Heart Failure

Ventricular Assist Device Implantation Corrects Myocardial Lipotoxicity, Reverses Insulin Resistance, and Normalizes Cardiac Metabolism in Patients With Advanced Heart Failure

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Background—Heart failure is associated with impaired myocardial metabolism with a shift from fatty acids to glucose use for ATP generation. We hypothesized that cardiac accumulation of toxic lipid intermediates inhibits insulin signaling in advanced heart failure and that mechanical unloading of the failing myocardium corrects impaired cardiac metabolism.

Methods and Results—We analyzed the myocardium and serum of 61 patients with heart failure (body mass index, 26.5±5.1 kg/m²; age, 51±12 years) obtained during left ventricular assist device implantation and at explantation (mean duration, 185±156 days) and from 9 control subjects. Systemic insulin resistance in heart failure was accompanied by decreased myocardial triglyceride and overall fatty acid content but increased toxic lipid intermediates, diacylglycerol, and ceramide. Increased membrane localization of protein kinase C isozymes, inhibitors of insulin signaling, and decreased activity of insulin signaling molecules Akt and Foxo were detectable in heart failure compared with control subjects. Left ventricular assist device implantation improved whole-body insulin resistance (homeostatic model of analysis–insulin resistance, 4.5±0.6–3.2±0.5; P<0.05) and decreased myocardial levels of diacylglycerol and ceramide, whereas triglyceride and fatty acid content remained unchanged. Improved activation of the insulin/phosphatidylinositol-3 kinase/Akt signaling cascade after left ventricular assist device implantation was confirmed by increased phosphorylation of Akt and Foxo, which was accompanied by decreased membrane localization of protein kinase C isozymes after left ventricular assist device implantation.

Conclusions—Mechanical unloading after left ventricular assist device implantation corrects systemic and local metabolic derangements in advanced heart failure, leading to reduced myocardial levels of toxic lipid intermediates and improved cardiac insulin signaling. (Circulation. 2012;125:2844-2853.)

Key Words: heart failure • lipids • metabolism • myocardium • ventricular assist device

A dvanced heart failure (HF) is associated with structural, functional, inflammatory, and metabolic derangements of the failing myocardium that develop and worsen during progression of the disease state.¹² Metabolic abnormalities of the failing myocardium are characterized by transcriptional changes with suppression of genes regulating fatty acid uptake and oxidation while the expression of genes controlling glucose metabolism remains relatively stable,²⁻⁴ a transcriptional program that is typical of embryogenesis.¹⁵⁶ The overall net result is a relative decrease in the metabolism and oxidation of fatty acids (FAs) and a shift toward glucose for primary ATP generation. These changes are also accompanied by profound mitochondrial dysfunction and impaired overall oxidative metabolism.⁷

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Besides this myocardial dysbalance of glucose and FA metabolism, patients with advanced HF also develop systemic insulin resistance (IR), and worse IR correlates with greater morbidity and mortality.⁸ Circulating proinflammatory cytokines such as tumor necrosis factor-α and interleukin-1β and dysregulation of adipokines have been
linked to the development and progression of IR in HF, which parallels metabolic derangements previously described in patients with abnormal glucose homeostasis indicative of early diabetes mellitus.

The complex network of cellular lipid metabolism pathways must supply substrates for the generation of ATP via mitochondrial β-oxidation, production of cellular structural components, and creation of signaling molecules that regulate a number of cellular processes, including insulin signaling. Less than 5% of total body triglycerides are stored in the nonadipose tissues such as skeletal muscle and myocardium; nevertheless, these neutral lipids can serve as an important energy storage form, whereas pathologic lipid accumulation in skeletal muscle can trigger IR. Several intermediates of lipid metabolism have toxic proapoptotic and proinflammatory actions. Skeletal muscle and myocardium of patients with diabetes mellitus have increased levels of diacylglycerol and ceramide, which have been directly linked to mitochondrial dysfunction and impaired intracellular glucose and FA oxidation. Intracellular lipid accumulation associated with such deleterious effects on cellular function has been called lipotoxicity. Proof that lipids themselves are toxic has come from studies showing that overexpression of enzymes that increase intracellular lipid levels leads to IR and that gene deletion of these enzymes, which reduces toxic levels of intracellular lipids, improves insulin sensitivity. In addition, induced lipid accumulation in the heart leads to cardiac dysfunction.

The aim of our study was to compare levels of intracellular lipids between patients with advanced HF and control subjects and to test the hypothesis that mechanical unloading through left ventricular assist device (LVAD) implantation leads to reversal of metabolic derangements in the failing myocardium.

Methods

Patient Cohort and Sample Collection

We retrospectively analyzed 61 patients (52 male, 9 female) with advanced HF undergoing LVAD implantation at Columbia University Medical Center. Patients received either pulsatile-flow LVADs (n = 30) or continuous-flow LVADs (n = 31). Clinical and laboratory characteristics of all patients and hemodynamic conditions within 5 days before LVAD implantation and explantation were collected. Myocardial specimens were collected from a subset of patients (n = 21), and blood samples were obtained from all patients (n = 61) at the time of LVAD implantation for end-stage HF as a bridge to transplantation and at the time of LVAD explantation during cardiac transplantation. Control blood samples were obtained from patients without cardiovascular disease recruited at the Columbia University Medical Center (n = 10). Control myocardial samples (n = 6) were obtained from a tissue bank of deidentified specimens collected from nonfailing hearts determined to be unusable for cardiac transplantation because of acute recipient issues or donor coronary artery disease but without evidence of previous infarction. The newly obtained heart samples were immediately snap-frozen, placed in liquid nitrogen for transport, and stored at −80°C until final analysis.

The present study was approved by the Institutional Review Board of Columbia University. All patients provided written informed consent before inclusion in the study.

Echocardiographic Analysis

Conventional echocardiograms were obtained from all patients within 5 days before LVAD implantation and at 1 month after the surgery (Sonos-5500 or Sonos-7500; Philips Healthcare Corp, Andover, MA). The routine standard echocardiographic examination included M-mode, 2-dimensional echocardiogram, and Doppler study for measurements of ventricular septal and posterior wall thickness and end-systolic and end-diastolic left ventricular diameters. Left ventricular ejection fraction was calculated by the biplane Simpson method from apical 4- and 2-chamber views. Mitral inflow was obtained by pulsed-wave Doppler echocardiography with the sample volume between mitral leaflet tips during diastole, and peak early (E) and late (A) transmitral filling velocities, their ratio (E/A), and deceleration time of E were measured. The peak positive dP/dt (dP/dtmax; the first derivative of left ventricular pressure with respect to time) as an index of contractility was calculated from continuous-wave Doppler determination of the velocities in mitral regurgitant jets. Early diastolic annular velocity (E') was obtained by placing a tissue Doppler sample volume at the septal and lateral mitral annulus in the apical 4-chamber view, and the E/E' ratio was calculated. Measurements were performed from 5 cardiac cycles and averaged.

Serum Analysis

Venous blood samples were collected and stored after centrifugation at −70°C until the assays were performed. Levels of insulin were measured with commercially available ELISA kits (CalBiotech, Spring Valley, CA). The homeostatic model of analysis–insulin resistance was used to described the levels of insulin resistance in all patients and control subjects. Circulating levels of brain natriuretic peptide were analyzed by the institutional core laboratory.

Tissue Culture

A human ventricular cardiomyocyte-derived cell line, designated AC-16, was kindly provided by M.M. Davidson. Cells were maintained in Dulbecco modified Eagle medium/nutrient mixture F-12 (Ham; Invitrogen, Carlsbad, CA). Experiments were performed on cells at 70% to 80% confluence after 4 hours of starvation. Cells were exposed to 0.4 mmol/L palmitic acid (PA; Sigma Aldrich) in methanol conjugated with 1% FA/free BSA overnight (16 hours). Control cells were incubated in the presence of 0.25% vehicle (methanol). After overnight treatment with PA (0.4 mmol/L for 16 hours), cells were incubated for different time intervals with 500 nm insulin (Sigma Aldrich) diluted in HEPES buffer for different time intervals in the presence or absence of protein kinase C (PKC) inhibitor (RO-318220; Sigma).

Gene Expression Analysis

Total RNA was extracted with standard methods, and the abundance of specific mRNAs was determined by reverse transcriptase–polymerase chain reaction with a Prism 7700 Sequence Detector (Perkin-Elmer, Foster City, CA). Sequences of primers and TaqMan probes specific for cluster of differentiation 36 (CD36), carnitine palmitoyltransferase-1 (CPT1), acyl-coenzyme A oxidase, glucose transporter type 1, glucose transporter type 4, insulin receptor pyruvate dehydrogenase kinase-4, adipose triglyceride lipase (ATGL), hormone-sensitive lipase, diglyceride acyltransferase 1 and 2 (DGAT1 and DGAT2), and brain natriuretic peptide were described previously. Gene expression was normalized by expression of 18S mRNA and expressed as relative expression.

Protein Analysis

Tissues and cells were lysed in 200 μL lysis buffer containing 20 mmol/L Tris-HCl (pH 8.0), 2 mmol/L EDTA, 2 mmol/L EGTA, 6 mmol/L β-mercaptoethanol, 0.1 mmol/L sodium vanadate, 50 mmol/L sodium fluoride, protease inhibitors, and phosphatase inhibitors (Roche, Indianapolis, IN). Protein extraction was performed with standard techniques. Protein lysates from cells were resolved on 4% to 15% SDS-PAGE-reducing gels (Bio-Rad), transferred to polyvinylidene difluoride membranes (Bio-Rad), blocked in 5% milk/TRIS-buffered saline, and probed with antibodies for detection of phosphorylated Akt, total Akt, phosphorylated Foxo, total Foxo, and GAPDH (all from Cell Signaling Technologies Inc). Membranes were washed, incubated with appropriate secondary antibodies conjugated to horse-
radish peroxidase, washed in TBS-Tween, and detected by use of ECL Western Blotting System Kit (Thermo Scientific).

Analysis of PKC isoform localization and activity was performed after protein isolation. Membrane and cytosolic fractions were separated by ultracentrifugation at 33,000 rpm for 1 hour. Then, 25 μg from each fraction was applied to SDS-PAGE and transferred onto nitrocellulose membranes, and PKC isoforms were detected with specific antibodies (PKCα from Millipore, Billerica, MA; PKCβ, PKCδ, and PKCε from Santa Cruz Biotechnology, Santa Cruz, CA). Specific PKC activity was measured with the nonradioactive PKC kinase activity kit (Assay Designs, Ann Arbor, MI) as previously described.15

Lipid Measurements
Total lipids were extracted by the Folch method of extraction. Briefly, 25 to 50 mg of tissue or cell pellet was homogenized in PBS and chloroform:methanol (2:1). The organic phase was separated twice and dried. The dried lipids were solubilized in 1% Triton X-100, dried, and resuspended in distilled water. Cardiac triglyceride content was measured by use of the Infinity Triglycerides Kit (Thermo Scientific) with the Matrix Plus Chemistry Reference Kit (Vericell Laboratory Inc) as standard. Total FAs were quantified as the sum of individual FAs detected by liquid chromatography—mass spectrometry—based lipidomics (Waters Xevo TQ MS ACQUITY UPLC System; Waters, Milford, MA). Total diacylglycerol and ceramide were detected with the diacylglycerol kinase method as previously described.24

Histology
Histopathology was assessed on myocardial specimens obtained at the time of LVAD implantation and at the time of explantation after paraffin fixation and hematoxylin and eosin and Masson trichrome staining. Myocardial fibrosis was quantified on serial sections with the use of Masson trichrome staining for the detection of collagen. Area of fibrosis was analyzed with the ImagePro software (National Institutes of Health) and expressed as percent of total section area. Myocyte cross-sectional area was analyzed after semiquantitative analysis with circumferential markings of the myocytes with ImagePro software and expression of average cross-sectional area and distribution of fiber sizes within each sample.

Statistical Analysis
Data are presented as mean±SD. Normality was evaluated for each variable from normal distribution plots and histograms and by the Kolmogorov-Smirnov test. The echocardiographic and laboratory variables, serum and myocardial gene expression before and after surgery, and percent changes of the variables through the surgery were compared between groups with the Student unpaired t test for groups with parametric distribution and the Mann-Whitney U test for samples with nonparametric distribution. The values before and after surgery in each group of patients were assessed with the Student paired t test for groups with parametric distribution and the Wilcoxon test for samples with nonparametric distribution. Comparisons of >2 groups were performed by use of ANOVA with appropriate post hoc testing. A value of P<0.05 was considered statistically significant. All data were analyzed with SPSS version 18 (IBM).

Results
Baseline Characteristics
We analyzed a total of 61 patients undergoing placement of a LVAD at our institution between 1998 and 2010. Clinical characteristics of all patients are summarized in Table 1. The duration of LVAD support ranged from 31 to 662 days (mean, 176 days). Patients with HF had generalized IR by the homeostatic model of analysis–insulin resistance (3.7±1.8 in HF versus 1.1±0.6 in control subjects; P<0.01).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Before LVAD</th>
<th>After LVAD</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Age at implantation (mean±SD), y</td>
<td>55±13.4</td>
<td>52/9</td>
<td></td>
</tr>
<tr>
<td>BMI (mean±SD), kg/m²</td>
<td>26.5±5.29</td>
<td>&lt;19</td>
<td>5</td>
</tr>
<tr>
<td>Type of heart failure, %</td>
<td>51</td>
<td>23</td>
<td>34</td>
</tr>
<tr>
<td>Ejection fraction (mean±SD), %</td>
<td>18±5.5</td>
<td>1255±1500</td>
<td>0.0001</td>
</tr>
<tr>
<td>LVAD duration (mean±SD), d</td>
<td>177±146</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

BMI indicates body mass index; LVAD, left ventricular assist device.

Echocardiographic Assessment
Echocardiographic parameters before and after LVAD placement are listed in Table 2. In short, left ventricular end-diastolic and end-systolic diameters after LVAD implantation were significantly smaller compared with the parameters before surgery in both groups. Left ventricular ejection fraction increased during mechanical support. Additional parameters are listed in Table 2.

Impaired Insulin Signaling in the Failing Human Myocardium
Next, we analyzed activation of the insulin signaling cascade and downstream targets of the insulin receptor, namely the insulin receptor substrate/Akt/Foxo signaling cascade in myocardial samples collected during LVAD implantation and explantation and in control samples. Samples of failing human myocardium revealed impaired phosphorylation of Akt and Foxo compared with control samples, indicating reduced myocardial activation of the insulin signaling cascade in advanced human HF, consistent with cardiac IR (Figure 1).

| Type of heart failure, % | 51 | 23 | 34 |
| Ejection fraction (mean±SD), % | 18±5.5 | 1255±1500 | 0.0001 |
| LVAD duration (mean±SD), d | 177±146 | NS |       |

LVAD indicates left ventricular assist device; LVESD, left ventricular end-systolic diameter; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; IVS, interventricular septum; and PWT, posterior wall thickness.
Reduction of FFA and triglycerides with accumulation of diacylglycerol and ceramide was accompanied by significant reduction in gene expression of CD36 and CPT1, markers of FA uptake and oxidation, as well as glucose transporter type 4, a marker of glucose uptake. Pyruvate dehydrogenase kinase-4 was suppressed, indicating increased glucose oxidation, consistent with previous findings of abnormal metabolic gene regulation in severe HF. Impaired mitochondrial oxidative metabolism was further indicated by reduced expression levels of PGC1α. Of note, expression of DGAT1, the rate-limiting enzyme catalyzing the conversion of diacylglycerol to monoacylglycerol, and ATGL, which catalyzes triglyceride to diacylglycerol degradation, was decreased in patients with advanced HF compared with control subjects (Figure 2B).

Lipid Overload Inhibits Cardiomyocyte Insulin Signaling Through PKC Activation
We next analyzed the impact of lipid overload on insulin signaling in cardiomyocytes in vitro. AC16 cells, a human cardiomyocyte cell line, was used to analyze the insulin signaling cascade. First, we confirmed that overnight incubation with PA increased overall intracellular lipid content using Oil Red O staining. Furthermore, we quantified the cellular content of triglycerides, diacylglycerol, and ceramide; they all showed a significant increase after overnight stimulation with PA (Figure 3A). We then tested whether PA affects insulin signaling through the phosphatidylinositol-3 kinase/Akt signaling cascade. Insulin stimulation of AC16 cells resulted in a strong activation of Akt that was inhibited by overnight incubation with PA, indicating that PA incubation and the associated increase in intracellular lipids block insulin-induced phosphorylation of Akt.

Next, we tested the impact of PA stimulation on PKC activity in AC16 cells. Incubation of AC16 cells with PA stimulated total PKC activity (51%; P<0.05 versus unstimulated cells; Figure 3C). Finally, inhibition of insulin-mediated Akt phosphorylation in cells preincubated with PA was reduced in the presence of a PKC inhibitor. These findings indicate that PKC is necessary for the inhibition of Akt phosphorylation in cardiomyocytes in the setting of high intracellular lipid levels (Figure 3D).

Mechanical Unloading of the Failing Myocardium Improves Systemic IR and Enhances Cardiac Insulin Signaling
LVAD implantation reduced fibrotic tissue accumulation (31.7±2.7% to 22.7±5.9%; P=0.016) and myocyte cross-sectional area (221.5±9.1 to 124.6±35.0 μm²; P<0.0001), indicating mechanical unloading of the failing myocardium. HF patients after LVAD implantation showed reduced fasting glucose and hemoglobin A1c levels (Table 3). This was accompanied by a reduction in circulating levels of insulin. These changes resulted in a significant decrease in calculated homeostatic model of analysis–insulin resistance, consistent with improved systemic insulin sensitivity, in patients with advanced HF after LVAD placement (3.7±1.8 in HF versus 2.5±0.8 after LVAD; P<0.05; Figure 4 and Figure I in the online-only Data Supplement).

Analysis of insulin signaling in myocardial samples obtained from patients before and after VAD implantation showed a dramatic increase in phosphorylation levels of Akt.
and Foxo (Figure 5). These findings indicate enhanced myocardial insulin signaling in response to mechanical unloading of the failing myocardium.

**Reduced Myocardial Toxic Lipids After Mechanical Unloading**

We next analyzed levels of intracellular lipids and FAs in myocardial samples obtained from patients before and after LVAD implantation. No changes were detectable in the triglyceride or FA content in response to LVAD implantation ($P=NS$). In contrast, levels of diacylglycerol and ceramide were reduced after LVAD placement (diacylglycerol: $-31\%, P<0.05$ versus before LVAD; ceramide: $-53\%, P<0.05$ versus before LVAD; Figure 6A). Surprisingly, these changes were accompanied by increased expression of CD36, indicating higher FA uptake. This appeared to be compensated for by greater FA oxidation because both CPT1 and pyruvate dehydrogenase kinase-4 mRNA levels increased after LVAD implantation (Figure 6B). Greater oxidation of incoming lipids might prevent their conversion to cellular toxic lipids with LVAD placement because DGAT1 and DGAT2 mRNA expression did not change after mechanical unloading. In line with previous reports on impaired mitochondrial function in advanced HF and the corrective impact of mechanical unloading, expression of PGC1α, a central regulator of oxidative metabolism, also increased after LVAD implantation (145%; $P<0.05$ versus before LVAD), consistent with an overall improvement of mitochondrial function and oxidative metabolism (Figure 6B). Furthermore, activation of adenosine monophosphate-activated protein kinase also increased in the myocardium after LVAD implantation compared with advanced HF (223%; $P<0.05$ versus pre-LVAD; Figure 7).

**Reduced PKC Activity in Response to VAD Implantation**

Finally, we analyzed levels of cytoplasmic and membrane-bound PKC isoforms to determine the activation. There was a clear reduction in membrane-associated PKCα, indicative of a reduced PKCα activation in the myocardium of patients after LVAD implantation ($-34\%; P<0.05$ versus before LVAD).
PKC showed a nonstatistically significant trend toward reduced activation (27%; \(P=0.08\) versus before LVAD). No differences were noted in PKC activity levels in response to LVAD implantation. These data indicate reduced activation of PKC after mechanical unloading of the failing myocardium (Figure 8).

**Discussion**

Although it is well known that HF alters glucose and lipid metabolism, the present study demonstrates for the first time that patients with advanced HF have a novel metabolic cardiac phenotype indicative of myocardial lipotoxicity accompanied by IR in nonobese patients. A central part of this phenotype is reduced insulin signaling, PKC activation, and accumulation of diacylglycerol and ceramide. Mechanical unloading corrects each of these abnormalities (Figure 9).

Advanced HF leads to an imbalance of catabolic and anabolic pathways favoring catabolism and loss of muscle mass in advanced stages of the disease.\(^{26}\) In part, this might result from IR, which has been characterized as a prognostic factor defining poor outcome.\(^{8,27}\) Impaired glucose homeostasis model assessment–insulin resistance.

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Insulin resistance in patients with advanced heart failure (HF) corrects after left ventricular assist device (LVAD) placement (white bar indicates control subjects, \(n=10\); black bar, HF before LVAD, \(n=36\); gray bar, HF after LVAD, \(n=30\); \(\star P<0.01\) vs control subjects; \(\#P<0.05\) vs HF before LVAD). HOMA-IR indicates homeostasis model assessment–insulin resistance.

![Figure 5](http://circ.ahajournals.org/)

**Figure 5.** Mechanical unloading of the failing myocardium increased cardiac phosphatidylinositol-3 kinase/Akt/Foxo signaling. **A,** Increased myocardial activation of Akt and Foxo after left ventricular assist device (LVAD) placement. **B,** Quantitative analysis of Akt and Foxo activation (open bars indicate before LVAD; solid bars, after LVAD; \(n=6\) individual patients before and after LVAD implantation; \(\ast P<0.05\) vs before LVAD).

![Figure 6](http://circ.ahajournals.org/)

**Figure 6.** Impact of mechanical unloading on myocardial lipid content, metabolic gene expression, and adenosine monophosphate-activated protein kinase (AMPK) activation in patients with advanced heart failure (HF). **A,** No changes are detectable in cardiac levels of triglycerides and free fatty acids. Increased levels of diacylglycerol and ceramide in advanced HF correct after mechanical unloading of the failing myocardium. **B,** Changes in myocardial metabolic gene expression in response to mechanical unloading (open bars, before left ventricular assist device [LVAD]; solid bars, after LVAD; \(\ast P<0.05\) vs before LVAD).

**Table 3. Laboratory Parameters Before and After Left Ventricular Assist Device Implantation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before LVAD</th>
<th>After LVAD</th>
<th>(P)</th>
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<tbody>
<tr>
<td>Complete blood count</td>
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<tr>
<td>Hematocrit, %</td>
<td>34.0±5.1</td>
<td>35.0±6.7</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>11.1±1.9</td>
<td>11.6±2.2</td>
<td>NS</td>
</tr>
<tr>
<td>MCV, fL</td>
<td>88±7.0</td>
<td>88±7.1</td>
<td>NS</td>
</tr>
<tr>
<td>Renal function</td>
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</tr>
<tr>
<td>BUN, mg/dL</td>
<td>40±21</td>
<td>27±17</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.7±0.8</td>
<td>1.4±0.6</td>
<td>&lt;0.05</td>
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<td>Hepatobiliary function</td>
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<td></td>
</tr>
<tr>
<td>AST, U/L</td>
<td>65±1.27</td>
<td>35±22</td>
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</tr>
<tr>
<td>ALT, U/L</td>
<td>67±12.3</td>
<td>31±24.7</td>
<td>0.06</td>
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<tr>
<td>Total bilirubin, mg/dL</td>
<td>2.3±5.1</td>
<td>0.8±0.4</td>
<td>0.06</td>
</tr>
<tr>
<td>Total protein, g/dL</td>
<td>6.5±1.0</td>
<td>7.4±1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>3.5±0.6</td>
<td>4.0±0.6</td>
<td>&lt;0.001</td>
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<td>Alkaline phosphatase, U/L</td>
<td>101±61</td>
<td>120±104</td>
<td>NS</td>
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<tr>
<td>Glucose and lipid metabolism</td>
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<td>Glucose, mg/dL</td>
<td>137±49</td>
<td>112±41</td>
<td>&lt;0.01</td>
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<tr>
<td>HbA1c, %</td>
<td>7.0±1.6</td>
<td>6.1±1.3</td>
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<td>Triglycerides, mg/dL</td>
<td>118±69</td>
<td>163±106</td>
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</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>129±36</td>
<td>170±50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>34±12</td>
<td>48±15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>71±27</td>
<td>104±42</td>
<td>&lt;0.001</td>
</tr>
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</table>

MCV indicates mean corpuscular volume; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

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**Figure 3.** Mean corpuscular volume (MCV) and hemoglobin (Hgb) in patients with advanced heart failure (HF) before and after left ventricular assist device (LVAD) placement. A, MCV and Hgb levels are lower before LVAD, but increase after LVAD. B, Hirudin prolongs activated partial thromboplastin time (APTT) and thrombin time (TT) before LVAD but fails to do so after LVAD.
stasis in HF has been linked to increased catecholamine levels, low-grade inflammation, reduced blood flow, and potentially immobilization. The use of LVADs in our patients corrected many of these abnormalities and improved homeostatic model of analysis–insulin resistance.

In the absence of HF, metabolic changes associated with IR are most commonly referred to as the metabolic syndrome, with obesity being the most prevalent. Metabolic risk factors established in individuals without overt disease such as lipid abnormalities and obesity do not carry the same risk in patients with advanced HF; in fact, some metabolic abnormalities (eg, high cholesterol levels or increased body mass index) carry a survival benefit in this patient population. This apparent paradox has been characterized as the lipid paradox or obesity paradox in patients with HF.

One characteristic of the metabolic syndrome and diabetes mellitus is accumulation of signaling lipids; these lipids are thought to cause IR and other types of cellular dysfunction. Lipotoxicity is a consequence of either increased lipid—especially FA—uptake or impaired lipid oxidation and storage, leading to accumulation of diacylglycerol and/or ceramide. Both diacylglycerol and ceramide have been implicated in IR, mitochondrial dysfunction, and apoptosis. Genetic alterations in mice that increase tissue diacylglycerol lead to IR, whereas prevention of ceramide synthesis negates saturated fat-induced IR. Analysis in hearts from patients with severe HF shows a marked suppression of genes associated with both FA oxidation and nontoxic storage of lipid in triglyceride. Although CD36, a well-established mediator of cardiac FA uptake, was reduced, it is likely that residual FAs entering the heart are rerouted into synthesis of diacylglycerol and ceramide, leading to accumulation of these signaling lipids.

Lipid accumulation can be a cause and a consequence of HF. A series of genetically altered mice have been created that have lipid accumulation owing to excess FA uptake or reduced oxidation. These include overexpression of genes that cause greater trapping of FAs, reduced activation of peroxisome proliferator-activated receptors (peroxisome proliferator–activated receptor-D knockout and ATGL knockout), and, paradoxically, peroxisome proliferator–activated receptor-α and peroxisome proliferator–activated receptor-γ, which must cause greater lipid uptake than oxidation. Several of these models have activation of PKC and development of hypertrophy. Therefore, lipid accumulation aside from altering metabolism might exacerbate HF.

Increased intracellular lipid accumulation in HF has been described in previous studies and was linked to obesity and diabetes mellitus. One prior human study showed increased levels of triglycerides in the myocardium of obese (body mass index >30 kg/m²) and diabetic patients with advanced HF undergoing heart transplantation. These findings are well in line with animal studies showing increased myocardial levels of triglycerides in murine models of diabetes mellitus. In contrast, animal models of HF secondary to transaortic banding under nondiabetic conditions are associated with reduced myocardial triglyceride content. Unlike in the present study, previous human studies have not differentiated between lipid subspecies. Limited data are available to ascertain whether a nondiabetic group with advanced HF also has an increase in various cardiac lipids. The present study found no increase in FAs or triglycerides whereas ceramide and diacylglycerol levels increased in the nonobese patient cohort. Perhaps this was due to a limited number of patients with diabetes mellitus and obesity;
the average body mass index of our patients was 26.5 kg/m². We also found a uniform decrease in gene expression of regulators of primarily lipid metabolism and oxidation. In particular, levels of DGAT1 and ATGL were reduced. We believe that the decreased levels of triglycerides and FAs represent a depletion of energy storage pools in the failing myocardium and the diversion of incoming FAs into diacylglycerol and ceramide. The underlying pathophysiological mechanism is likely a failure to oxidize FAs owing to mitochondrial dysfunction, a phenomenon that has been well described in advanced HF. Alternatively, impaired myocardial lipolysis might be a primary mechanism leading to reduced FA oxidation. Our patients showed reduced ATGL expression, and ATGL deficiency in mice leads to decreased peroxisome proliferator–activated receptor-α activation and defective lipid use.

One of the central findings of this study is the beneficial effect of mechanical unloading on cardiac metabolism in the failing myocardium. The advent of VADs has improved the therapeutic options for patients with advanced HF, and VADs are now an established intervention in patients awaiting cardiac transplantation. We show here that improved hemodynamics after VAD implantation is accompanied by reduced whole-body and cardiac IR and improved glucose homeostasis, along with a reduction in the levels of the toxic lipid intermediates diacylglycerol and ceramide. Activation of PKC by toxic lipid intermediates with subsequent inhibition of Akt signaling represents a direct molecular link between HF-associated lipotoxicity and myocardial IR through accumulation of the toxic lipid intermediates diacylglycerol and ceramide. PKC activation may be a crucial mediator of this pathophenomenon. Further studies should focus on potential therapeutic interventions correcting impaired cardiac metabolism and IR through PKC inhibition or alterations in lipid metabolism that prevent the accumulation of toxic lipid intermediates.

Conclusions
This study represents the first analysis of cellular pathways of lipotoxicity and IR in patients with advanced HF. Our study suggests a novel pathway of lipotoxic cellular damage contributing to myocardial IR through accumulation of the toxic lipid intermediates diacylglycerol and ceramide. PKC activation may be a crucial mediator of this pathophenomenon. Further studies should focus on potential therapeutic interventions correcting impaired cardiac metabolism and IR through PKC inhibition or alterations in lipid metabolism that prevent the accumulation of toxic lipid intermediates.

Sources of Funding
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Disclosures
None.

References


**CLINICAL PERSPECTIVE**

The failing human heart develops metabolic derangements characterized by a shift from fatty acids to glucose use for ATP generation. These metabolic changes are accompanied by transcriptional changes typically seen during embryogenesis. The aim of our study was to investigate myocardial levels of toxic lipid intermediates in samples from patients with advanced heart failure compared with control subjects. Furthermore, we analyzed whether mechanical unloading of the failing myocardium corrects impaired cardiac metabolism. For this purpose, we analyzed myocardium and serum of patients with advanced heart failure obtained during left ventricular assist device implantation and at explantation and from control subjects. Systemic insulin resistance in heart failure was accompanied by decreased myocardial triglyceride and overall fatty acid content but increased toxic lipid intermediates, diacylglycerol, and ceramide. Several downstream signaling molecules known to regulate insulin signaling (Akt, Foxo, and protein kinase C) were also found to be dysregulated in the failing myocardium, favoring an insulin-resistant state compared with control subjects. Left ventricular assist device implantation improved insulin resistance and decreased myocardial levels of the toxic lipid intermediates diacylglycerol and ceramide, whereas triglyceride and free fatty acid content remained unchanged. Therefore, abnormal myocardial metabolism, insulin resistance, and lipotoxicity develop in human heart failure. Mechanical unloading through left ventricular assist device implantation corrects systemic and local metabolic derangements in advanced heart failure. This study characterizes metabolic changes in human heart failure with the development of cardiac lipotoxicity. Furthermore, these findings suggest potential therapeutic implications of mechanical unloading of the failing myocardium for the correction of metabolic derangements in advanced human heart failure.
Ventricular Assist Device Implantation Corrects Myocardial Lipotoxicity, Reverses Insulin Resistance, and Normalizes Cardiac Metabolism in Patients With Advanced Heart Failure


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Supplemental Material
Supplemental Figure 1
Individual changes of HOMA-IR in patients undergoing LVAD placement.
좌심실 보조 장치는 심부전 환자의 심장 대사 변화를 호전시킨다

강석민 교수 세브란스병원 심장내과

Summary

배경
심부전 환자의 심장 내에서는 효과적인 ATP(adenosine triphosphate) 생산을 위해 지방산에서 포도당으로의 대사 변화를 일으킨다. 저자들은 독성 지방(toxic lipid)의 심장 내 축적이 인슐린 대사를 방해하고, 심장에 대한 물리적인 과부하의 경감은 심장의 대사 변화를 호전 시킨다고 가정하였다.

방법 및 결과
61명의 심부전 환자(체질량지수, 26.5±5.1kg/m²; 연령, 51±12세)의 심근 조직과 혈액을 좌심실 보조장치 삽입 시와 제거 시(평균, 185±156일)에 채취하여 9명의 대조군과 비교하였다. 심부전 환자의 인슐린 저항성은 심근 내 중성지방과 지방산 함량의 감소를 동반하였으나, 독성 지방산물인 diacylglycerol과 ceramide는 심근 내 함량이 증가하였다. 또한, protein kinase C isoforms, 인슐린 신호 전달체계 저해제들의 membrane localization의 증가, Akt와 Foxo 등의 인슐린 신호 전달체계 물질들의 감소 등이 심부전 환자들에서 관찰되었다. 좌심실 보조장치의 삽입은 전체 인슐린 저항성(homeostatic model of analysis–insulin resistance, 4.5±0.6–3.2±0.5; P<0.05)을 향상시켰고, 심근 내 diacylglycerol과 ceramide의 함량을 감소시켰지만, 중성지방과 지방산 함량은 변화가 없었다. 좌심실 보조장치 삽입 후 insulin/phosphatidylinositol-3 kinase/Akt 신호 전달체계의 향상은 Akt와 Foxo의 인산화 증가로 확인할 수 있었으며, 이는 protein kinase C isoforms의 membrane localization의 감소를 동반하였다.

결론
심부전 환자에서 좌심실 보조장치 삽입으로 인한 물리적인 과부하의 경감은 독성 지방 산물의 심근 내 축적을 감소시키고, 인슐린 신호 전달체계를 향상시키는 심장의 대사 변화를 호전시킨다.
Commentary

말기 심부전 환자는 심근에서 지방산을 흡수하고 산화하는 데 관여하는 유전자들이 억제되어 있고, 포도당 대사에 관여하는 유전자들의 발현은 상대적으로 변화가 없다고 알려져 있다. 즉, 상대적으로 지방산 대사의 과정은 억제된 상태이다. 특히, 독성 지방 산물인 diacylglycerol과 ceramide의 심근 내 축적은 미토콘드리아의 기능 저하와 세포 내 포도당, 지방 대사의 이상 및 세포 자멸 등을 일으킨다고 알려져 있다. 이러한 지방 독성(lipotoxicity)이 심부전의 유발 요인이라거나 결과산물일 수 있다.

동물실험 외에 심부전 환자에서 이러한 지방 독성은 당뇨병이나 비만한 환자들에게 보고되어 왔으며, 본 연구에서는 비교적 비만하지 않은 심부전 환자군에서 이러한 지방 독성에 대한 증거를 제시하고 있다. 아쉬운 점은 환자 수가 적어서 당뇨병군과 비당뇨병군, 허혈성 심부전 환자군과 비허혈성 심부전 환자군, 비만군과 비만하지 않은 군 등의 비교 분석이 없는 점이며, 좌심실 보조장치의 type(pulsatile-flow vs. continuous-flow)에 따른 심장 대사의 변화 분석도 흥미로울 것이다. 본 연구의 결과를 통해 좌심실 보조장치가 심장에 대한 물리적인 과부하를 감소시킴으로써 전체적인 인슐린 저항성의 호전과 더불어 심근 내의 대사성 변화의 호전을 보인다는 사실은 향후 말기 심부전 환자의 대사성 변화를 고정하는 데 있어 중요한 정보를 제공한다고 말할 수 있었다. 이러한 지방 독성 유발에 있어 protein kinase C의 활성화가 중요한 역할을 하므로, 이를 조절할 수 있는 치료 방법이 향후 지방 독성을 예방할 수 있는 하나의 방법이 될 수도 있었다.

우리나라에서도 1992년 이후 심장이식 수술이 가능해지고 날로 증가하는 말기 심부전 환자들 때문에 좌심실 보조장치에 많은 관심을 가지게 되었다. 현재 우리나라는 매년 약 70-80명 정도의 심장이식이 시행되고 있으며, 향후 이러한 좌심실 보조장치에 대한 연구 결과들이 말기 심부전 환자들에게 한 줄기 희망의 메시지가 되기 위해서는 보다 더 많은 임상연구 결과가 앞받침되어야 할 것이다.

Figure 1. Insulin resistance in patients with advanced HF corrects after LVAD placement (white bar indicates control subjects, n=10; black bar, HF before LVAD, n=36; gray bar, HF after LVAD, n=30; *P<0.01 vs. control subjects; #P<0.05 vs. HF before LVAD).

HF, heart failure; LVAD, left ventricular assist device; HOMA-IR, homeostasis model assessment-insulin resistance.
Figure 2. Impact of mechanical unloading on myocardial lipid content, metabolic gene expression, and AMPK activation in patients with advanced HF. **A**, No changes are detectable in cardiac levels of triglycerides and free fatty acids. Increased levels of diacylglycerol and ceramide in advanced HF correct after mechanical unloading of the failing myocardium. **B**, Changes in myocardial metabolic gene expression in response to mechanical unloading (open bars, before LVAD; solid bars, after LVAD; *P*<0.05 vs. before LVAD).

**A**

<table>
<thead>
<tr>
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<td>600</td>
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<tr>
<td>Ceramide</td>
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<td>800</td>
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**B**

<table>
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<th>Gene</th>
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</tr>
<tr>
<td>CPT1</td>
<td>*</td>
</tr>
<tr>
<td>ACO</td>
<td>*</td>
</tr>
<tr>
<td>DGAT1</td>
<td>*</td>
</tr>
<tr>
<td>DGAT2</td>
<td>*</td>
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<tr>
<td>ATGL</td>
<td>*</td>
</tr>
<tr>
<td>PDK4</td>
<td>*</td>
</tr>
<tr>
<td>GLUT4</td>
<td>*</td>
</tr>
<tr>
<td>InsR</td>
<td>*</td>
</tr>
<tr>
<td>PGC1a</td>
<td>*</td>
</tr>
</tbody>
</table>

AMPK, adenosine monophosphate-activated protein kinase; HF, heart failure; LVAD, left ventricular assist device.

References
Ventricular Assist Device Implantation Corrects Myocardial Lipotoxicity, Reverses Insulin Resistance, and Normalizes Cardiac Metabolism in Patients With Advanced Heart Failure

Aalap Chokshi, MD; Konstantinos Drosatos, PhD; Faisal H. Cheema, MD; Ruiping Ji, MD; Tuba Khawaja, MD; Shuqiong Yu, BS; Tomoko Kato, MD; Raffay Khan, MD; Hiroo Takayama, MD; Ralph Knöll, MD, PhD; Hendrik Milting, PhD; Christine S. Chung, MD; Ulrich Jorde, MD; Yoshifumi Naka, MD, PhD; Donna M. Mancini, MD; Ira J. Goldberg, MD; P. Christian Schulze, MD, PhD

Background—Heart failure is associated with impaired myocardial metabolism with a shift from fatty acids to glucose use for ATP generation. We hypothesized that cardiac accumulation of toxic lipid intermediates inhibits insulin signaling in advanced heart failure and that mechanical unloading of the failing myocardium corrects impaired cardiac metabolism.

Methods and Results—We analyzed the myocardium and serum of 61 patients with heart failure (body mass index, 26.5 ± 5.1 kg/m²; age, 51 ± 12 years) obtained during left ventricular assist device implantation and at explantation (mean duration, 185 ± 156 days) and from 9 control subjects. Systemic insulin resistance in heart failure was accompanied by decreased myocardial triglyceride and overall fatty acid content but increased toxic lipid intermediates, diacylglycerol, and ceramide. Increased membrane localization of protein kinase C isoforms, inhibitors of insulin signaling, and decreased activity of insulin signaling molecules Akt and Foxo were detectable in heart failure compared with control subjects. Left ventricular assist device implantation improved whole-body insulin resistance (homeostatic model of analysis–insulin resistance, 4.5 ± 0.6–3.2 ± 0.5; *P* < 0.05) and decreased myocardial levels of diacylglycerol and ceramide, whereas triglyceride and fatty acid content remained unchanged. Improved activation of the insulin/phosphatidylinositol-3 kinase/Akt signaling cascade after left ventricular assist device implantation was confirmed by increased phosphorylation of Akt and Foxo, which was accompanied by decreased membrane localization of protein kinase C isoforms after left ventricular assist device implantation.

Conclusions—Mechanical unloading after left ventricular assist device implantation corrects systemic and local metabolic derangements in advanced heart failure, leading to reduced myocardial levels of toxic lipid intermediates and improved cardiac insulin signaling. (Circulation. 2012;125:2844-2853.)

Key Words: heart failure ■ lipids ■ metabolism ■ myocardium ■ ventricular assist device

Advanced heart failure (HF) is associated with structural, functional, inflammatory, and metabolic derangements of the failing myocardium that develop and worsen during progression of the disease state.1,2 Metabolic abnormalities of the failing myocardium are characterized by transcriptional changes with suppression of genes regulating fatty acid uptake and oxidation while the expression of genes controlling glucose metabolism remains relatively stable,3–4 a transcriptional program that is typical of embryogenesis.1,5,6 The overall net result is a relative decrease in the metabolism and oxidation of fatty acids (FAs) and a shift toward glucose for primary ATP generation. These changes are also accompanied by profound mitochondrial dysfunction and impaired overall oxidative metabolism.7

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Besides this myocardial dysbalance of glucose and FA metabolism, patients with advanced HF also develop systemic insulin resistance (IR), and worse IR correlates with greater morbidity and mortality.8 Circulating proinflammatory cytokines such as tumor necrosis factor-α and interleukin-1β and dysregulation of adipokines have been
linked to the development and progression of IR in HF,9 which parallels metabolic derangements previously described in patients with abnormal glucose homeostasis indicative of early diabetes mellitus.10

The complex network of cellular lipid metabolism pathways must supply substrates for the generation of ATP via mitochondrial β-oxidation, production of cellular structural components, and creation of signaling molecules that regulate a number of cellular processes, including insulin signaling.11 Less than 5% of total body triglycerides are stored in the nonadipose tissues such as skeletal muscle and myocardium; nevertheless, these neutral lipids can serve as an important energy storage form, whereas pathological lipid accumulation in skeletal muscle can trigger IR.12 Several intermediates of lipid metabolism have toxic proapoptotic and proinflammatory actions.13–15 Skeletal muscle and myocardium of patients with diabetes mellitus have increased levels of diacylglycerol and ceramide, which have been directly linked to mitochondrial dysfunction and impaired intracellular glucose and FA oxidation.14,16 Intracellular lipid accumulation associated with such deleterious effects on cellular function has been called lipotoxicity. Proof that lipids themselves are toxic has come from studies showing that overexpression of enzymes that increase intracellular lipid levels leads to IR and that gene deletion of these enzymes, which reduces toxic lipids, improves insulin sensitivity.13,17–20 In addition, induced lipid accumulation in the heart leads to cardiac dysfunction.13,15,21

The aim of our study was to compare levels of intracellular lipids between patients with advanced HF and control subjects and to test the hypothesis that mechanical unloading through left ventricular assist device (LVAD) implantation leads to reversal of metabolic derangements in the failing myocardium.

Methods

Patient Cohort and Sample Collection

We retrospectively analyzed 61 patients (52 male, 9 female) with advanced HF undergoing LVAD implantation at Columbia University Medical Center. Patients received either pulsatile-flow LVADs (n=30) or continuous-flow LVADs (n=31). Clinical and laboratory characteristics of all patients and hemodynamic conditions within 5 days before LVAD implantation and explantation were collected.

Myocardial specimens were collected from a subset of patients (n=21), and blood samples were obtained from all patients (n=61) at the time of LVAD implantation for end-stage HF as a bridge to transplantation and at the time of LVAD explantation during cardiac transplantation. Control blood samples were obtained from patients without cardiovascular disease recruited at the Columbia University Medical Center (n=10). Control myocardial samples (n=6) were obtained from a tissue bank of deidentified specimens collected from nonfailing hearts determined to be unusable for cardiac transplantation because of acute recipient issues or donor coronary artery disease but without evidence of previous infarction. The newly obtained heart samples were immediately snap-frozen, placed in liquid nitrogen for transport, and stored at −80°C until final analysis.

The present study was approved by the Institutional Review Board of Columbia University. All patients provided written informed consent before inclusion in the study.

Echocardiographic Analysis

Conventional echocardiograms were obtained from all patients within 5 days before LVAD implantation and at 1 month after the surgery (Sonos-5500 or Sonos-7500, Philips Healthcare Corp, Andover, MA). The routine standard echocardiographic examination included M-mode, 2-dimensional echocardiogram, and Doppler study for measurements of ventricular septal and posterior wall thickness and end-systolic and end-diastolic left ventricular diameters. Left ventricular ejection fraction was calculated by the biplane Simpson method from apical 4- and 2-chamber views. Mitral inflow was obtained by pulsed-wave Doppler echocardiography with the sample volume between mitral leaflet tips during diastole, and peak early (E) and late (A) transmitral filling velocities, their ratio (E/A), and deceleration time of E were measured. The peak positive dP/dt (dP/dtmax; the first derivative of left ventricular pressure with respect to time) as an index of contractility was calculated from continuous-wave Doppler determination of the velocities in mitral regurgitant jets.19 Early diastolic annular velocity (E′) was obtained by placing a tissue Doppler sample volume at the septal and lateral mitral annulus in the apical 4-chamber view, and the E/E′ ratio was calculated. Measurements were performed from 5 cardiac cycles and averaged.

Serum Analysis

Venous blood samples were collected and stored after centrifugation at −70°C until the assays were performed. Levels of insulin were measured with commercially available ELISA kits (CalBiotech, Spring Valley, CA). The homeostatic model of analysis–insulin resistance was used to described the levels of insulin resistance in all patients and control subjects.22 Circulating levels of brain natriuretic peptide were analyzed by the institutional core laboratory.

Tissue Culture

A human ventricular cardiomyocyte-derived cell line, designated AC-16, was kindly provided by M.M. Davidson.23 Cells were maintained in Dulbecco modified Eagle medium/nutrient mixture F-12 (Ham; Invitrogen, Carlsbad, CA). Experiments were performed on cells at 70% to 80% confluence after 4 hours of starvation. Cells were exposed to 0.4 mmol/L palmitic acid (PA; Sigma Aldrich) in methanol conjugated with 1% FA/free BSA overnight (16 hours). Control cells were incubated in the presence of 0.25% vehicle (methanol). After overnight treatment with PA (0.4 mmol/L for 16 hours), cells were incubated for different time intervals with 500 nm insulin (Sigma Aldrich) diluted in HEPES buffer for different time intervals in the presence or absence of protein kinase C (PKC) inhibitor (RO-318220; Sigma).

Gene Expression Analysis

Total RNA was extracted with standard methods, and the abundance of specific mRNAs was determined by reverse transcriptase–polymerase chain reaction with a Prism 7700 Sequence Detector (Perkin–Elmer, Foster City, CA). Sequences of primers and TaqMan probes specific for cluster of differentiation 36 (CD36), carnitine palmitoyltransferase-1 (CPT1), acyl-Coenzyme A oxidase, glucose transporter type 1, glucose transporter type 4, insulin receptor pyruvate dehydrogenase kinase-4, adipose triglyceride lipase (ATGL), hormone-sensitive lipase, diacylglycerol acyltransferase 1 and 2 (DGAT1 and DGAT2), and brain natriuretic peptide were described previously.6,13,15,18 Gene expression was normalized by expression of 18S mRNA and expressed as relative expression.

Protein Analysis

Tissues and cells were lysed in 200 µL lysis buffer containing 20 mmol/L Tris-HCl (pH 8.0), 2 mmol/L EDTA, 2 mmol/L EGTA, 6 mmol/L β-mercaptoethanol, 0.1 mmol/L sodium vanadate, 50 mmol/L sodium fluoride, protease inhibitors, and phosphatase inhibitors (Roche, Indianapolis, IN). Protein extraction was performed with standard techniques. Protein lysates from cells were resolved on 4% to 15% SDS-PAGE–reducing gels (Bio-Rad), transferred to polyvinylidene difluoride membranes (Bio-Rad), blocked in 5% milk/TRIS-buffered saline, and probed with antibodies for detection of phosphorylated Akt, total Akt, phosphorylated Foxo, total Foxo, and GAPDH (all from Cell Signaling Technologies Inc). Membranes were washed, incubated with appropriate secondary antibodies conjugated to horse-
radish peroxidase, washed in TBS-Tween, and detected by use of ECL Western Blotting System Kit (Thermo Scientific).

Analysis of PKC isofom localization and activity was performed after protein isolation. Membrane and cytosolic fractions were separated by ultracentrifugation at 33 000 rpm for 1 hour. Then, 25 μg from each fraction was applied to SDS-PAGE and transferred onto nitrocellulose membranes, and PKC isofoms were detected with specific antibodies (PKCα from Millipore, Billerica, MA; PKCβ, PKCδ, and PKCε from Santa Cruz Biotechnology, Santa Cruz, CA). Specific PKC activity was measured with the nonradioactive PKC kinase activity kit (Assay Designs, Ann Arbor, MI) as previously described.15

Lipid Measurements
Total lipids were extracted by the Folch method of extraction. Briefly, 25 to 50 mg of tissue or cell pellet was homogenized in PBS and chloroform:methanol (2:1). The organic phase was separated twice and dried. The dried lipids were solubilized in 1% Triton X-100, dried, and resuspended in distilled water. Cardiac triglyceride content was measured by use of the Infinity Triglycerides Kit (Thermo Scientific) with the Matrix Plus Chemistry Reference Kit (Vercichem Laboratory Inc) as standard. Total FAs were quantified as the sum of individual FAs detected by liquid chromatography–mass spectrometry—based lipomics (Waters Xevo TQ MS ACQUITY UPLC System; Waters, Milford, MA). Total diacylglycerol and ceramide were detected with the diacylglycerol kinase method as previously described.24

Histology
Histopathology was assessed on myocardial specimens obtained at the time of LVAD implantation and at the time of explantation after paraffin fixation and hematoxylin and eosin and Masson trichrome staining. Myocardial fibrosis was quantified on serial sections with the use of Masson trichrome staining for the detection of collagen. Area of fibrosis was analyzed with the ImagePro software (National Institutes of Health) and expressed as percent of total section area. Myocyte cross-sectional area was analyzed after semiquantitative analysis with circumferential markings of the myocytes with ImagePro software and expression of average cross-sectional area and distribution of fiber sizes within each sample.

Statistical Analysis
Data are presented as mean±SD. Normality was evaluated for each variable from normal distribution plots and histograms and by the Kolmogorov-Smirnov test. The echocardiographic and laboratory variables, serum and myocardial gene expression before and after surgery, and percent changes of the variables through the surgery were compared by use of ANOVA with appropriate post hoc testing. A value of P<0.05 was considered statistically significant. All data were analyzed with SPSS version 18 (IBM).

Results
Baseline Characteristics
We analyzed a total of 61 patients undergoing placement of a LVAD at our institution between 1998 and 2010. Clinical characteristics of all patients are summarized in Table 1. The duration of LVAD support ranged from 31 to 662 days (mean, 176 days). Patients with HF had generalied IR by the homeostatic model of analysis–insulin resistance (3.7±1.8 in HF versus 1.1±0.6 in control subjects; P<0.01).

<table>
<thead>
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<th>Characteristic</th>
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<th>After LVAD</th>
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<tr>
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<td>25.4±5.20</td>
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</tr>
<tr>
<td>&lt;19</td>
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<tr>
<td>19–29</td>
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<td>25–30</td>
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<tr>
<td>&gt;30</td>
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</tr>
<tr>
<td>Ischemic cardiomyopathy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ejection fraction (mean±SD), %</td>
<td>18±5.5</td>
<td>16±4.3</td>
<td></td>
</tr>
<tr>
<td>LVAD duration (mean±SD), d</td>
<td>177±146</td>
<td>172±150</td>
<td></td>
</tr>
</tbody>
</table>

BMI indicates body mass index; LVAD, left ventricular assist device.

Echocardiographic Assessment
Echocardiographic parameters before and after LVAD placement are listed in Table 2. In short, left ventricular end-diastolic and end-systolic diameters after LVAD implantation were significantly smaller compared with the parameters before surgery in both groups. Left ventricular ejection fraction increased during mechanical support. Additional parameters are listed in Table 2.

Impaired Insulin Signaling in the Failing Human Myocardium
Next, we analyzed activation of the insulin signaling cascade and downstream targets of the insulin receptor, namely the insulin receptor substrate/Akt/Foxo signaling cascade in myocardial samples collected during LVAD implantation and explantation and in control samples. Samples of failing human myocardium revealed impaired phosphorylation of Akt and Foxo compared with control samples, indicating reduced myocardial activation of the insulin signaling cascade in advanced human HF, consistent with cardiac IR (Figure 1).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Before LVAD</th>
<th>After LVAD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD, mm</td>
<td>69±12</td>
<td>55±15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>63±13</td>
<td>48±17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>17±2</td>
<td>25±15</td>
<td>0.003</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>10±3</td>
<td>11±2</td>
<td>NS</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>10±2</td>
<td>11±2</td>
<td>NS</td>
</tr>
</tbody>
</table>

LVEDD indicates left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; IVS, interventricular septum; and PWT, posterior wall thickness.
Reduced activation indicated by decreased phosphorylation (p) status of Akt and Foxo in samples from patients with advanced HF compared with control subjects (3 representative samples per group). Decreased myocardial phosphorylation levels of Akt and Foxo in patients with advanced HF compared with control subjects (P<0.05; n=6–8 samples per group).

Figure 1. Impairment of phosphatidylinositol-3 kinase/Akt signaling in human advanced heart failure (HF). A, Reduced activation indicated by decreased phosphorylation (p) status of Akt and Foxo in samples from patients with advanced HF compared with control subjects (3 representative samples per group). B, Decreased myocardial phosphorylation levels of Akt and Foxo in patients with advanced HF compared with control subjects (P<0.05; n=6–8 samples per group).

Increased Levels of Diacylglycerol and Ceramide in the Failing Human Myocardium

We further determined levels of intracellular lipids in failing human myocardium for comparison with nonfailing cardiac samples. Significantly decreased levels of triglycerides and free FAs were noted in failing human myocardium compared with control samples. In contrast, a strong increase in intracellular levels of the toxic lipid intermediates diacylglycerol and ceramide was found in samples from patients with advanced HF compared with control samples (Figure 2A). These findings demonstrate myocardial accumulation of toxic lipid intermediates in patients with advanced HF whereas neutral lipids and free FAs remained decreased.

Reduction of FFA and triglycerides with accumulation of diacylglycerol and ceramide was accompanied by significant reduction in gene expression of CD36 and CPT1, markers of FA uptake and oxidation, as well as glucose transporter type 4, a marker of glucose uptake. Pyruvate dehydrogenase kinase-4 was suppressed, indicating increased glucose oxidation, consistent with previous findings of abnormal metabolic gene regulation in severe HF. Impaired mitochondrial oxidative metabolism was further indicated by reduced expression levels of PGC1α. Of note, expression of DGAT1, the rate-limiting enzyme catalyzing the conversion of diacylglycerol to monoacylglycerol, and ATGL, which catalyzes triglyceride to diacylglycerol degradation, was decreased in patients with advanced HF compared with control subjects (Figure 2B).

Lipid Overload Inhibits Cardiomyocyte Insulin Signaling Through PKC Activation

We next analyzed the impact of lipid overload on insulin signaling in cardiomyocytes in vitro. AC16 cells, a human cardiomyocyte cell line, was used to analyze the insulin signaling cascade. First, we confirmed that overnight incubation with PA increased overall intracellular lipid content using Oil Red O staining. Furthermore, we quantified the cellular content of triglycerides, diacylglycerol, and ceramide; they all showed a significant increase after overnight stimulation with PA (Figure 3A). We then tested whether PA affects insulin signaling through the phosphatidylinositol-3 kinase/Akt signaling cascade. Insulin stimulation of AC16 cells resulted in a strong activation of Akt that was inhibited by overnight incubation with PA, indicating that PA incubation and the associated increase in intracellular lipid block insulin-induced phosphorylation of Akt.

Next, we tested the impact of PA stimulation on PKC activity in AC16 cells. Incubation of AC16 cells with PA stimulated total PKC activity (51%; P<0.05 versus unstimulated cells; Figure 3C). Finally, inhibition of insulin-mediated Akt phosphorylation in cells preincubated with PA was reduced in the presence of a PKC inhibitor. These findings indicate that PKC is necessary for the inhibition of Akt phosphorylation in cardiomyocytes in the setting of high intracellular lipid levels (Figure 3D).

Mechanical Unloading of the Failing Myocardium Improves Systemic IR and Enhances Cardiac Insulin Signaling

LVAD implantation reduced fibrotic tissue accumulation (31.7±2.7% to 22.7±5.9%; P=0.016) and myocyte cross-sectional area (221.5±9.1 to 124.6±35.0 μm²; P<0.0001), indicating mechanical unloading of the failing myocardium.

HF patients after LVAD implantation showed reduced fasting glucose and hemoglobin A1c levels (Table 3). This was accompanied by a reduction in circulating levels of insulin. These changes resulted in a significant decrease in calculated homeostatic model of analysis–insulin resistance, consistent with improved systemic insulin sensitivity, in patients with advanced HF after LVAD placement (3.7±1.8 in HF versus 2.5±0.8 after LVAD; P<0.05; Figure 4 and Figure I in the online-only Data Supplement).

Analysis of insulin signaling in myocardial samples obtained from patients before and after VAD implantation showed a dramatic increase in phosphorylation levels of Akt.
These findings indicate enhanced myocardial insulin signaling in response to mechanical unloading of the failing myocardium.

Reduced Myocardial Toxic Lipids After Mechanical Unloading

We next analyzed levels of intracellular lipids and FAs in myocardial samples obtained from patients before and after LVAD implantation. No changes were detectable in the triglyceride or FA content in response to LVAD implantation \((P=NS)\). In contrast, levels of diacylglycerol and ceramide were reduced after LVAD placement \((\text{diacylglycerol: } -31\%, \ P<0.05 \text{ versus before LVAD}; \text{ceramide: } -53\%, \ P<0.05 \text{ versus before LVAD}; \text{Figure 6A})\). Surprisingly, these changes were accompanied by increased expression of CD36, indicating higher FA uptake. This appeared to be compensated for by greater FA oxidation because both CPT1 and pyruvate dehydrogenase kinase-4 mRNA levels increased after LVAD implantation \((\text{Figure 6B})\). Greater oxidation of incoming lipids might prevent their conversion to cellular toxic lipids with LVAD placement because DGAT1 and DGAT2 mRNA expression did not change after mechanical unloading. In line with previous reports on impaired mitochondrial function in advanced HF and the corrective impact of mechanical unloading, \(P_{GCl}\), a central regulator of oxidative metabolism, also increased after LVAD implantation \((145\%; \ P<0.05 \text{ versus before LVAD})\), consistent with an overall improvement of mitochondrial function and oxidative metabolism \((\text{Figure 6B})\). Furthermore, activation of adenosine monophosphate-activated protein kinase also increased in the myocardium after LVAD implantation compared with advanced HF \((223\%; \ P<0.05 \text{ versus pre-LVAD}; \text{Figure 7})\).

Reduced PKC Activity in Response to VAD Implantation

Finally, we analyzed levels of cytoplasmic and membrane-bound PKC isoforms to determine the activation. There was a clear reduction in membrane-associated PKC\(\alpha\), indicative of a reduced PKC\(\alpha\) activation in the myocardium of patients after LVAD implantation \((-34\%; \ P<0.05 \text{ versus before LVAD})\).
PKC showed a nonstatistically significant trend toward reduced activation (−27%; \( \text{NS} \)) versus before LVAD. No differences were noted in PKC activity levels in response to LVAD implantation. These data indicate reduced activation of PKC after mechanical unloading of the failing myocardium (Figure 8).

**Discussion**

Although it is well known that HF alters glucose and lipid metabolism, the present study demonstrates for the first time that patients with advanced HF have a novel metabolic cardiac phenotype indicative of myocardial lipotoxicity accompanied by IR in nonobese patients. A central part of this phenotype is reduced insulin signaling, PKC activation, and accumulation of diacylglycerol and ceramide. Mechanical unloading corrects each of these abnormalities (Figure 9).

Advanced HF leads to an imbalance of catabolic and anabolic pathways favoring catabolism and loss of muscle mass in advanced stages of the disease.26 In part, this might result from IR, which has been characterized as a prognostic factor defining poor outcome.8,27 Impaired glucose homeo-

**Table 3. Laboratory Parameters Before and After Left Ventricular Assist Device Implantation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before LVAD</th>
<th>After LVAD</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete blood count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>34.0±5.1</td>
<td>35.0±6.7</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>11.1±1.9</td>
<td>11.6±2.2</td>
<td>NS</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>88±7.0</td>
<td>88±7.1</td>
<td>NS</td>
</tr>
<tr>
<td>Renal function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>40±21</td>
<td>27±17</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.7±0.8</td>
<td>1.4±0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hepatobiliary function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST, U/L</td>
<td>65±127</td>
<td>35±22</td>
<td>0.12</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>67±123</td>
<td>31±247</td>
<td>0.06</td>
</tr>
<tr>
<td>Total bilirubin, mg/dL</td>
<td>2.3±5.1</td>
<td>0.8±0.4</td>
<td>0.06</td>
</tr>
<tr>
<td>Total protein, g/dL</td>
<td>6.5±1.0</td>
<td>7.4±1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>3.5±0.6</td>
<td>4.0±0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/L</td>
<td>101±61</td>
<td>120±104</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose and lipid metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>137±49</td>
<td>112±41</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>7.0±1.6</td>
<td>6.1±1.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>118±69</td>
<td>163±106</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>129±36</td>
<td>170±50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>34±12</td>
<td>48±15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>71±27</td>
<td>104±42</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

MCV indicates mean corpuscular volume; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

Figure 4. Insulin resistance in patients with advanced heart failure (HF) corrects after left ventricular assist device (LVAD) placement (white bar indicates control subjects, \( n=10 \); black bar, HF before LVAD, \( n=36 \); gray bar, HF after LVAD, \( n=30 \); *\( P<0.01 \) vs control subjects; #\( P<0.05 \) vs HF before LVAD). HOMA-IR indicates homeostasis model assessment–insulin resistance.

Figure 5. Mechanical unloading of the failing myocardium increased cardiac phosphatidylinositol-3 kinase/Akt/Foxo signaling. A, Increased myocardial activation of Akt and Foxo after left ventricular assist device (LVAD) placement. B, Quantitative analysis of Akt and Foxo activation (open bars indicate before LVAD; solid bars, after LVAD; \( n=6 \) individual patients before and after LVAD implantation; *\( P<0.05 \) vs before LVAD).

Figure 6. Impact of mechanical unloading on myocardial lipid content, metabolic gene expression, and adenosine monophosphate-activated protein kinase (AMPK) activation in patients with advanced heart failure (HF). A, No changes are detectable in cardiac levels of triglycerides and free fatty acids. Increased levels of diacylglycerol and ceramide in advanced HF correct after mechanical unloading of the failing myocardium. B, Changes in myocardial metabolic gene expression in response to mechanical unloading (open bars, before left ventricular assist device [LVAD]; solid bars, after LVAD; *\( P<0.05 \) vs before LVAD).
stasis in HF has been linked to increased catecholamine levels, low-grade inflammation, reduced blood flow, and potentially immobilization. The use of LVADs in our patients corrected many of these abnormalities and improved homeostatic model of analysis–insulin resistance.

In the absence of HF, metabolic changes associated with IR are most commonly referred to as the metabolic syndrome, with obesity being the most prevalent. Metabolic risk factors established in individuals without overt disease such as lipid abnormalities and obesity do not carry the same risk in patients with advanced HF; in fact, some metabolic abnormalities (eg, high cholesterol levels or increased body mass index) carry a survival benefit in this patient population.

One characteristic of the metabolic syndrome and diabetes mellitus is accumulation of signaling lipids; these lipids are thought to cause IR and other types of cellular dysfunction. Lipotoxicity is a consequence of either increased lipid—especially FA—uptake or impaired lipid oxidation and storage, leading to accumulation of diacylglycerol and/or ceramide. Both diacylglycerol and ceramide have been implicated in IR, mitochondrial dysfunction, and apoptosis. Genetic alterations in mice that increase tissue diacylglycerol lead to IR, whereas prevention of ceramide synthesis negates fat-induced IR. Analysis in hearts from patients with severe HF shows a marked suppression of genes associated with both FA oxidation and nontoxic storage of lipid in triglyceride. Although CD36, a well-established mediator of cardiac FA uptake, was reduced, it is likely that residual FAs entering the heart are rerouted into synthesis of diacylglycerol and ceramide, leading to accumulation of these signaling lipids.

Lipid accumulation can be a cause and a consequence of HF. A series of genetically altered mice have been created that have lipid accumulation owing to excess FA uptake or reduced oxidation. These include overexpression of genes that cause greater trapping of FAs, reduced activation of peroxisome proliferator–activated receptors, and paradoxically, peroxisome proliferator–activated receptor-α and peroxisome proliferator–activated receptor-γ, which must cause greater lipid uptake than oxidation. Several of these models have activation of PKC and development of hypertrophy. Therefore, lipid accumulation aside from altering metabolism might exacerbate HF.

Increased intracellular lipid accumulation in HF has been described in previous studies and was linked to obesity and diabetes mellitus. One prior human study showed increased levels of triglycerides in the myocardium of obese (body mass index >30 kg/m²) and diabetic patients with advanced HF undergoing heart transplantation. These findings are well in line with animal studies showing increased myocardial levels of triglycerides in murine models of diabetes mellitus. In contrast, animal models of HF secondary to transaortic banding under nondiabetic conditions are associated with reduced myocardial triglyceride content. Unlike in the present study, previous human studies have not differentiated between lipid subspecies. Limited data are available to ascertain whether a nondiabetic group with advanced HF also has an increase in various cardiac lipids. The present study found no increase in FAs or triglycerides whereas ceramide and diacylglycerol levels increased in the nonobese patient cohort. Perhaps this was due to a limited number of patients with diabetes mellitus and obesity;
the average body mass index of our patients was 26.5 kg/m². We also found a uniform decrease in gene expression of regulators of primarily lipid metabolism and oxidation. In particular, levels of DGAT1 and ATGL were reduced. We believe that the decreased levels of triglycerides and FAs represent a depletion of energy storage pools in the failing myocardium and the diversion of incoming FAs into diacylglycerol and ceramide. The underlying pathophysiological mechanism is likely a failure to oxidize FAs owing to mitochondrial dysfunction, a phenomenon that has been well described in advanced HF. Alternatively, impaired myocardial lipolysis might be a primary mechanism leading to reduced FA oxidation. Our patients showed reduced ATGL expression, and ATGL deficiency in mice leads to decreased peroxisome proliferator–activated receptor-α activation and defective lipid use.

One of the central findings of this study is the beneficial effect of mechanical unloading on cardiac metabolism in the failing myocardium. The advent of VADs has improved the therapeutic options for patients with advanced HF, and VADs are now an established intervention in patients awaiting cardiac transplantation. We show here that improved hemodynamics after VAD implantation is accompanied by reduced whole-body and cardiac IR and improved glucose homeostasis, along with a reduction in the levels of the toxic lipid intermediates diacylglycerol and ceramide. Activation of PKC by toxic lipid intermediates with subsequent inhibition of Akt signaling represents a direct molecular link between HF-associated lipotoxicity and myocardial IR. It remains to be clarified which molecular mechanisms connect biomechanical cardiac stress with impaired metabolism. However, the finding of reversible myocardial metabolic derangement after mechanical unloading might uncover novel therapeutic strategies for the treatment of advanced HF patients. Although we found increased activation of adenosine monophosphate-activated protein kinase and enhanced expression of PGC1α after VAD implantation, partially correcting the decreased activation state found in HF samples compared with control subjects, other mechanisms might contribute to the improvement in metabolic state. Increased expression of PGC1α after mechanical unloading also suggests an improvement in oxidative capacity and mitochondrial dysfunction of the failing myocardium.

Our study has several limitations. First, we have only incomplete clinical and laboratory data on the control patients, which is related to the use of samples from a deidentified tissue depository. Furthermore, we could not analyze subgroups of patients such as diabetic versus nondiabetic patients, obese versus nonobese patients, and ischemic versus nonischemic cardiomyopathies owing to the limited number of patients in our tissue substudy. Furthermore, the effects of aging and sex differences are unclear because of the small number of patients in our study.

Conclusions
This study represents the first analysis of cellular pathways of lipotoxicity and IR in patients with advanced HF. Our study suggests a novel pathway of lipotoxic cellular damage contributing to myocardial IR through accumulation of the toxic lipid intermediates diacylglycerol and ceramide. PKC activation may be a crucial mediator of this pathophenomenon. Further studies should focus on potential therapeutic interventions correcting impaired cardiac metabolism and IR through PKC inhibition or alterations in lipid metabolism that prevent the accumulation of toxic lipid intermediates.

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Disclosures
None.

References


**CLINICAL PERSPECTIVE**

The failing human heart develops metabolic derangements characterized by a shift from fatty acids to glucose use for ATP generation. These metabolic changes are accompanied by transcriptional changes typically seen during embryogenesis. The aim of our study was to investigate myocardial levels of toxic lipid intermediates in samples from patients with advanced heart failure compared with control subjects. Furthermore, we analyzed whether mechanical unloading of the failing myocardium corrects impaired cardiac metabolism. For this purpose, we analyzed myocardium and serum of patients with advanced heart failure obtained during left ventricular assist device implantation and at explantation and from control subjects. Systemic insulin resistance in heart failure was accompanied by decreased myocardial triglyceride and overall fatty acid content but increased toxic lipid intermediates, diacylglycerol, and ceramide. Several downstream signaling molecules known to regulate insulin signaling (Akt, Foxo, and protein kinase C) were also found to be dysregulated in the failing myocardium, favoring an insulin-resistant state compared with control subjects. Left ventricular assist device implantation improved insulin resistance and decreased myocardial levels of the toxic lipid intermediates diacylglycerol and ceramide, whereas triglyceride and free fatty acid content remained unchanged. Therefore, abnormal myocardial metabolism, insulin resistance, and lipotoxicity develop in human heart failure. Mechanical unloading through left ventricular assist device implantation corrects systemic and local metabolic derangements in advanced heart failure. This study characterizes metabolic changes in human heart failure with the development of cardiac lipotoxicity. Furthermore, these findings suggest potential therapeutic implications of mechanical unloading of the failing myocardium for the correction of metabolic derangements in advanced human heart failure.