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Persistent Fetal Gene Expression Patterns During Assist Device Support

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Abstract

Background—Heart failure is associated with reversal to a fetal gene expression pattern of contractile and metabolic genes. Substantial recovery of ventricular function with assist devices is rare. Our goal was to evaluate the effects of assist devices on fetal gene expression and hypoxia inducible factor 1 alpha (HIF-1 α , a transcriptional factor in hypoxic signaling.

Methods—Human heart tissue was obtained from the left ventricular apex at the time of assist device implantation and again from the left ventricular free wall during cardiac transplantation. Non-failing tissue was obtained from unutilized hearts from human donors. Gene expression was measured with the Affymetrix 133 plus 2 Array. HIF-1 α was measured by Western blotting with commercially available antibodies.

Results—Heart failure was associated with a decrease in α -myosin heavy chain and sarcoplasmic reticulum-Ca++ ATPase mRNA expression along with an increase in skeletal tropomyosin. This pattern persisted after assist device therapy. Heart failure was also associated with abnormalities in regulatory metabolic genes including GLUT1. These patterns also persisted after assist device therapy despite a reduction in ANP expression and normalization of HIF-1 α .

Conclusions—Failure of assist devices to produce sustained recovery of myocardial contractile function maybe due in part to persistent fetal transcriptional patterns of contractile and metabolic genes.

Introduction

Cardiac assist devices are effective for bridging patients to transplantation and are increasingly being utilized as destination therapy.^{1,2} Unloading the heart with a left ventricular assist device results in immediate improvement in diastolic and systolic dimensions.³ Despite this remodeling, myocardial recovery to the degree that device explantation occurs successfully without transplant is highly variable ranging from 5% to 78% of patients.^{4–7} Unloading the heart with an assist device leads to an immediate decrease in cardiac work but may also lead

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to myocardial atrophy.^{8–10} Animal models suggest that extreme myocardial unloading can contribute to a fetal gene expression profile.¹¹

Classically, fetal expression of contractile proteins and associated genes refers to a pattern of myosin heavy chain (MHC) shifts (decrease in the “adult” α -MHC, increase in “fetal” β -MHC, and increase in the α/β ratio), increased atrial natriuretic peptide (ANP), and downregulation of sarcoplasmic reticulum calcium ATPase, (SRCaATPase). For metabolic genes, a fetal pattern in heart failure consists predominately of downregulation in adult isoforms of glucose transporters, and pyruvate dehydrogenase kinase.^{12,13} The fetal heart exists in a relatively hypoxic environment and the reversal of an adult heart to a pattern of fetal gene expression likely represents an adaptive response to stress. Heart failure is associated with a switch to a fetal gene expression pattern of key genes involved in myocardial contraction and metabolism.^{13,14} The fetal gene programs can be activated in multiple conditions including pressure overload, hypothyroidism, cytokines, hypoxia, norepinephrine, diabetes, and extreme myocardial unloading.^{13,15–22}

β -blockers are known to diminish myocardial oxygen demand, reverse remodeling, improve cardiac contractility, and ultimately decrease mortality in heart failure patients.^{23–25} Serial analysis of myocardial gene expression in patients with heart failure indicate that β -blockers reverse components of the contractile protein fetal gene expression program in association with improving cardiac contractility and reverse remodeling.^{26,27} Increasingly, in addition to standard heart failure medication regimens, β -blockers are being used in patients with left-ventricular assist devices despite the lack of well designed clinical trials in this population. Our goal was to evaluate the effects of assist devices in conjunction with standard heart failure medical therapy on fetal gene expression and HIF-1 α levels.

Methods

This protocol was approved by the Institutional Review Board at the University of Colorado. This is a single center observational study. Patients with idiopathic dilated cardiomyopathies undergoing assist device placement were eligible for inclusion. Patients with ischemic, infiltrative or inflammatory cardiomyopathies were excluded from the analysis. In all patients assist devices were placed for heart failure not responsive to inotropic therapy in association with evidence of end organ damage secondary to low cardiac output. Medical therapy post-assist device placement consisted of β -blockers, ACE-I, and aldosterone antagonists as tolerated. Loop diuretics were used as needed for volume management. Control nonfailing left ventricular myocardium was obtained from unutilized hearts from organ donors. Gene expression analysis was conducted on 8 non-failing hearts, along with 4 patient’s pre and post LVAD. HIF-1 α measurements were conducted on samples from these same 4 patients plus two additional patients pre and post LVAD.

Tissue sampling and RNA extraction

Tissue was obtained at the time of assist device placement from the apex of the left ventricle. At the time of assist device explantation and transplantation tissue was obtained from the left ventricular free wall. Samples were frozen in liquid nitrogen.

Total RNA is extracted from myocardial tissue by the guanidinium thiocyanate phenol-chloroform method using RNA STAT-60. We use a double extraction to eliminate small amounts of DNA contamination.

Reverse Transcription

Reverse transcription was performed with total RNA as the starting template. One to five μg of total RNA was converted to double stranded cDNA (ds-cDNA) using the Superscript Choice System (Life Technologies). An oligo-dT primer containing a T7 RNA polymerase promoter (Geneset) was used and cRNA generated. Following second strand synthesis, the reaction mixture was extracted with phenol-chloroform-isoamyl alcohol and ds-cDNA recovered by ethanol precipitation.

In Vitro transcription

In vitro transcription was performed to generate biotin-labeled cRNA. *In vitro* transcription was performed using a T7 Megascript Kit by Ambion. 1.5 mL of ds-cDNA template is transcribed in the presence of a mixture of unlabeled ATP, CTP, GTP and UTP. In addition, biotin-labeled CTP and UTP (bio- 11 -CTP and bio- 16-UTP) from Enzo was added to the mixture. Biotin-labeled cRNA was purified using an RNeasy affinity column by Qiagen. To ensure optimal hybridization to the oligonucleotide array, the cRNA was fragmented such that the cRNA fragments were between 35–200 bases in length, accomplished by incubating the cRNA at 94° C for 35 minutes. The sample was then added to a hybridization solution containing 100 mM MES, 1 M Na⁺ and 20 mM EDTA in the presence of 0.01% Tween 20. The final concentration of the fragmented cRNA was 0.05 $\mu\text{g}/\mu\text{L}$.

Hybridization

Hybridization was performed by incubating 200 μL of the sample with the U133 plus 2 Affymetrix GeneChip. Hybridization was carried out for 18–20 hours. After hybridization, the hybridization solutions were removed and the GeneChip was washed and stained with Streptavidin-phycoerythrin, and read at a resolution of 6 microns with an HP Gene Array Scanner.

Microarray Analysis

The University of Colorado Health Sciences Center has established a Microarray Core facility in its Cancer Center. This Core analyzes expression arrays hybridized with RNA from human tissues, as well as from a variety of animal models. The Affymetrix data analysis software includes links to the public domain expression databases. The data was normalized to the total mRNA intensity and analyzed in GeneSifter. A Wilcoxon rank sum test was utilized and a p value of less than 0.05 was considered significant. A 1.5 fold change threshold and the Benjamini and Hochberg correction were imposed on the genomic wide comparison between groups.

Western Blotting—Samples from frozen tissue were homogenized in NP-40 buffer with protease and phosphatase inhibitors. Equal amounts of homogenate were fractionated on a 7.5% SDS-PAGE gel and Western blotted onto nitrocellulose membrane. The blot was probed for HIF 1 α (Transduction laboratories), Hsp-90 (Upstate). Equal protein loading was monitored by probing for GAPDH on the same blot.

Results

Patient characteristics are shown in table 1. The mean age at the time of LVAD placement was 45 years. All patients carried a diagnosis of dilated cardiomyopathy (DCM) and were all NYHC class 4 at the time of LVAD placement. The mean duration of assist device therapy was 230 days. Medical therapy while on assist devices is outlined in Table 2.

Transcriptional profiles of fetal/adult genes are shown in figures 1–3. In heart failure ANP expression is elevated relative to normal controls ($p < .05$). Post assist device ANP levels decrease to a level that is no longer significantly different than normal. Expression of α -MHC ($p < .05$) and SRCaATPase ($p < .1$) were diminished in heart failure and remained diminished relative to normal after assist device placement. There were no significant changes in β -MHC expression in this study in heart failure or post assist device. Skeletal tropomyosin is elevated in heart failure ($p < .05$) and remains elevated despite assist device therapy ($p < .05$).

The transcriptional profiles of metabolic genes are shown in Figure 3. GLUT1 is diminished in heart failure ($p < .05$) and remains persistently diminished with assist devices. There were trends for GLUT4 and PDK2 towards a decrease in heart failure but these did not reach significance. Post assist device both GLUT4 ($p < .05$) and PDK2 ($p < .05$) were diminished relative to nonfailing controls. CPT-1 was not significantly altered in heart failure but increased post assist device ($p < .05$) when compared to the nonfailing heart. The Western Blots of HIF1 α are shown in figure 4 along with the integrated density values. GAPDH was utilized as a loading control. Normalizing for GAPDH, HIF-1 α and HSP90 were elevated in heart failure. Assist device therapy normalized both these proteins.

Broad analysis of changes in gene expression post assist device revealed that 2058 genes are differentially expressed. An ontology report of these genes is shown in Figure 5. There is a predominance of downregulated genes (70%) post device placement. Downregulation of genes involved in biological and developmental processes were significant (Z-score > 2).

Discussion

Our results suggest that fetal gene expression patterns of contractile proteins may persist after assist device placement despite standard heart failure therapy. Classically, heart failure is characterized by diminished myocyte velocity of shortening in association with shifts in myosin heavy chain isoforms, specifically, a decrease in α -MHC. While α -MHC only represents a small proportion of total MHC it's importance is emphasized by the observations that mutations in this gene can lead to a dilated cardiomyopathy in humans; in rats, small changes in α -MyHC are associated with measurable changes in contractile function; and that improvements in expression of this gene are associated with improvements in cardiac contractility in humans in response to beta blocker therapy.^{27,28} Persistent down-regulation of α -MHC likely contributes to ongoing contractile dysfunction in patients treated with left ventricular assist devices. Such ongoing down-regulation of α -MHC, and reinforced with down-regulation of SRCaATPase and increased expression of skeletal tropomyosin is consistent with extreme unloading of the left ventricle causing myocardial atrophy as suggested previously in animal models.¹¹ This pattern of fetal gene expression persists despite concurrent treatment with standard heart failure therapy in the setting of the unloaded ventricle.

Maladaptive changes in key metabolic genes also persist after assist device therapy despite medical treatment. Previous studies have shown that glucose transporters do not improve with assist devices and our results support this finding.²⁹ Our results also indicate the PDK2, a key regulatory enzyme in glucose metabolism, is not normalized by assist device therapy. The failing heart is increasingly reliant on glucose metabolism and abnormalities in glucose uptake or processing may contribute to disease progression in heart failure. Our results differ from prior work in that we were not able to show a decrease in carnitine palmitoyl transferase-1 (CPT-1), a regulatory enzyme in fatty acid metabolism, in heart failure relative to nonfailing controls.^{13,29} Our results suggest that assist devices may not have a negative interaction on expression of this gene but that abnormalities in glucose metabolism persist. Recent data in mice suggests that sustained downregulation of genes involved in fatty acid metabolism is not characteristic of heart failure.³⁰

Hypoxia inducible factor 1 α (HIF 1 α) is an important transcription factor for the regulation of numerous genes involved in glycolytic pathways and cell growth.^{31–32} HIF-1 α levels are regulated by hypoxia, mitogen activated protein kinase (MAPK), and phosphatidylinositol 3-kinase signaling pathways.³¹ In rat models of volume overloaded heart failure, increased HIF-1 α expression showed reversal with the addition of β -blockers.³³ Our data reinforces the finding of increased HIF-1 α signaling in heart failure. Assist devices in combination with standard medical therapy diminish HIF-1 α signaling as expected. HSP-90 is known to stabilize HIF-1 α .^{34–35} We previously have shown that β -blocker therapy in heart failure is associated with a decrease in HSP-90 mRNA suggesting a possible mechanism for elevated HIF-1 α in heart failure.²⁶ GLUT1 is a hypoxia sensitive gene. In rat models hypoxia increases GLUT1 expression.³⁶ Diminished GLUT 1 in heart failure and the absence of significant changes in GLUT1 expression despite changes in HIF 1 α suggest that either there are other factors affecting the transcriptional control of this gene or that HIF-1 α activity is diminished. Several factors may affect HIF-1 α activity including TSGA10 which inhibits HIF-1 α translocation to the nucleus.³⁷ Our gene array results indicate that TSGA10 expression is elevated 12 fold in heart failure compared to nonfailing hearts (not shown, $p < .005$).

Gene array studies are beginning to provide a broad understanding of the transcriptional changes that occur in response to assist devices.^{38–43} An overview of our results may be seen in Table 3. These results indicate that assist devices decrease the transcription of the majority of genes undergoing differential expression consistent with extreme unloading contributing to myocardial atrophy. Downregulation of genes involved in developmental and biological process suggest the importance of these pathways in adverse remodeling due to assist devices. Previous work with DNA arrays suggests that myocardial recovery in patients on assist devices is associated with specific alterations in sarcomeric and nonsarcomeric cytoskeletal proteins.^{44, 45} Our study further characterizes the gene expression patterns that occur in patients who fail to reverse remodel with assist devices despite standard heart failure medications. Particular patterns of gene expression are emerging in heart failure and failed response to assist device therapy. Using small inhibitory mRNA molecules it may become feasible to tailor therapies to alter gene expression in a fashion that improves clinical outcomes.^{46–48}

This study has several limitations. The small size limits the ability to detect small changes in gene expression. As an observational study, it is subject to bias. Non-failing cardiac tissue obtained from organ donors does not represent “normal tissue” as these hearts are often subjected to increased catecholamine and cytokine signaling. Post assist device therapy tissue was only available from subjects who failed to improve cardiac function necessitating the need for cardiac transplantation. By definition, subjects in this study had failed the combination of medical and assist device therapy. Other studies suggest that at least in a certain percentage of patients, standard medical therapy improves expression of certain genes including SRCaATPase.⁴⁹ Finally, transcriptional changes are only one mechanism regulating the biology of the myocyte. Numerous genes including HIF-1 α are regulated at a post-transcriptional level. Other studies suggest that assist devices actually improve myocardial contractility by phosphorylation states suggesting that further studies are needed to truly understand the effects of assist devices and their interactions with medical therapies in this patient population.⁵⁰

There is increasing evidence that excessive withdrawal of adrenergic stimuli may have adverse effects on outcomes. Clinical trials utilizing moxonidine and bucindolol in patients with advanced heart failure have associated sympatholysis with increased mortality.^{51,52} The β_2 pathway is thought to be important to physiological myocyte growth or hypertrophy. Increased β_2 signaling by over-expression of β_2 receptors or by β_2 agonists has been shown to have beneficial effects on cardiac contractility, remodeling and apoptosis in animal models of heart failure.^{53,54} Recently a small observational study in patients receiving assist devices suggested

that clenbuterol, a β_2 agonist, could prevent myocardial atrophy in this population.⁵⁵ The effects of β_2 agonists on fetal gene expression remain unknown.

As the population ages and more patients are surviving myocardial infarctions the prevalence of heart failure is increasing. Due to limitations in donor supply, transplantation is not an option for the majority of people with advanced disease. As technology advances, assist devices likely will become increasingly utilized. Assist device therapy by itself appears inadequate to reverse myocardial failure in the majority of patients. Well designed clinical trials are necessary to determine appropriate medical therapy for this population. Understanding the interactions between assist devices and medical therapy at a molecular level is necessary to facilitate new treatment strategies. Advances in microarray technology allow for genome wide expression studies to be conducted in combination with focused hypothesis testing.

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References

1. Rose EA, Gelijns AC, Moskowitz AJ, Heitjan DF, Stevenson LW, Dembitsky W, Long JW, Ascheim DD, Tierney AR, Levitan RG, Watson JT, Ronan NS, Shapiro PA, Lazar RM, Miller LW, Gupta L, Frazier OH, Desvigne-Nickens P, Oz MC, Poirier VL, Meier P. The Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) Study Group, Long-Term Use of a Left Ventricular Assist Device for End-Stage Heart Failure. *N Engl J Med* 2001;345:1435–1443. [PubMed: 11794191]
2. Long JW, Kfoury AG, Slaughter MS, Silver M, Milano C, Rogers J, Delgado R, Frazier OH. Long-term destination therapy with the HeartMate XVE left ventricular assist device: improved outcomes since the REMATCH study. *Congestive Heart Failure* 2005;11(3):133–8. [PubMed: 15947534]
3. Nakatani S, McCarthy PM, Kottke-Marchant K, Harasaki H, James KB, Savage RM, Thomas JD. Left ventricular echocardiographic and histologic changes: impact of chronic unloading by an implantable ventricular assist device. *Journal of the American College of Cardiology* 1996;27(4):894–901. [PubMed: 8613620]
4. Muller J, Wallukat G, Weng YG, Dandel M, Spiegelsberger S, Semrau S, Brandes K, Theodoridis V, Loebe M, Meyer R, Hetzer R. Weaning from mechanical cardiac support in patients with idiopathic dilated cardiomyopathy. *Circulation* 1997;96(2):542–9. [PubMed: 9244223]
5. Mancini D, Goldsmith R, Levin H, Beniaminovitz A, Rose E, Catanese K, Flannery M, Oz M. Comparison of exercise performance in patients with chronic severe heart failure versus left ventricular assist devices. *Circulation* 1998;98(12):1178–83. [PubMed: 9743508]
6. Dandel M, Weng Y, Siniawski H, Potapov E, Lehmkuhl HB, Hetzer R. Long-term results in patients with idiopathic dilated cardiomyopathy after weaning from left ventricular assist devices. *Circulation* 2005;112(9 Suppl):I37–45. [PubMed: 16159848]
7. Mann DL, Willerson JT. Left ventricular assist devices and the failing heart: a bridge to recovery, a permanent assist device, or a bridge too far? *Circulation* 1998 Dec 1;98(22):2367–9. [PubMed: 9832479]
8. Kinoshita M, Takano H, Takaichi S, Taenaka Y, Nakatani T. Influence of prolonged ventricular assistance on myocardial histopathology in intact heart. *Annals of Thoracic Surgery* 1996;61(2):640–5. [PubMed: 8572780]
9. Milting H, Jacob M, Kassner A, Heimann P, Mannherz HG, Becker G, Meyer HE, Bothig D, Arusoglu L, Morshuis M, Korfer R, El Banayosy A. The structural examination of myocardial samples from patients with end-stage heart failure supported by ventricular assist devices using electron microscopy and amino acid analysis reveals low degree of reverse remodeling. *Journal of Heart & Lung Transplantation* 2004;23(4):396–404. [PubMed: 15063398]
10. Soloff LA. Atrophy of myocardium and its myocytes by left ventricular assist device. *Circulation* 1999;100(9):1012. [PubMed: 10469428]

11. Galinanes M, Zhai X, Hearse DJ. The effect of load on atrophy, myosin isoform shifts and contractile function: studies in a novel rat heart transplant preparation. *Journal of Molecular & Cellular Cardiology* 1995;27(1):407–17. [PubMed: 7760361]
12. Parker TG, Packer SE, Schneider MD. Peptide growth factors can provoke “fetal” contractile protein gene expression in rat cardiac myocytes. *J Clin Invest* 1990;85(2):507–514. [PubMed: 1688886]
13. Razeghi P, Young ME, Alcorn JL, Moravec CS, Frazier OH, Taegtmeier H. Metabolic gene expression in fetal and failing human heart. *Circulation* 2001 Dec 11;104(24):2923–31. [PubMed: 11739307]
14. Lowes BD, Minobe W, Abraham WT, Rizeq MN, Bohlmeier TJ, Quaife RA, Roden RL, Dutcher DL, Robertson AD, Voelkel NF, Badesch DB, Groves BM, Gilbert EM, Bristow MR. Changes in gene expression in the intact human heart. Downregulation of alpha-myosin heavy chain in hypertrophied, failing ventricular myocardium. *Journal of Clinical Investigation* 1997;100(9):2315–24. [PubMed: 9410910]
15. Haddad F, Bodell PW, Baldwin KM. Pressure-induced regulation of myosin expression in rodent heart. *Journal of Applied Physiology* 1995;78(4):1489–95. [PubMed: 7615460]
16. Swynghedauw B. Remodeling of the heart in chronic pressure overload. *Basic Research in Cardiology* 1991;86 (Suppl 1):99–105. [PubMed: 1645166]
17. Kinugawa K, Minobe WA, Wood WM, Ridgway EC, Baxter JD, Ribeiro RC, Tawadrous MF, Lowes BA, Long CS, Bristow MR. Signaling pathways responsible for fetal gene induction in the failing human heart: evidence for altered thyroid hormone receptor gene expression. *Circulation* 2001;103(8):1089–94. [PubMed: 11222471]
18. Klein I, Ojamaa K. Thyroid hormone and the cardiovascular system. *New England Journal of Medicine* 2001;344(7):501–9. [PubMed: 11172193]
19. Mann DL. Inflammatory mediators and the failing heart: past, present, and the foreseeable future. *Circulation Research* 2002;91(11):988–98. [PubMed: 12456484]
20. Razeghi P, Essop MF, Huss JM, Abbasi S, Manga N, Taegtmeier H. Hypoxia-induced switches of myosin heavy chain iso-gene expression in rat heart. *Biochemical & Biophysical Research Communications* 2003;303(4):1024–7. [PubMed: 12684037]
21. Rundell VL, Geenen DL, Buttrick PM, de Tombe PP. Depressed cardiac tension cost in experimental diabetes is due to altered myosin heavy chain isoform expression. *American Journal of Physiology - Heart & Circulatory Physiology* 2004;287(1):H408–13. [PubMed: 15001437]
22. Waspe LE, Ordahl CP, Simpson PC. The cardiac beta-myosin heavy chain isogene is induced selectively in alpha 1-adrenergic receptor-stimulated hypertrophy of cultured rat heart myocytes. *Journal of Clinical Investigation* 1990;85(4):1206–14. [PubMed: 2156896]
23. Lowes BD, Gill EA, Abraham WT, Larrain JR, Robertson AD, Bristow MR, Gilbert EM. Effects of carvedilol on left ventricular mass, chamber geometry, and mitral regurgitation in chronic heart failure. *American Journal of Cardiology* 1999 Apr 15;83(8):1201–5. [PubMed: 10215284]
24. Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, Shusterman NH. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. U.S. Carvedilol Heart Failure Study Group. *New England Journal of Medicine* 1996;334(21):1349–55. [PubMed: 8614419]
25. Hjalmarson A, Goldstein S, Fagerberg B, Wedel H, Waagstein F, Kjekshus J, Wikstrand J, El Allaf D, Vitovec J, Aldershvile J, Halinen M, Dietz R, Neuhaus KL, Janosi A, Thorgeirsson G, Dunselman PH, Gullestad L, Kuch J, Herlitz J, Rickenbacher P, Ball S, Gottlieb S, Deedwania P. Effects of controlled-release metoprolol on total mortality, hospitalizations, and well-being in patients with heart failure: the Metoprolol CR/XL Randomized Intervention Trial in congestive heart failure MERIT-HF Study Group. *JAMA* 2000;283(10):1295–302. [PubMed: 10714728]
26. Lowes BD, Zolty R, Minobe WA, Robertson AD, Leach S, Hunter L, Bristow MR. Serial gene expression profiling in the intact human heart. *Journal of Heart & Lung Transplantation* 2006;25(5):579–88. [PubMed: 16678038]
27. Lowes BD, Gilbert EM, Abraham WT, Minobe WA, Larrabee P, Ferguson D, Wolfel EE, Lindenfeld J, Tsvetkova T, Robertson AD, Quaife RA, Bristow MR. Myocardial gene expression in dilated cardiomyopathy treated with beta-blocking agents. *New England Journal of Medicine* 2002;346(18):1357–65. [PubMed: 11986409]

28. Carniel E, Taylor MR, Sinagra G, Di Lenarda A, Ku L, Fain PR, Boucek MM, Cavanaugh J, Miodic S, Slavov D, Graw SL, Feiger J, Zhu XZ, Dao D, Ferguson DA, Bristow MR, Mestroni L. Alpha-myosin heavy chain: a sarcomeric gene associated with dilated and hypertrophic phenotypes of cardiomyopathy. *Circulation* 2005 Jul 5;112(1):54–9. [PubMed: 15998695]
29. Razeghi P, Young ME, Ying J, Depre C, Uray IP, Kolesar J, Shipley GL, Moravec CS, Davies PJ, Frazier OH, Taegtmeyer H. Downregulation of metabolic gene expression in failing human heart before and after mechanical unloading. *Cardiology* 2002;97(4):203–9. [PubMed: 12145475]
30. de Brouwer KF, Degens H, Aartsen WM, Lindhout M, Bitsch NJ, Gilde AJ, Willemsen PH, Janssen BJ, van der Vusse GJ, van Bilsen M. Specific and sustained down-regulation of genes involved in fatty acid metabolism is not a hallmark of progression to cardiac failure in mice. *Journal of Molecular & Cellular Cardiology* 2006;40(6):838–45. [PubMed: 16697005]
31. Lopez-Lazaro M. HIF-1: hypoxia-inducible factor or dysoxia-inducible factor? *FASEB Journal* 2006;20(7):828–32. [PubMed: 16675839]
32. Wykoff CC, Pugh CW, Maxwell PH, Harris AL, Ratcliffe PJ. Identification of novel hypoxia dependent and independent target genes of the von Hippel-Lindau (VHL) tumour suppressor by mRNA differential expression profiling. *Oncogene* 2000;19(54):6297–305. [PubMed: 11175344]
33. Shyu KG, Liou JY, Wang BW, Fang WJ, Chang H. Carvedilol prevents cardiac hypertrophy and overexpression of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in pressure-overloaded rat heart. *Journal of Biomedical Science* 2005;12(2):409–20. [PubMed: 15942707]
34. Minet E, Mottet D, Michel G, Roland I, Raes M, Remacle J, Michiels C. Hypoxia-induced activation of HIF-1: role of HIF-1alpha-Hsp90 interaction. *FEBS Letters* 1999;460(2):251–6. [PubMed: 10544245]
35. Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, Neckers LM. Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway. *Journal of Biological Chemistry* 2002;277(33):29936–44. [PubMed: 12052835]
36. Ebert BL, Firth JD, Ratcliffe PJ. Hypoxia and mitochondrial inhibitors regulate expression of glucose transporter-1 via distinct Cis-acting sequences. *Journal of Biological Chemistry* 1995;270(49):29083–9. [PubMed: 7493931]
37. Hagele S, Behnam B, Bortner E, Wolfe J, Paasch U, Lukashev D, Sitkovsky M, Wenger RH, Katschinski DM. TSGA10 prevents nuclear localization of the hypoxia-inducible factor (HIF)-1alpha. *FEBS Letters* 2006;580(15):3731–8. [PubMed: 16777103]
38. Tan FL, Moravec CS, Li J, Apperson-Hansen C, McCarthy PM, Young JB, Bond M. The gene expression fingerprint of human heart failure. *Proceedings of the National Academy of Sciences of the United States of America* 2002 Aug 20;99(17):11387–92. [PubMed: 12177426]
39. Barrans JD, Allen PD, Stamatiou D, Dzau VJ, Liew CC. Global gene expression profiling of end-stage dilated cardiomyopathy using a human cardiovascular-based cDNA microarray. *American Journal of Pathology* 2002;160(6):2035–43. [PubMed: 12057908]
40. Yang J, Moravec CS, Sussman MA, DiPaola NR, Fu D, Hawthorn L, Mitchell CA, Young JB, Francis GS, McCarthy PM, Bond M. Decreased SLIM1 expression and increased gelsolin expression in failing human hearts measured by high-density oligonucleotide arrays. *Circulation* 2000;102(25):3046–52. [PubMed: 11120693]
41. Blaxall BC, Tschannen-Moran BM, Milano CA, Koch WJ. Differential gene expression and genomic patient stratification following left ventricular assist device support. *Journal of the American College of Cardiology* 2003;41(7):1096–106. [PubMed: 12679207]
42. Wohlschlaeger J, Schmitz KJ, Schmid C, Schmid KW, Keul P, Takeda A, Weis S, Levkau B, Baba HA. Reverse remodeling following insertion of left ventricular assist devices (LVAD): a review of the morphological and molecular changes. *Cardiovascular Research* 2005;68(3):376–86. [PubMed: 16024006]
43. Burkhardt D, Klotz S, Mancini DM. LVAD-induced reverse remodeling: basic and clinical implications for myocardial recovery. *Journal of Cardiac Failure* 2006;12(3):227–39. [PubMed: 16624689]

44. Birks EJ, Hall JL, Barton PJ, Grindle S, Latif N, Hardy JP, Rider JE, Banner NR, Khaghani A, Miller LW, Yacoub MH. Gene profiling changes in cytoskeletal proteins during clinical recovery after left ventricular-assist device support. *Circulation* 2005;112(9 Suppl):I57–64. [PubMed: 16159866]
45. Rodrigue Way A, Burkhoff D, Geesaman BJ, Golden S, Xu J, Pollman MJ, Donoghue M, Jeyaseelan R, Houser S, Breitbart RE, Marks A, Acton S. Sarcomeric genes involved in reverse remodeling of the heart during left ventricular assist device support. *Journal of Heart & Lung Transplantation* 24 (1):73–80.
46. Watanabe A, Arai M, Yamazaki M, Koitabashi N, Wuytack F, Kurabayashi M. Phospholamban ablation by RNA interference increases Ca²⁺ uptake into rat cardiac myocyte sarcoplasmic reticulum. *Journal of Molecular & Cellular Cardiology* 2004;37(3):691–8. [PubMed: 15350842]
47. Fechner H, Suckau L, Kurreck J, Sipo I, Wang X, Pinkert S, Loschen S, Reikittke J, Weger S, Dekkers D, Vetter R, Erdmann VA, Schultheiss HP, Paul M, Lamers J, Poller W. Highly efficient and specific modulation of cardiac calcium homeostasis by adenovector-derived short hairpin RNA targeting phospholamban. *Gene Therapy* 2007;14(3):211–8. [PubMed: 17024101]
48. Arnold AS, Tang YL, Qian K, Shen L, Valencia V, Phillips MI, Zhang YC. Specific beta1-adrenergic receptor silencing with small interfering RNA lowers high blood pressure and improves cardiac function in myocardial ischemia. *Journal of Hypertension* 2007;25(1):197–205. [PubMed: 17143192]
49. Heerdt PM, Holmes JW, Cai B, Barbone A, Madigan JD, Reiken S, Lee DL, Oz MC, Marks AR, Burkhoff D. Chronic unloading by left ventricular assist device reverses contractile dysfunction and alters gene expression in end-stage heart failure. *Circulation* 2000;102(22):2713–9. [PubMed: 11094037]
50. Noguchi T, Hunlich M, Camp PC, Begin KJ, El-Zaru M, Patten R, Leavitt BJ, Ittleman FP, Alpert NR, LeWinter MM, VanBuren P. Thin-filament-based modulation of contractile performance in human heart failure. *Circulation* 2004;110(8):982–7. [PubMed: 15302786]
51. Bristow MR, Krause-Steinrauf H, Nuzzo R, et al. Effect of baseline or changes in adrenergic activity on clinical outcomes in the beta-blocker evaluation of survival trial. *Circulation* 2004;110(11):1437–1442. [PubMed: 15337700]
52. Swedberg K, Bristow MR, Cohn JN, et al. Effects of sustained-release moxonidine, an imidazoline agonist, on plasma norepinephrine in patients with chronic heart failure. *Circulation* 2002;105(15):1797–1803. [PubMed: 11956122]
53. Tevaearai HT, Eckhart AD, Walton GB, et al. Myocardial gene transfer and overexpression of beta2-adrenergic receptors potentiates the functional recovery of unloaded failing hearts. *Circulation* 2002;106(1):124–129. [PubMed: 12093781]
54. Ahmet I, Lakatta EG, Talan MI. Pharmacological stimulation of beta2-adrenergic receptors (beta2AR) enhances therapeutic effectiveness of beta1 AR blockade in rodent dilated ischemic cardiomyopathy. *Heart Fail Rev* 2005;10(4):289–296. [PubMed: 16583177]
55. Birks EJ, Tansley PD, Hardy J, George RS, Bowles CT, Burke M, Banner NR, Khaghani A, Yacoub MH. Left ventricular assist device and drug therapy for the reversal of heart failure. *New England Journal of Medicine* 2006;355(18):1873–84. [PubMed: 17079761]

Figure 1.
ANP transcriptional profiles, * = $p < .05$ vs nonfailing

Figure 2.
Transcriptional Profiles of Contractile Genes, * = $p < .05$ vs nonfailing, # = $p < .1$

Figure 3. Transcriptional Profiles of Metabolic Genes, * = p<.05, # = p<.1

HIF1 α

Figure 4.

Western blot of HIF 1 α (NF= non-failing, F= Failing, Fp=Failing post LVAD human heart tissue)

Table 1

Patient Characteristics.

Patient	Age	Race	Diagnosis (mo c HF)	NYHC	Days on LVAD	LVAD IAP (#days)	Inotrope at time of LVAD (mck/kg/min)	EF %	Renal failure	CO (PCWP)
1	43	white	DCM (1)	4	352	Yes (18)	Dobutamine (7)	10-15	No (Cr 0.8)	4.07 (14)
2	47	white	DCM (128)	4	160	Yes (10)	Dobutamine (8)	10-15	No (Cr 1.1)	4.0 (10)
3	51	AA	DCM (143)	4	298	Yes (8)	Dobutamine (6)	18	Yes (Cr 3.5)	4.0 (23)
4	39	AA	DCM (48+)	4	71	None	Dobutamine (2); Milrinone (0.35)	10	Yes (Cr 2.5)	3.22 (29)
5	56	AA	DCM (24+)	4	266	Yes (14)	Dobutamine (12) Milrinone (0.4)	10	No (1.2)	3.07 (21)
6	35	Hispanic	DCM (120+)	4	232	Yes (2)	Dobutamine (8) Milrinone (0.25)	15	Yes (3.6)	4.07 (28)

Table 2

Meds at time of Transplant. (Y=yes, N=no)

Patient	BB	Ace	ARB	Nitrate	Hydralazine	Spiro	lactone	Amio	Digoxin
1	Y	Y	N	N	N	Y	Y	N	N
2	Y	N	N	N	Y	N	N	N	Y
3	N	Y	N	Y	N	Y	N	Y	Y
4	N	Y	N	N	N	N	N	N	N
5	Y	Y	Y	N	N	Y	Y	N	Y
6	Y	Y	Y	N	N	Y	Y	Y	Y

Table 3**Ontology Report of Differentially Regulated Genes Post LVAD**

Ontology	# Up regulated genes (Z-score)	# Down regulated genes (Z-score)
Cellular process	217 (-1.14)	514 (-0.95)
Physiological process	205 (-0.83)	481 (-1.20)
Regulation of biological processes	87 (1.82)	203 (2.76)
Development	36 (-0.33)	110 (2.54)
Response to Stimulus	35 (-0.83)	86 (-0.83)
Growth	6 (1.12)	11 (0.69)
Reproduction	5 (-0.28)	12 (-0.35)
Other*	17	33

* Other includes interaction between organisms, viral cycles, unknown biological process, and pigmentation.