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Left Ventricular Assist Device Implantation Augments Nitric Oxide Dependent Control of Mitochondrial Respiration in Failing Human Hearts

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OBJECTIVES The objective of the study was to evaluate nitric oxide (NO) mediated regulation of mitochondrial respiration after implantation of a mechanical assist device in end-stage heart failure.

BACKGROUND Ventricular unloading using a left ventricular assist device (LVAD) can improve mitochondrial function in end-stage heart failure. Nitric oxide modulates the activity of the mitochondrial electron transport chain to regulate myocardial oxygen consumption (MVO_2).

METHODS Myocardial oxygen consumption was measured polarographically using a Clark-type oxygen electrode in isolated left ventricular myocardium from 26 explanted failing human hearts obtained at the time of heart transplantation.

RESULTS The rate of decrease in oxygen concentration was expressed as a percentage of baseline. Results of the highest dose of drug are shown. Decrease in MVO_2 was greater in LVAD hearts ($n = 8$) compared with heart failure controls ($n = 18$) in response to the following drugs: bradykinin ($-34 \pm 3\%$ vs. $-24 \pm 5\%$), enalaprilat ($-37 \pm 5\%$ vs. $-23 \pm 5\%$) and amlodipine ($-43 \pm 13\%$ vs. $-16 \pm 5\%$; $p < 0.05$ from controls). The decrease in MVO_2 in LVAD hearts was not significantly different from controls in response to diltiazem ($-22 \pm 5\%$ in both groups) and exogenous NO donor, nitroglycerin ($-33 \pm 7\%$ vs. $-30 \pm 3\%$). N^w-nitro-L-arginine methyl ester, inhibitor of NO synthase, attenuated the response to bradykinin, enalaprilat and amlodipine. Reductions in MVO_2 in response to diltiazem and nitroglycerin were not altered by inhibiting NO.

CONCLUSIONS Chronic LVAD support potentiates endogenous NO-mediated regulation of mitochondrial respiration. Use of medical or surgical interventions that augment NO bioavailability may promote myocardial recovery in end-stage heart failure. (J Am Coll Cardiol 2000;36: 1897–902) © 2000 by the American College of Cardiology

Structural and functional mitochondrial abnormalities have been described in heart failure with evidence of impaired oxidative phosphorylation and energy production (1–3). Nitric oxide (NO) released from vascular endothelium is an important regulator of mitochondrial respiration and tissue oxygen consumption (4). Nitric oxide binds reversibly at low micromolar concentrations to the oxygen binding site of cytochrome oxidase, the terminal enzyme complex of the electron transport chain, to decrease tissue oxygen consumption. Under conditions of lowered oxygen availability, NO can bind with greater affinity to the enzyme and may help decrease oxygen consumption to match tissue oxygen supply (5). Heart failure is associated with impaired endothelial NO production (6). Recchia et al. (7) showed that loss of endogenous NO in canine heart failure is associated with loss of regulation of myocardial oxygen consumption (MVO_2) and progression from compensated to decompensated heart failure.

Angiotensin-converting enzyme (ACE) inhibitors and amlodipine can regulate MVO_2 by promoting kinin-dependent NO production in coronary microcirculation (8,9). However, despite pharmacologic therapy some patients progress to severely decompensated heart failure, requiring the implantation of a mechanical assist device as a bridge to transplant. Ventricular unloading using a left ventricular assist device (LVAD) has been shown to decrease myocardial workload and improve exercise performance (10) as well as improve efficiency of mitochondrial metabolism (11). The role of NO in mediating this regulation has not been previously studied. We proposed the hypothesis that improved mitochondrial function after mechanical circulatory support in heart failure is related, in part, to augmentation of endogenous NO production. This hypothesis was tested by measuring MVO_2 at baseline and in response to NO stimulating drugs in LVAD-supported hearts and by comparing them with hearts with end-stage heart failure but without LVAD support.

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Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
L-NAME = N^w-nitro-L-arginine methyl ester
LVAD = left ventricular assist device
MVO_2 = myocardial oxygen consumption
NO = nitric oxide

METHODS

Tissue preparation. Myocardial tissue was isolated from the left ventricular free wall of hearts harvested from 26 patients with end-stage heart failure at the time of orthotopic cardiac transplantation and transported immediately in iced normal saline. Studies of the human heart were approved by the institutional review board, and the investigators were blinded to the identity of the patients. The myocardium was freed of epicardium, endocardium, connective tissue, fat and large arteries and was cut into 30 to 50 mg segments. The muscle slices were incubated for 2 h in Krebs-bicarbonate buffer (containing the following in mM: 118 NaCl, 4.7 KCl, 1.5 $CaCl_2$, 25 $NaHCO_3$, 1.1 $MgSO_4$, 1.2 KH_2PO_4 and 5.6 glucose) through which 21% O_2/5% CO_2/74% N_2 (room air) was bubbled continuously.
Experimental protocols. Myocardial oxygen consumption was measured polarographically using a Clark-type oxygen electrode (YSI 5331, Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio) at 37°C in a stirred bath (YSI 5301) containing 3 ml Krebs solution buffered with 10 mMol/L HEPES (pH 7.4). Tissue respiration was calculated as the rate of decrease in oxygen concentration after the addition of muscle slices, assuming an initial oxygen concentration of 224 nmol/ml and was expressed as nanomoles of oxygen consumed per minute per gram of tissue. After measurement of baseline MVO_2, cumulative dose-response curves were generated by the addition of various pharmacologic agents to separate tissue baths in increasing concentrations. Approximate observation time for each dose of agent was 5 to 7 min. Succinate, a substrate for the electron transport chain (1 mMol/L) followed by sodium cyanide, an inhibitor of cytochrome oxidase, (1 mMol/L) were added at the end of each experiment to confirm that the change in MVO_2 was reversible and was from mitochondrial sources.

The following agents were tested: bradykinin, a stimulator of endogenous NO production (10^{-7} to 10^{-4} M, Sigma, St Louis, Missouri), enalaprilat, an ACE inhibitor (10^{-7} to 10^{-4} M, Sigma, St. Louis, Missouri), amlodipine and diltiazem, calcium-channel blockers (10^{-7} to 10^{-5} M, Pfizer, Groton, Connecticut and Sigma, St. Louis, Missouri, respectively) and nitroglycerin, an exogenous NO donor (2.5×10^{-6} to 2.5×10^{-3} M, Parke-Davis, Morris Plains, New Jersey). To assess the role of endogenous NO, the above experiments were repeated after preincubation of the muscle segments with N^w-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase (10^{-4} M, Aldrich Chemical Co., Chicago, Illinois).

Table 1. Clinical Characteristics

	HF	LVAD-HF
Number	18	8
Age (yrs)	30 ± 6	46 ± 6
Gender		
Female	4	2
Male	14	6
Etiology		
Ischemic cardiomyopathy	3	3
Nonischemic cardiomyopathy	15	5
Ejection fraction (%)	18 ± 6	21 ± 1
Cardiac index (L/min/m^2)	2.2 ± 0.3	1.6 ± 0.2
Mixed venous saturations (%)	53 ± 3	44 ± 5
Pretransplant ACE inhibitors	11 (61%)	6 (75%)
Pretransplant inotropes	12/18	0/8

HF = heart failure; LVAD = left ventricular assist device.

Statistical analysis. All results are expressed as mean ± SEM of percent change in MVO_2 from baseline. Data were analyzed using a two-way repeated measures analysis of variance, with a Student-Neuman-Keuls post-hoc analysis to identify which means were different (Sigma Stat, Version 2.03, Jandel Scientific, San Rafael, California). A p value < 0.05 was considered significant.

RESULTS

Clinical profile. Left ventricular myocardium was obtained from 26 failing hearts at the time of cardiac transplantation between March 1997 and July 1999. Eight patients had LVAD support for a mean period of 96 ± 15 days before cardiac transplantation. The remaining 18 patients without ventricular assist devices comprised the control heart failure group. Patients included 20 men and 6 women. Age range was 18 to 64 years (mean age = 47 ± 4 years). Diagnoses included idiopathic dilated cardiomyopathy (n = 11), ischemic cardiomyopathy (n = 6), hypertrophic cardiomyopathy (n = 2), end-stage complex congenital heart disease (n = 5), postpartum cardiomyopathy (n = 1) and primary pulmonary hypertension (n = 1). All patients were in New York Heart Association class IV and had hemodynamics consistent with end-stage heart failure. Average values were as follows: ejection fraction, 20 ± 1%, cardiac index, 2 ± 0.2 L/min/m^2; mixed venous hemoglobin saturations, 50 ± 3%.

Clinical characteristics of the patients in the two groups are shown in Table 1. Hemodynamic data in the LVAD group reflect hemodynamics before LVAD implantation. Hemodynamics measured before transplant or LVAD implantation were consistent with severe heart failure in both groups of patients. After LVAD implantation follow-up cardiac catheterization data were available in two patients (one to two months after LVAD). Both these patients showed hemodynamics consistent with improved cardiac output, including decrease in pulmonary capillary wedge pressure from 28 to 3, increase in mixed venous hemoglobin saturations from 44% to 57% and increase in cardiac index from 2 to 3 L/min/m^2. Further, inotropic support was successfully discontinued in all eight patients after LVAD

placement. Six of eight patients were continued on an ACE inhibitor as the only cardiac medication. Therefore, ventricular unloading with a mechanical assist device was characterized by both clinical and hemodynamic improvement.

MVO_2. *MVO_2 in LVAD and heart failure controls.* Baseline MVO_2 was not significantly different in the LVAD group compared with the control group (195 ± 17 nmol/g/min in LVAD vs. 196 ± 11 nmol/g/min in control heart failure). Cumulative doses of bradykinin, enalaprilat, amlodipine, diltiazem and nitroglycerin caused concentration-dependent decreases in oxygen consumption in myocardium from both groups. However, drugs that promote endogenous NO synthesis caused a greater reduction in MVO_2 in the LVAD group compared with the control heart failure group. The rates of decrease in oxygen consumption at the highest dose in LVAD versus the control group, respectively, with these drugs were as follows: bradykinin (−34 ± 3% vs. −24 ± 5%, $p < 0.05$) enalaprilat (−37 ± 5% vs. −23 ± 5%, $p < 0.05$), amlodipine (−43 ± 13% vs. −16 ± 5%, $p < 0.001$) (Fig. 1). Responses to NO-independent calcium channel blocker, diltiazem and to exogenous NO donor, nitroglycerin, were not significantly different in LVAD versus the heart failure group: diltiazem (−22 ± 5% in both groups), nitroglycerin (−33 ± 7% vs. −30 ± 3%) (Fig. 2).

Effect of inhibiting NO on oxygen consumption. Responses to bradykinin, enalaprilat and amlodipine were significantly attenuated by L-NAME, an inhibitor of NO. Responses to nitroglycerin and diltiazem were not altered by L-NAME. Results for the LVAD group are shown in Figure 3A.

Effect of succinate and cyanide on MVO_2. Succinate is an NAD-independent/FAD-dependent substrate that donates electrons to the mitochondrial electron transport chain to stimulate mitochondrial respiration and oxygen consumption. Succinate was added at the end of the highest dose of each drug. As seen in Figure 3B, the addition of succinate at the end of each experiment increased oxygen consumption in both groups of hearts confirming that the drugs were acting on the electron transport chain and that their effect was fully reversible. Sodium cyanide, an inhibitor of cytochrome oxidase, consistently decreased oxygen consumption by 60% to 80% in both groups confirming that the changes in oxygen consumption in response to the drugs were of mitochondrial origin.

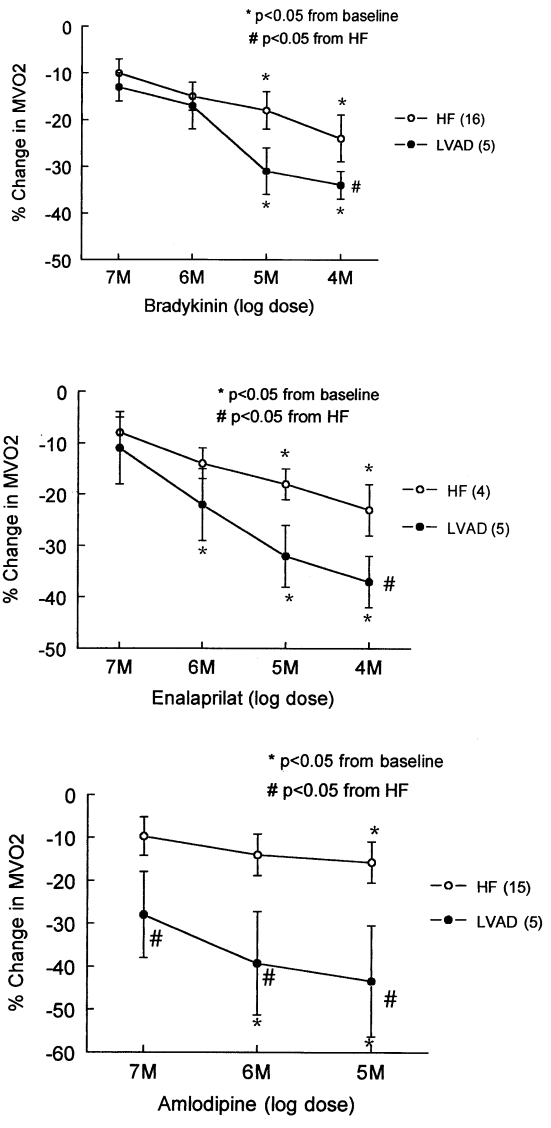

Figure 1. Bradykinin, enalaprilat and amlodipine caused a dose-dependent reduction in myocardial oxygen consumption in failing human hearts. Left ventricular assist device HF group **(solid circles)** showed a greater reduction in oxygen consumption compared with the control HF group **(open circles)**. *$p < 0.05$ from baseline; #$p < 0.05$ from HF. HF = heart failure; LVAD = left ventricular assist device; MVO_2 = myocardial oxygen consumption.

DISCUSSION

This study demonstrated that NO regulates oxygen consumption in failing explanted human hearts. The most important new finding of the study is that chronic LVAD support in end-stage heart failure is associated with enhanced NO mediated regulation of mitochondrial respiration.

Mitochondrial function in heart failure. Mitochondrial function may be reduced in heart failure (1–3). Studies from our laboratory have shown an important role for NO in regulating mitochondrial respiration in the heart. By regulating mitochondrial respiration, NO may help match tissue oxygen consumption to availability. This is important in both physiologic and pathophysiologic states. Inhibition of NO increases whole body, as well as cardiac, oxygen consumption (12,13). The ability of blood vessels to synthesize NO is markedly reduced in heart failure secondary to decreased expression of NO synthase (6,14), and this may contribute to the progression of heart failure (7).

Recent evidence suggests that chronic ventricular unloading using a mechanical assist device can improve mitochondrial function and promote myocardial recovery with reversal of left ventricular remodeling and improvement in myocyte contraction (15,16). Our results indicate that the

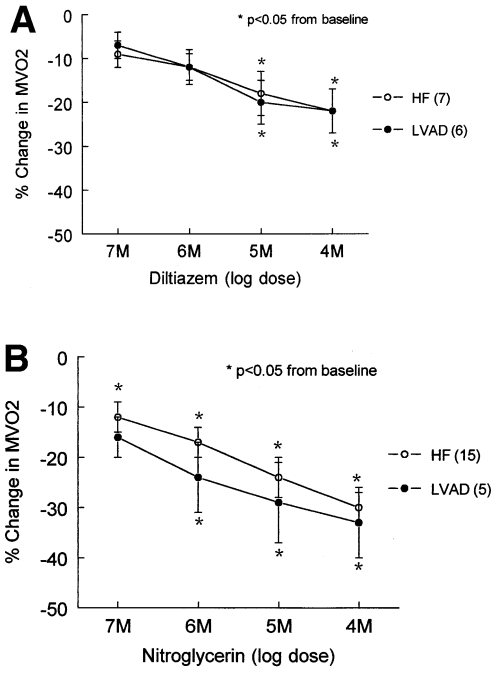

Figure 2. (A) Diltiazem and **(B)** nitroglycerin caused dose-dependent reductions in MVO_2 in failing human hearts. There was no difference in the response of the LVAD **(solid circles)** and HF **(open circles)** groups to diltiazem or nitroglycerin. *$p < 0.05$ from baseline. HF = heart failure; LVAD = left ventricular assist device; MVO_2 = myocardial oxygen consumption.

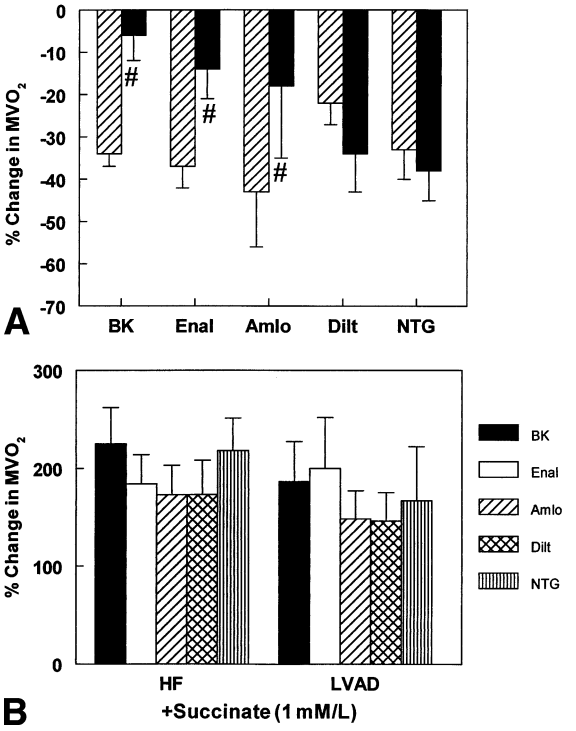

Figure 3. (A) The effect of L-NAME, inhibitor of nitric oxide synthesis, on MVO_2 at the highest doses of drug in the LVAD group is shown. N^w-nitro-L-arginine methyl ester attenuated the effect of bradykinin, enalaprilat and amlodipine on oxygen consumption. N^w-nitro-L-arginine methyl ester had no effect on decrease in oxygen consumption in response to diltiazem and nitroglycerin. **Striped bar** = LVAD-HF; **solid bar** = +L-NAME. #$p < 0.05$ from control. **(B)** Effect of succinate on myocardial oxygen consumption. Succinate, NAD-independent substrate of the electron transport chain, increased MVO_2 in both HF and LVAD-HF groups, and this response was not different between the two groups. Amlo = amlodipine; BK = bradykinin; Dilt = diltiazem; Enal = enalaprilat; HF = heart failure; L-NAME = N^w-nitro-L-arginine methyl ester; LVAD = left ventricular assist device; MVO_2 = myocardial oxygen consumption; NTG = nitroglycerin.

improvement in mitochondrial function after LVADs may be related, in part, to restoration of NO biosynthesis in failing hearts.

MVO_2 in failing hearts after LVAD. We have previously shown that ACE inhibitors and amlodipine, a calcium channel blocker, cause kinin-dependent NO production in coronary microvessels to regulate MVO_2 (17,18). This study showed that all the NO dependent agonists: bradykinin, enalaprilat and amlodipine, caused significantly larger reductions in MVO_2 in LVAD hearts compared with controls. This augmentation of response in LVAD-supported hearts was seen in myocardium from both ischemic and nonischemic cardiomyopathy and may have been secondary to either increased NO availability or to improved mitochondrial function. However, there was no augmentation of response when a NO-independent agent, diltiazem, was used or when NO was supplied exogenously using nitroglycerin. This indicates that baseline mitochondrial function was comparable between the two groups and that enhancement of response in the LVAD group was secondary to increased NO availability. This ability to alter mitochondrial function also suggests that mitochondria may not be irreversibly damaged in all heart failure and that restoring NO availability can at least partially restore regulation of MVO_2. It is important to note that, despite augmented response to NO agonists, baseline MVO_2 in the LVAD group was not significantly different from that in non-LVAD hearts. These estimations were made in isolated myocardium, which is independent of preload and afterload. In the absence of vascular shear stress, one of the most potent physiologic stimulators of NO production, differences in basal NO levels between the two groups may not have been large enough to cause a measurable change in basal oxygen consumption.

One of the potential mechanisms of the increase in NO production after ventricular unloading may be the amelioration of endothelial dysfunction secondary to a decrease in wall stress. Although we did not measure NO synthase expression, the rapid (within minutes) decrease in oxygen consumption and the response to kinin agonists suggests the involvement of endothelial, rather than inducible, NO synthase (19).

Lee et al. (11) studied myocardial mitochondrial function by comparing mitochondrial respiratory rates after LVAD implantation in heart failure. They reported that the respiratory control index was greater in the LVAD group compared with controls when NAD-dependent substrates were used but not with succinate, a NAD-independent substrate, which represents a less efficient use of oxygen.

Our study differs in that we did not investigate different respiratory states since the primary focus of our study was to evaluate the effect of NO on mitochondrial respiration as a whole. However, our results with succinate, which demonstrated a comparable increase in oxygen consumption in both LVAD and control heart failure groups, are consistent with the findings of Lee et al. (11). They also indicate that the decrease in oxygen consumption seen with NO is at the level of the electron transport chain and is fully reversible, confirming a modulatory role for NO.

Clinical implications. The ability of LVADs to improve NO-mediated regulation of MVO_2 in failing human hearts may have important clinical implications. The potential of LVADs to cause myocardial recovery has prompted research into the use of VADs as permanent palliation rather than as a bridge to transplantation. Clinical studies evaluating the outcome of LVAD explantation, however, have reported mixed results. While Muller et al. (20) identified 5 of 17 patients with dilated cardiomyopathy in whom LVADs were successfully explanted with maintenance of normal cardiac function, Mancini et al. (21) identified only 5 of 111 patients from whom LVADs were successfully explanted. None of these patients could sustain normal cardiac function. Therefore, the extent of myocardial recovery after LVADs is highly variable. Of note, the patients in the German study not only had a longer duration of LVAD support but were also maintained on maximal medical regimen including ACE inhibitors and coenzyme Q10, an electron transport carrier, both of which have the potential to improve mitochondrial function. Our study suggests that if increasing NO production improves regulation of mitochondrial function, then concomitant use of NO agonist drugs with mechanical assist devices may accelerate myocardial recovery in heart failure.

Study limitations. The major limitation of the study was the lack of suitable controls and the heterogeneity of the patient population studied. The inability to obtain sufficient ventricular tissue from the failing hearts at the time of LVAD implantation prevented paired analysis of the same heart at the time of transplantation, that is, before and after ventricular unloading. We, therefore, compared LVAD supported hearts with non-LVAD hearts. The major concern using this comparison is the heterogeneity of the patient population studied, especially with regard to etiology of heart failure and pretransplant exposure to inotropes and medications. It is important to recognize, however, that, despite different etiologies, all patients were in decompensated, end-stage heart failure at the time of study. The pretransplant use of ACE inhibitors and inotropes may potentially alter NO availability in failing hearts. However, most patients in both groups were receiving ACE inhibitors. Therefore, ACE inhibitor use was unlikely to account for the differences between the two groups (Table 1). The majority of patients with heart failure in the control group were on inotropic support. Beta-adrenergic stimulation has been shown to increase NO release in cardiomyocytes (22). However, since none of the patients in the LVAD group were on inotropic support after LVAD placement, inotropic support is unlikely to account for the increased NO availability in LVAD hearts.

Conclusions. This study demonstrated that ventricular unloading augments NO-mediated regulation of mitochondrial respiration in end-stage heart failure. Use of medical or surgical interventions that augment NO availability may act synergistically to optimize myocardial recovery and mitochondrial function in end-stage heart failure.

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