Myocardial Insulin-Like Growth Factor-I Gene Expression During Recovery From Heart Failure After Combined Left Ventricular Assist Device and Clenbuterol Therapy

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Background—Patients who undergo mechanical support with a left ventricular assist device (LVAD) exhibit reverse remodeling and in some cases recover from heart failure. We have developed a combination therapy using LVAD support combined with pharmacological therapy to maximize reverse remodeling, followed by the β2 adrenergic agonist clenbuterol. We recently found that clenbuterol induces insulin-like growth factor I (IGF-I) in cardiac myocytes in vitro. The purpose of this study is to examine IGF-I expression in recovery patients after combination therapy.

Methods and Results—Myocardial mRNA levels were determined by real-time quantitative polymerase chain reaction in 12 recovery patients (at LVAD implantation, explantation, and 1 year after explantation). IGF-I mRNA was elevated at the time of LVAD explantation relative to donors, with 2 groups distinguishable: Those with low IGF-I mRNA at implantation who showed significant increase during recovery and those with high IGF-I mRNA at implantation who remained high. Levels returned to normal by 1 year after explantation. Microarray analysis of implantation and explantation samples of recovery patients further revealed elevated IGF-II and IGF binding proteins IGFBP4 and IGFBP6. IGF-I levels correlated with stromal cell-derived factor mRNA measured both in LVAD patients and in a wider cohort of heart failure patients.

Conclusions—The data suggest involvement of elevated myocardial IGF-I mRNA in recovery. IGF-I may act to limit atrophy and apoptosis during reverse remodeling and to promote repair and regeneration in concert with stromal cell derived factor. (Circulation. 2005;112[suppl I]:I-46–I-50.)

Key Words: polymerase chain reaction • myocardium • heart failure • growth substances • ventricles

It is becoming increasingly recognized that mechanical support of the failing human heart can result in recovery from heart failure.1 The overall rate of recovery remains low, however, with an estimated frequency of approximately 5%.2 In an attempt to maximize the rate and durability of recovery, we have developed a combination therapy whereby mechanical unloading using left ventricular assist device (LVAD) support is combined with pharmacological therapy aimed at maximizing reverse remodeling and then followed by the use of the β2 adrenergic agonist clenbuterol to stimulate physiological hypertrophy and improve cardiac function (the Harefield Protocol).3,4 To date, this has resulted in approximately two thirds of patients who received the combination therapy showing sufficient recovery to allow device removal without the need for transplantation.5 The mechanisms underlying the process of recovery are unknown.

Clenbuterol can induce physiological hypertrophy in experimental models6 and has the unique ability to reverse pathological, functional, and molecular markers in the pressure-overloaded heart toward normality by normalizing systolic and diastolic function and SERCA2a mRNA levels and reducing collagen deposition.7,8 We recently found that clenbuterol induces insulin-like growth factor I (IGF-I) gene expression in cultured cardiac myocytes in vitro,9 suggesting that local IGF-I may be an autocrine/paracrine mediator of the salutary effects of clenbuterol on the heart. IGF-I is known to exert many beneficial effects on the heart and can improve cardiac function in the failing heart in vivo,10 for example by attenuating the progression of heart failure in a model of dilated cardiomyopathy.11 Transgenic mice with cardiac overexpression of IGF-I initially develop physiological-type hypertrophy,12 and local IGF-I expression has been linked to stem cell recruitment in skeletal muscle13 and to the regenerative capacity of myocardium.11 In humans, IGF-I mRNA is elevated in compensated cardiac hypertrophy.14,15 In this study, we sought to test the hypothesis that myocardial IGF-I gene expression is elevated in patients undergoing combined LVAD and clenbuterol therapy and may therefore
contribute to the recovery process. In addition, we investigated potential interactions between IGF-I and other gene families previously analyzed in these patients.

Methods

Patient Groups
Fifteen dilated cardiomyopathy patients who required LVAD implantation because of deteriorating clinical status with evidence of secondary organ dysfunction in the context of low cardiac output were analyzed. Inclusion criteria were severe heart failure (New York Heart Association functional class IV) due to dilated cardiomyopathy, eligibility for heart transplantation (or expected eligibility after LVAD), and deterioration despite optimal medical treatment leading to the development acute heart failure (cardiac index ≤ 2 L·min⁻¹·m⁻²) associated with high filling pressure (pulmonary capillary wedge pressure ≥ 20 mm Hg) despite inotropic support. Exclusion criteria were evidence of irreversible multigorgan failure, incurable disease (e.g., cancer or metabolic disease), fixed elevated peripheral vascular resistance in presence of severe right ventricular failure, cerebrovascular disease, peripheral vascular disease with trophic lesions, or previous prosthetic replacement of aortic or mitral valves. For the patients studied here, mean duration of heart failure symptoms before LVAD implantation was 43.9 months. All patients were receiving inotropic support at the time of implantation. During LVAD support, patients received a combination therapy composed of mechanical support and administration of β-blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, and spironolactone, followed by administration of clenbuterol to stimulate hypertrophy and improve cardiac function.³ ⁴ Mean ejection fraction at time of implantation was 10.3 ± 5.2%. Mean duration of support was 387 days. Twelve of the 15 patients showed sufficient myocardial recovery to allow removal of the LVAD and were re-analyzed both at the time of device removal and 1 year later. Immediately before explantation, ejection fraction measured after the pump had been turned off for 15 minutes was 65±6%. The study was approved by the Royal Brompton and Harefield ethical review committee and informed consent obtained from patients.

Myocardial Sample Analysis
Myocardial samples were obtained from the LV core taken at time of device implantation (n =15) and LV endomyocardial biopsies taken at time of device removal in the recovery patients (n =12). Where available, LV biopsy samples taken 1 year after device removal were also analyzed (n =8). RNA was extracted using methods adapted for maximal RNA recovery from endomyocardial biopsies¹⁶ and accurately quantified using RiboGreen (Invitrogen Ltd) to ensure equal loading of polymerase chain reactions (PCRs).¹⁶ A control group of RV biopsies from 10 used cardiac transplant donors with normal hemodynamic function was also analyzed, as well as 3 pairs of implant and explant samples from non-recovery LVAD patients after the combination therapy protocol. Reverse-transcription quantitative PCR was performed essentially as described¹⁷ using ABI PRISM 7700 and TaqMan chemistry. Expression levels were normalized using 18S rRNA with output data analyzed as described in figure legends. The IGF-I TaqMan assay used a minor groove binding probe that straddles an obligate splice site between exons 2 and 3 (Genbank accession # NM_000618. Probe: 5’-tggtgtgtgagtaaat-3’, forward primer: 5’-tcgaagctgtgagctgtaa-3’, reverse primer: 5’-gtgtgtgtagctctgcga-3’). The stromal cell derived factor-1 (SDF-1) assay was purchased as a pre-optimized kit from Applied Biosystems (Hs00171022_m1).

HG-U133A Affymetrix microarray analysis was performed essentially as described¹⁸ ¹⁹ using RNA isolated from paired implant LV cores and single LV biopsies from each of 6 patients from the recovery group. Data were analyzed with Gene Expressionist (GeneData AG) using a hierarchical clustering algorithm to assess similarity. Normalized expression values were analyzed using Student’s paired t test.

Results
Initially we compared IGF-I mRNA levels during the time course of recovery (Figure 1). IGF-I mRNA levels were significantly higher at the time of LVAD explantation compared with both donor levels and levels 1 year after explantation. IGF-I mRNA levels showed some elevation relative to those of donors at the time of implantation. There was a further increase in IGF-I mRNA at explantation, although this failed to reach significance relative to the level at implantation. IGF-I mRNA levels were variable at implantation, and 2 expression profiles could be identified in the recovery group (Figure 2). The groups were those with low IGF-I mRNA levels at the time of implantation (1.11 ± 0.16 versus 1 ± 0.26 [donors], P = NS) who showed significant increase during recovery (3.74 ± 0.89-fold increase relative to implant, P < 0.02) (Figure 2A) and those with high IGF-I at the time of implantation (4.25 ± 0.5 versus 1 ± 0.26 [donors], P < 0.001) who showed no significant change with recovery (Figure 2B).

Levels of IGF-I mRNA at implantation were found to be inversely related to time from onset of symptoms (Figure 3a). Patients with high initial IGF-I levels had a significantly shorter time from onset of first symptoms to the time of LVAD support than patients with low IGF-I levels (14 ± 9.8 months versus 70 ± 22 months, P < 0.05). Conversely, levels of IGF-I mRNA at implantation did not correlate with time to recovery (Figure 3B), nor was the degree of change in IGF-I mRNA expression during recovery related to the time of LVAD support (Figure 3C).

We used an unbiased microarray approach to further identify potential alterations in IGF-I signaling that take place during recovery in 6 paired implant and explant samples. This confirmed upregulation of IGF-I (2.38-fold increase at explantation, n = 6, P < 0.05) and further identified changes in IGF-II (1.88-fold increase, P < 0.02) and IGF binding proteins IGFBP4 and IGFBP6 (2.13- and 2.10-fold increase respectively, P < 0.02). There were no significant changes in the IGF receptor, the receptor regulatory subunits IRS-1 and IRS-2, or
the downstream targets phosphoinositide 3-kinase or PKB/Akt.

To identify potential regulatory pathways involving IGF-I, we compared implantation and explantation IGF-I levels with expression levels of other genes previously determined by real-time PCR in the same patient samples. Positive correlation was found with matrix metalloproteinases MMP11 ($r=0.49$, $P<0.001$) and MMP14 ($r=0.73$, $P<0.001$), the tissue inhibitors of MMPs TIMP1 ($r=0.42$, $P<0.01$) and TIMP2 ($r=0.43$, $P<0.01$), and the stem cell factor stromal cell derived factor 1 ($r=0.52$, $P<0.001$) (Figure 4).

**Discussion**

This study is the first to examine IGF-I in LVAD patients in the context of myocardial recovery after mechanical and pharmacological therapy. IGF-I mRNA levels were elevated at the time of LVAD explantation and returned to normal 1 year later (after device removal and cessation of clenbuterol treatment). Alterations in other parts of the IGF-I signaling pathway were also evident at explantation, including elevated IGF-II and IGF binding proteins IGFBP4 and IGFBP6. Close inspection of IGF-I mRNA levels at the time of implantation identified 2 groups of patients defined by IGF-I level at implant. Those with low IGF-I mRNA levels had a longer time from onset of symptoms to the point of requiring LVAD support, suggesting that IGF-I expression may be induced after the onset of heart failure but that levels decline with time. In these patients, the effect of the combination therapy would appear to be related to maintaining high levels rather than elevating IGF-I further. Patients with low IGF-I at implantation showed marked increase between levels at implantation and explantation. The mechanisms leading to elevated IGF-I expression in these patients remain unknown. However, we recently demonstrated that clenbuterol, an integral part of the Harefield bridge to recovery protocol, can

**Figure 2.** IGF-I mRNA levels in individual patients during recovery. Real-time PCR analysis of myocardial samples obtained from recovery patients at implantation, at explantation and 1 year after explant. mRNA levels were normalized to 18S rRNA and are shown on an arbitrary relative scale (calculated as $2^{-\Delta\Delta C_T}$) for patients with (A) low or (B) high levels of IGF-I at implant.

**Figure 3.** IGF-I mRNA levels at time of implantation in relation to time. A, LVAD patients ($n=15$) were grouped according to high (>10) or low (<10) IGF-I as defined in Figure 2 and analyzed in relation to time from onset of symptoms to LVAD. B, Recovery patients were grouped as above and analyzed in relation to time from implantation to explantation. C, Change in IGF-I mRNA abundance in recovery patients relative to time on LVAD support.
induce IGF-I gene expression in cultured cardiomyocytes in vitro and may therefore contribute to inducing IGF-I in vivo. The contribution of mechanical unloading is currently unknown but appears unlikely to contribute directly to IGF-I expression, as previous studies using real-time PCR and microarray analysis have failed to detect elevated IGF-I in LVAD patients (those not taking clenbuterol and who do not recover) and mechanical unloading of skeletal muscle does not induce IGF-I.

IGF-I is known to exert a number of potentially beneficial effects on the myocardium, including countering anti-apoptotic signaling, inducing adaptive hypertrophy, and reducing fibrosis. The data presented here are therefore consistent with the hypothesis that elevated IGF-I plays a positive role in the process of recovery. Our study differs from the only previous study on IGF-I in LVAD patients because we have shown that structural remodeling and reversal of myocyte hypertrophy seen in many LVAD patients during mechanical unloading is not in itself an indicator of recovery. Our patient group is also distinguished by the use of clenbuterol. In the study by Razeghi et al., no change in IGF-I mRNA was observed during LVAD support and there was no evidence for IGF-I pathway activation (absence of Akt and IRS1 phosphorylation). This further suggests that elevated IGF-I may be specific to the recovery process. Our data derived from microarray analysis of paired implant and explant samples also point to recovery-specific changes in mRNA levels of the IGF binding proteins and the stem cell recruitment factor SDF-1 (see Figure 4), which has previously been shown to play a role in the myocardial response to damage. Recent studies have shown that local IGF-I production may act as a stem cell recruitment factor in skeletal muscle and cardiac over-expression of IGF-I in heart has been shown to elevate markers of cell division and stem cell recruitment. It is therefore possible that elevated IGF-I acts as part of a survival and regeneration program in recovery. Our data show that SDF-1 and IGF-I mRNA levels correlate both in LVAD patients and in a wider cohort, suggesting this correlation may indeed be a general feature of heart failure.

### Conclusion

The data presented here demonstrate that elevated IGF-I mRNA at explantation is a feature of LVAD patients who receive the HeartWare protocol and who recover from heart failure. The mechanisms leading to elevated IGF-I expression remain unknown but may relate to the use of clenbuterol.

### Acknowledgments

The authors are grateful to the British Heart Foundation, the Magdi Yacoub Institute, The Royal Brompton and Harefield Charitable Trustees, The Lillehei Heart Institute University of Minnesota, and Thoratec Corporation for supporting this work.

### References


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**Figure 4.** IGF-I and SDF-1 mRNA levels correlate in a wide cohort of patients. Individual mRNA levels of IGF-I and SDF-1 were determined by real-time PCR in a large cohort of patients, including LVAD recovery patients and donor organs as described here, and end-stage heart failure as previously described. Data were normalized to 18S rRNA, plotted as raw ΔCt values and analyzed by Spearman rank correlation (n = 83, r = 0.51, P < 0.0001).


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_Circulation_. 2005;112:I-46-I-50
doi: 10.1161/01.CIRCULATIONAHA.105.525873

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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