiltrating round cells exhibited positive strong expression of HO-1. After LVAD treatment, the expression of HO-1 recedes to levels as low as measured in control hearts (Fig. 4).

In the same study, cultured rat cardiomyocytes were exposed to hypoxia. In this experimental setting, a significant increase of HO-1 was measured, which declined to normal levels after restoration of normoxic conditions [68].

The downregulation of HO-1 especially in the subendocardial region can be possibly explained by the reduction of tissue hypoxia and decrease of cardiomyocyte hypertrophy following reduced wall stress and improved oxygen supply by mechanical unloading.

3.4.2. Metallothionein

Metallothionein (MT) constitutes a cell stress protein, which inactivates free reactive oxygen species (ROS) and protects cells against the effects of oxygen radicals [70].

We examined MT in hearts before and after unloading by LVAD with regard to possible reversible expression and localized pattern of expression.

Cardiomyocytes with vacuolated cytoplasm and loss of contractile filaments showed strong immunostaining accentuated in the subendocardium.

LVAD has been shown to reduce MT-positive cardiomyocytes and vascular endothelial and smooth muscle cells (in a time-dependent manner).

These data point to a reversal of MT gene-expression after hemodynamic unloading of the left ventricle. The subendocardial distribution of MT-positive vacuolated cardiomyocytes, indicating (reversible) hypoxic damage, and their decreasing numbers underscore the concept of improved oxygen supply after mechanical unloading [17].
4. Conclusion

When a normal heart is affected by noxious stimuli, it responds rather uniformly with compensatory and adaptive changes. When excessive, they themselves may lead to volume and pressure overload and impaired cardiac function. The concomitantly increased mechanical wall stress accompanied by local ischemia may be two of the mechanisms that activate molecular and cellular responses. As CHF proceeds, protective thresholds are overrun with increases in apoptotic rates and the architecture of the heart cannot further adapt. Together with the altered neurohormonal regulation these changes further deteriorate the cardiac function.

LVAD lead to considerable improvement of cardiac performance in a subset of patients. These clinical observations are reflected by both morphological and molecular changes in the myocardium. Some of the mechanisms associated with the pathogenesis of CHF were identified as being reversible. However, the master molecular switches orchestrating the process of “reverse remodeling” are still unknown despite the identification of several highly dynamic molecular changes involved. Better understanding of the basic biology and pathophysiology of this process may help initiate the development of novel pharmacological approaches in the future. The heterogeneity of patients receiving LVAD causes some limitations to scientific interpretation. First of all, the etiologies underlying the development of CHF differ. However, the vast majority of patients eventually develop terminal heart failure against the background of either ischemic heart disease or DCM sharing common final paths with other etiologies.

Interestingly, mechanical support appears not only to reverse many of the adaptive changes at the cellular and molecular level but is also able to restore basic cardiac function. Associated with this is the decrease in apoptosis, hypertrophy, and fibrosis. The beneficial effects of LVAD are also reflected at the molecular level, with changes in the expression of proteins such as NFκB, β1-adrenoceptor density, and SERCA2a.

Fig. 5. Schematic synopsis of morphological and molecular events in heart failure before (A) and after (B) mechanical support.
function in a subset of patients (Fig. 5A and B). This interesting subgroup of patients (which may even mirror a special cardiac state thinkable in any heart failure patient) would extremely profit from LVAD treatment if it could be better characterized and clinically screened for. Maybe some of the factors already identified so far could help in the challenging work towards the development of such screens in the future.

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