Adipose Tissue Inflammation and Adiponectin Resistance in Patients With Advanced Heart Failure
Correction After Ventricular Assist Device Implantation

Raffay S. Khan, MD; Tomoko S. Kato, MD, PhD; Aalap Chokshi, MD; Michael Chew, MS; Shuqing Yu, MS; Christina Wu, MS; Parvati Singh, MS; Faisal H. Cheema, MD; Hiroo Takayama, MD, PhD; Collette Harris, MS; Gissette Reyes-Soffer, MD; Ralph Knöll, MD, PhD; Hendrik Milting, PhD; Yoshifumi Naka, MD, PhD; Donna Mancini, MD; P. Christian Schulze, MD, PhD

Background—Heart failure (HF) is characterized by inflammation, insulin resistance, and progressive catabolism. We hypothesized that patients with advanced HF also develop adipose tissue inflammation associated with impaired adipokine signaling and that hemodynamic correction through implantation of ventricular assist devices (VADs) would reverse adipocyte activation and correct adipokine signaling in advanced HF.

Methods and Results—Circulating insulin, adiponectin, leptin, and resistin levels were measured in 36 patients with advanced HF before and after VAD implantation and 10 healthy control subjects. Serum adiponectin was higher in HF patients before VAD implantation compared with control subjects (13.3±4.9 versus 6.4±2.1 μg/mL, P=0.02). VAD implantation (mean, 129±99 days) reduced serum adiponectin (7.4±3.4 μg/mL, P<0.05) and improved insulin resistance (Homeostasis Assessment Model of insulin resistance: 6.3±5.8–3.6±2.9; P<0.05). Adiponectin expression in adipose tissue decreased after VAD implantation (−65%; P<0.03). Adiponectin receptor expression was suppressed in the failing myocardium compared with control subjects and increased after mechanical unloading. Histomorphometric analysis of adipose tissue specimens revealed reduced adipocyte size in patients with advanced HF compared with control subjects (1999±24 μm² versus 5583±142 μm² in control subjects; P<0.05), which increased after VAD placement. Of note, macrophage infiltration in adipose tissue was higher in advanced HF patients compared with control subjects (+25%; P<0.01), which normalized after VAD implantation.

Conclusions—Adipose tissue inflammation and adiponectin resistance develop in advanced HF. Mechanical unloading of the failing myocardium reverses adipose tissue macrophage infiltration, inflammation, and adiponectin resistance in patients with advanced HF. (Circ Heart Fail. 2012;5:340-348.)

Key Words: adiponectin ♦ ventricular assist device ♦ heart failure ♦ metabolism ♦ insulin resistance

Advanced heart failure (HF) is associated with local and systemic metabolic derangements and the development of a progressive catabolic state. These changes are linked to systemic inflammation and insulin resistance, which have been shown to influence survival of patients with advanced HF. 1,2 Although several groups have analyzed changes in systemic, myocardial, and skeletal muscle metabolism and inflammation, no study has investigated the specific effects of advanced HF on local adipose tissue histomorphology, metabolism, and inflammation.

Clinical Perspective on p 348

Secreted products of adipose tissue (adipokines) are regulated in HF. 3-5 Adiponectin, an adipokine with insulin-sensitizing effects increases AMP kinase (AMPK) signaling, inhibits cardiac hypertrophy and protects the heart from ischemia-reperfusion injury. 6,7 In human HF, increased levels of adiponectin have been interpreted as a counterregulatory response to increased metabolic stress. These increased levels, however, are insufficient to reverse the insulin resistance associated with HF. 8 Recent evidence suggests that this may be due to the development of a functional adiponectin resistance in the failing heart as well as in the periphery. 5,9 The specific regulation of adipokine signaling in the failing myocardium as well as peripheral tissues such as skeletal muscle and adipose tissue is widely unknown.

The aim of the current study was to characterize adipose tissue histomorphology and inflammation in patients with...
advanced HF and describe the impact of hemodynamic correction through ventricular assist device (VAD) placement in patients with advanced HF. Further, we investigated the regulation of adipokine signaling in patients with advanced HF, before and after mechanical unloading with a VAD.

Methods

Study Cohort

We analyzed 36 patients (29 male, 7 female) with advanced HF who underwent implantation of a VAD at Columbia University Medical Center between January 2002 and May 2010. Of the 36 VADs implanted, 14 were pulsatile and 22 were continuous flow devices.

Blood samples were collected from all patients at the time of VAD implantation and explantation. Blood was centrifuged and serum stored in aliquots at −80°C until final analysis. Myocardial samples from the VAD core of the myocardial apex and from the explanted heart, along with subcutaneous adipose tissue from the abdominal wall, were collected during VAD implantation and at the time of VAD explantation with subsequent cardiac transplantation. Care was taken to avoid using myocardial tissue samples that contained significant scar tissue.

Blood samples were obtained from 10 healthy individuals without renal, hepatic, metabolic, or cardiovascular disease, who had never smoked. Subcutaneous adipose tissue samples were obtained from a tissue database of a cohort of 10 normal subjects who did not have cardiovascular disease. Control myocardial samples 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.

Baseline characteristics, including medical history, medication, and clinical and laboratory information were collected for all patients from electronic medical records. Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg. The Institutional Review Board of Columbia University Medical Center approved the study protocol, and written informed consent was obtained from all patients.

Serum Analysis

Serum samples were analyzed for circulating levels of adiponectin, leptin, resistin (all from Millipore, St Charles, MO), tumor necrosis factor (TNF-α) (R&D Systems, Minneapolis, MN), and insulin (CalBiotech, Spring Valley, CA) according to the manufacturer’s protocol. Brain natriuretic peptide (BNP) values were collected from institutional records. Insulin resistance (IR) was calculated by means of the previously validated Homeostasis Assessment Model (HOMA) of IR according to the formula HOMA-IR = (baseline glucose concentration (mmol/L) × baseline insulin concentration (μU/mL))/22.5.10

RNA Extraction and Real-Time RT-PCR

Total RNA was extracted from the adipose tissue with the use of the phenol guanidine isothiocyanate method (TRIZOL kit, Invitrogen) as per the manufacturer’s instructions. Total RNA (0.7 μg) was reverse-transcribed for 1 hour at 50°C with the THERMOScript RT-PCR system (Invitrogen). The reverse-transcribed cDNA was then amplified with the use of iCycler (Biorad) with Brilliant SYBR Green QPCR master mix (Stratagene). Relative quantification of gene expression for adiponectin (forward primer: GCC GAG GTG ATA GTG TGG TT, reverse primer: TGA GG TGA TGT CCT CGT CTG) was performed using the 2−ΔΔCt method with β-actin (forward primer: AAC GGC AGA AGA GAG AAC CA, reverse primer: AAG ATG ACC CAG GTG AGT GG) as the housekeeping gene.

Protein Expression Analysis

Tissue was homogenized in the presence of protease and phosphatase inhibitors; 30 μg from each fraction was applied to SDS-PAGE and transferred onto nitrocellulose membranes. Antibodies directed against human adiponectin, adiponectin receptor-2 (Phoenix Pharmaceuticals, Burlingame, CA), adiponectin receptor-1 (Cambridge, MA), phospho-AMPK, and AMPK (both Cell Signaling Technology, Danvers, MA) were used for quantification of protein expression. GAPDH (Cell Signaling Technology) was used as a loading control. Biotinylated secondary antibodies were used for detection of specific bands, which were then visualized by incubation of the membrane with enhanced chemiluminescence reagents and exposure to x-ray film. Densitometry was carried out using a Medical Dynamics Personal Densitometer and analyzed using ImageJ software.

Histology for Adipocyte Cross-Sectional Area and Macrophage Content

Adipocyte size was measured on hematoxylin and eosin–stained sections of adipose tissue specimens. Scanned images were analyzed using ImageJ software for cross-sectional area measurements. Immunohistochemical detection of macrophages was performed on 6-μm-thick human adipose tissue paraffin sections and fixed in 4% paraformaldehyde for 5 minutes, followed by methanol for 5 minutes. The sections were blocked for endogenous peroxidase activity by incubation in 0.3% H2O2 in PBS for 10 minutes at room temperature. For CD68 immunostaining, mouse anti-human CD68 monoclonal antibody (Abcam) was used at a dilution of 1:400 in 5% goat serum with 0.1% BSA for 30 minutes, followed by incubation with a biotinylated anti-mouse IgG secondary antibody (Vector Laboratories, Burlingame, CA) for 30 minutes at room temperature. The slides were rinsed with water and washed in methanol for 10 minutes to remove the lipid droplets. Sections were visualized with a Nikon Eclipse E200 microscope. For macrophage quantification, macrophages were counted from 5 to 7 fields per slide and expressed as number of macrophages per high-power field.

Statistical Analysis

Data are presented as mean±SD. Normality was evaluated for each variable from normal distribution plots and histograms. Logarithmic transformation of the variables was performed as needed to improve normality. For data showing a bimodal distribution such as non-Gaussian distribution or positive or negative skewness, we square root–transformed the data to meet assumptions of normality and get an approximately normal distribution before performing statistical analysis. Clinical characteristics of control and HF patients were compared by using the Student unpaired t test for continuous variables or χ2 analysis for noncontinuous variables. Analysis of variance, with Scheffe F adjustment for multiple comparisons, was used to assess differences among control and the values before and after left VAD (LVAD) implantation in HF patients. The change in values in individual patients during LVAD support was analyzed by Student paired t test. Logarithmic transformation of the variables was applied before comparative analysis and nonparametric analysis using the Mann-Whitney U test, and Wilcoxon signed rank test was performed as appropriate. The relation between serum adiponectin and BNP levels was quantified with the Pearson correlation coefficient. Data were analyzed using Prism 5.0 software. Data were considered statistically significant at P<0.05.

Results

Clinical Characteristics

Clinical characteristics of patients with advanced HF who underwent VAD implantation and those who provided their blood samples are shown in Table 1. The mean age was higher and the body mass index (BMI) was lower in patients with HF compared with control subjects. Average duration of LVAD implantation was 129±99 days.

The comparison of laboratory results among control subjects and patients before and after VAD surgery is summarized in Table 2. Both renal function, as reflected by serum
creatinine and blood urea nitrogen levels, and liver function, as reflected by total bilirubin concentration as well as direct bilirubin concentrations, were improved after VAD implantation. Both total protein and albumin levels increased and lipid panels significantly improved after VAD implantation, although total cholesterol levels showed only a trend toward an increase. Anemia was also improved by VAD support. The statistical comparison of values in control and HF patients before and after VAD implantation showed a significant difference except for aspartate aminotransferase, alanine aminotransferase, and total cholesterol levels (Table 2). Nonparametric analysis of the Mann-Whitney test and Wilcoxon signed rank test were performed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and BNP values, which had relatively large standard deviation. The Mann-Whitney test for comparison between control and HF patients revealed probability values for AST, ALT, and BNP of 0.0491, 0.0321, and <0.0001, respectively. The Wilcoxon test revealed that the changes in AST, ALT, and BNP before and after LVAD were associated with probability values of 0.7059, 0.3189, and 0.0018.

In the HF cohort, BMI at the time of VAD explantation was 26.4±3.9 kg/m², which was not significantly different from the value at the time of VAD implantation.

Serum Adiponectin and IR

Mean serum adiponectin levels were significantly higher in advanced HF patients before VAD surgery compared with the value in control subjects. These values normalized after VAD implantation (Figure 1A). RT-PCR analyses of the adipose

Table 1. Baseline Demographics

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>Patients With HF</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean±SD</td>
<td>39.2±9.5</td>
<td>59.4±10.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex, male (%)</td>
<td>7 (70)</td>
<td>29 (81)</td>
<td>0.474</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.4</td>
<td>27.0±5.6</td>
<td>0.485</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>20 (57)</td>
<td>10 (29)</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetes mellitus, type II, n (%)</td>
<td>None</td>
<td>10 (29)</td>
<td>NA</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>14 (40)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>16 (46)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Etiology of heart failure, n (%)</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ischemic</td>
<td>12 (33)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>24 (67)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Left ventricular ejection, %</td>
<td>&gt;60%</td>
<td>17±5.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VAD duration, d</td>
<td>None</td>
<td>129±99</td>
<td>NA</td>
</tr>
</tbody>
</table>

HF indicates heart failure; BMI, body mass index; VAD, ventricular assist device.

Serum Adiponectin and IR

Mean serum adiponectin levels were significantly higher in advanced HF patients before VAD surgery compared with the value in control subjects. These values normalized after VAD implantation (Figure 1A). RT-PCR analyses of the adipose

Table 2. Laboratory Changes After VAD Implantation

<table>
<thead>
<tr>
<th></th>
<th>HF Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-VAD</td>
</tr>
<tr>
<td>Blood count</td>
<td></td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>413±3.7</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>13.8±1.3</td>
</tr>
<tr>
<td>Renal function</td>
<td></td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>126±2.7</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>Liver function</td>
<td></td>
</tr>
<tr>
<td>AST, U/L</td>
<td>19±7</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>20±11</td>
</tr>
<tr>
<td>Total bilirubin, mg/dL</td>
<td>0.6±0.5</td>
</tr>
<tr>
<td>Total protein, g/dL</td>
<td>6.9±0.3</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>4.3±0.2</td>
</tr>
<tr>
<td>Lipid panel</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>110±29</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>136±28</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>57±8</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>89±12</td>
</tr>
<tr>
<td>Cardiac biomarker</td>
<td></td>
</tr>
<tr>
<td>BNP, ng/L</td>
<td>12±4</td>
</tr>
</tbody>
</table>

VAD indicates ventricular assist device; HF, heart failure; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BNP, brain natriuretic peptide.

*P values based on paired t test between pre-VAD and post-VAD values in HF patients.
†P values based on ANOVA of values of control, pre-VAD, and post-VAD groups.
‡P<0.0001, §P<0.05, ¶P<0.01, †P<0.001, versus control subjects, based on ANOVA and subsequent post hoc analysis.
analyzed by real-time PCR and normalized to tissue after VAD implantation. Adiponectin mRNA levels were measured by ELISA.

Figure 1. Regulation of the serum adiponectin levels in advanced heart failure (HF). A, Increased circulating levels of adiponectin in patients with HF corrected after ventricular assist device (VAD) implantation. Serum levels of adiponectin were measured by ELISA. B, Adiponectin gene expression in adipose tissue after VAD implantation. Adiponectin mRNA levels were analyzed by real-time PCR and normalized to β-actin. Data are mean±SEM; n=7 to 21 per group. *P<0.05 versus control (Con) subjects; †P<0.05 versus HF.

tissue revealed that levels of adiponectin mRNA expression were also reduced in HF patients after VAD implantation (Figure 1B).

Table 3 describes the comparison of parameters associated with IR among control subjects and patients before and after LVAD. Compared with control subjects, patients with advanced HF before VAD demonstrated higher fasting blood glucose and an increase in HOMA-IR. Although 10 of the 36 patients with HF had preexisting diabetes, fasting glucose and HOMA-IR were reduced significantly after VAD implantation (Table 3). The analysis of variance of all parameters associated with IR among the values in control and those before and after VAD showed significant differences.

We further measured serum levels of 2 other adipokines, leptin and resistin. Serum levels of leptin in HF patients before VAD tended to be higher than in control subjects (P=0.17) and serum levels of resistin in HF patients before VAD were elevated compared with that in control subjects (P<0.0001). Serum leptin and resistin levels remained elevated after VAD implantation (Table 3).

The correlation analysis revealed that serum adiponectin was positively correlated with serum BNP levels (r=0.4, P=0.004). Serum levels of TNFα, which is marker for a systemic proinflammatory state, were higher in patients with HF before VAD implantation compared with that in control subjects. Serum TNFα levels significantly decreased after VAD implantation (Table 3).

**Myocardial Expression of Adiponectin Receptors**

To study the myocardial regulation of the adiponectin system, we analyzed expression of the adiponectin receptors AdipoR1 and AdipoR2 in myocardial samples obtained from HF patients undergoing VAD implantation and explantation (Figure 2). Expression levels were further analyzed in samples of nondiseased hearts that were obtained to be possible donor hearts but could not be used for transplantation because of recipient-specific causes. Myocardial samples from patients with advanced HF before VAD surgery demonstrated significant reduction in the protein expression of AdipoR1 (Figure 2B) and AdipoR2 (Figure 2C) compared with that in control subjects. However, both AdipoR1 expression and AdipoR2 expression were significantly increased after VAD support (Figure 2B and 2C).

**Myocardial Expression and Activation of AMPK**

Myocardial levels of phosphorylated and total AMPK were also significantly decreased in HF patients before VAD implantation (Figure 2C).

Table 3. Markers of Insulin Resistance, Adipokines, and TNFα

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Subjects</th>
<th>Pre-VAD</th>
<th>Post-VAD</th>
<th>P Value*</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, μIU</td>
<td>3.7±1.9</td>
<td>25.2±21.7†</td>
<td>22.7±24.0‡</td>
<td>0.0486</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.1±0.9</td>
<td>6.7±1.9§</td>
<td>5.5±1.2</td>
<td>0.0495</td>
<td>0.0027</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>NA</td>
<td>6.4±0.9</td>
<td>5.8±0.6</td>
<td>0.0043</td>
<td>NA</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.7±1.9</td>
<td>6.3±5.8‡</td>
<td>3.6±2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin, μg/mL</td>
<td>6.4±2.1</td>
<td>13.3±4.9§</td>
<td>7.4±3.4</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Resistin, ng/mL</td>
<td>8.6±1.2</td>
<td>57.4±21.6‡</td>
<td>53.2±56.3‡</td>
<td>0.1942</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>7.7±2.3</td>
<td>10.2±2.8§</td>
<td>10.1±2.4§†</td>
<td>0.6354</td>
<td>0.0004</td>
</tr>
<tr>
<td>TNFα, pg/mL</td>
<td>1.0±0.3</td>
<td>4.6±0.9‡</td>
<td>2.3±0.4</td>
<td></td>
<td>0.0431</td>
</tr>
</tbody>
</table>

TNF indicates tumor necrosis factor; HF, heart failure; VAD, ventricular assist device; and HOMA-IR, Homeostasis Assessment Model of insulin resistance.

*P values based on paired t test between pre-LVAD and post-LVAD values in HF patients.
†P values based on ANOVA of values of control, pre-VAD, and post-VAD groups.
‡P<0.0001, §P<0.05, ||P<0.01 versus control subjects, based on ANOVA and subsequent post hoc analysis.
implantation compared with that in control subjects (Figure 2A and 2D). Mechanical unloading by VAD increased phosphorylated AMPK levels (Figure 2D) and increased total AMPK expression (Figure 2A).

**Histological Analysis of Adipocyte Size and Adipose Tissue Macrophage Infiltration**

Analysis of adipocyte cross-sectional area revealed that the size of adipocytes obtained from patients with HF before VAD surgery was smaller than that in control subjects (2105±585 μm² versus 5583±757 μm² in control subjects; P<0.05) (Figure 3A). In addition, the size of adipocytes in control subjects tended to be more heterogeneous compared with that in patients with HF (Figure 3B). After mechanical unloading and hemodynamic correction through VAD implantation, adipocyte cross-sectional area increased by around 50% to 3018±727 μm² (P<0.05 versus pre-VAD) but remained lower than that in control adipose tissue.

In the subgroup of individuals who provided adipose tissue samples for the histological analysis, the mean BMI of control subjects was 33.4±1.6 kg/m². The BMI of patients with advanced HF in this subgroup was 28.5±4.3 kg/m².

![Figure 2](image-url) Regulation of myocardial adiponectin signaling in advanced heart failure (HF). Representative immunoblots (A) and quantification of AdipoR1 (B) and AdipoR2 (C) and their downstream target AMPK (D) are shown. Tissue was homogenized in the presence of protease and phosphatase inhibitors. Thirty micrograms of protein extract was applied to SDS-PAGE and transferred onto nitrocellulose membranes. Antibodies against AdipoR1, AdipoR2, p-AMPK, AMPK, and GAPDH were applied. Bands were then visualized by incubation with chemiluminescence reagents and exposure to x-ray. *P<0.05 versus control (Con) subjects; †P<0.05 versus HF; n=6 to 8 per group. VAD indicates ventricular assist device.

![Figure 3](image-url) Adipose tissue area and inflammation in advanced heart failure (HF). A, Mean adipocyte cross-sectional area (CSA), and B, adipocyte frequency histogram in control (Con) subjects (white bars), advanced HF (black bars), and after ventricular assist device (VAD) implantation (gray bars) was made using ImageJ software. Quantification was performed at 200× magnification using 42 to 56 high-power fields (HPF) per group. C, Macrophage infiltration (black arrow) was detected using antibodies directed against CD68. Quantification was performed at a magnification of 200× in 42 to 56 HPF per group. Data are mean±SEM. *P<0.05 versus control subjects; †P<0.05 versus HF; n=6 to 8 per group.
without changes after VAD implantation (post-VAD: 28.2 ± 3.8 kg/m²). To normalize adipocyte cross-sectional area for differences in BMI between the control subjects and the cohort with advanced HF before and after VAD implantation, we performed a mathematical correction of adipocyte cross-sectional area for individual BMI. Consistent with the uncorrected results, adipocyte cross-sectional area corrected for BMI was lower in patients with advanced HF compared with control subjects (169.6 ± 28.1 in control subjects versus 76.2 ± 25.6 · 10⁻¹² m⁴/kg in HF; P < 0.001). VAD implantation increased adipocyte cross-sectional area in patients with advanced HF by around 30% compared with pre-LVAD (post-LVAD: 108.9 ± 30.3 · 10⁻¹² m⁴/kg; P < 0.05 versus pre-VAD). Compared with control subjects, there was a 25% increase in macrophage infiltration in the subcutaneous adipose tissue of HF patients before VAD support, which normalized after mechanical unloading and subsequent hemodynamic correction (Figure 3C).

**Discussion**

HF has traditionally been characterized by hemodynamic and neurohormonal perturbations. Recent studies suggest that immune activation and inflammation as well as disturbed adipokine signaling and insulin resistance play a role in the progression of this disorder. The present study shows for the first time that patients with advanced HF develop adipose tissue inflammation, abnormal systemic and cardiac adiponectin signaling, and functional adiponectin resistance in the failing myocardium and that these abnormalities are corrected by mechanical unloading through VAD support.

Increased levels of several adipokines have been described in advanced HF and elevated circulating levels of adiponectin portend poor survival. These findings are counterintuitive because adiponectin, through its actions on its receptors AdipoR1 and AdipoR2 and subsequent activation of AMPK, mediates insulin-sensitizing and cardioprotective effects. This paradox, though interpreted as a compensatory effort by adipose tissue to correct the energy deficient state of the failing myocardium, probably reflects the development of functional adiponectin resistance. This argument is supported by recent studies that demonstrated a reduction in AdipoR1 expression in infarcted mouse hearts and in skeletal muscles of patients with advanced HF. Increased serum adiponectin levels were linked to increased adiponectin expression in skeletal muscles. Downstream signaling of the adiponectin receptor has been linked to activation of AMPK. Impairment of adiponectin/AMPK signaling has been shown to be associated with increased transition to myocardial failure and susceptibility to ischemia in animal studies of HF. Consistent with these data, we demonstrate for the first time reduced AdipoR1 and AdipoR2 protein expression in the myocardium of patients with advanced HF associated with a concomitant increase in serum adiponectin levels. In our study, adiponectin protein expression in failing myocardium showed no differences from that in control subjects, suggesting that the myocardium is not contributing to the higher levels of serum adiponectin seen in our patient cohort.

Mechanical unloading through VAD implantation reversed the downregulation of both adiponectin receptors and reduced levels of serum adiponectin, indicating the reversal of adiponectin resistance in advanced HF. We speculate that the correction of adiponectin receptor expression and the decrease in serum adiponectin levels are related to the normalization of myocardial as well as systemic metabolic and inflammatory processes by mechanical unloading. This is supported by reduced levels of TNFα after VAD surgery. HF is a systemic inflammatory condition associated with increased circulating levels of cytokines. Mechanical unloading of the failing heart is associated with the changes of molecular markers of inflammation. Previous studies reported that adipose tissue macrophages contribute to systemic inflammation in disease states such as obesity. In the present study, we assessed the effect of VAD implantation on the size of adipocytes and changes in adipose tissue macrophage infiltration in patients with HF and demonstrated an increase in adipocyte size and reduction of macrophage infiltration by mechanical unloading.

Of note, insulin resistance found in patients with advanced HF was also improved after mechanical unloading by the VAD. We speculate that anti-inflammatory processes induced by mechanical unloading would also lead to normalization of IR in these patients. The improvement of renal and liver function parameters after VAD implantation shown in this study implicates improved end-organ perfusion provided by VAD support. This is consistent with a decrease in BNP, mainly due to a decrease in ventricular filling pressure by mechanical unloading through VAD support. We believe that the changes in serum adiponectin and myocardial adiponectin receptors as well as IR after mechanical unloading are not only due to improvement of end-organ perfusion and decreased myocardial stretch but also due to a combination of anti-inflammatory processes and improvement of the patient’s overall morbidity and nutritional status.

In the present observation, serum levels of other adipokines such as leptin and resistin did not change in response to mechanical unloading by the VAD. Further investigation in a larger cohort of patients would be required to clarify the relation between adiponectin resistance and systemic adipokine signaling.

Adipose tissue has long been considered a nonfunctional storage pool of energy without any direct impact on organ function. Recently, however, it has been increasingly recognized as a highly reactive and responsive organ system. In obese patients, increased accumulation of macrophages is a hallmark of a proinflammatory state that links obesity with systemic inflammation. This present study provides the first documentation of adipose tissue inflammation in patients with advanced HF. This was supported by macrophage infiltration in the subcutaneous adipose tissue of patients with advanced HF. In addition, adipocyte size was significantly smaller in patients with HF, consistent with systemic and local catabolic derangements that include the adipose tissue. Adipocyte size and number in general tend to follow overall fat mass. In healthy individuals, adipocyte cross-sectional area and number are approximately 20% to 30% lower in patients with a BMI around 25 to 30 kg/m², compared with...
subjects with a BMI >30 kg/m². In our histological sub-
study analyzing adipocyte cross-sectional area, the group of
patients with advanced HF with a mean BMI of 28.5 kg/m²
had a 62% lower adipocyte cross-sectional area compared
with the control group with a mean BMI of 33.4 kg/m², a
decrease clearly out of proportion for the differences in BMI
noted in our cohorts. This suggests that additional mecha-
nisms may be contributing to this wasting phenotype. VAD
placement resulted in a 50% increase in adipocyte size
compared with pre-VAD placement without changes in BMI.
The differences in adipocyte cross-sectional area remained
significant when adipocyte size was corrected for BMI. These
changes indicate a profound abnormality of adipose tissue in
HF patients that might be related to impaired tissue oxygen-
ation levels secondary to reduced cardiac output. Several
recent studies in obese animals and patients have demon-
strated that local adipose tissue hypoxia contributes to adi-
pose tissue inflammation. The reversal of systemic in-
flammatory processes and adequate blood flow to the adipose
tissue through VAD support might therefore also lead to
normalization of adipocyte size and resolution of macrophage
infiltration.

In contrast to obesity, however, HF is associated with
increased levels of circulating adiponectin. This has been
linked to increased circulating levels of natriuretic peptides
such as atrial natriuretic peptide and BNP that correlate with
systemic adiponectin levels in HF patients and are known to
stimulate adiponectin expression and secretion from adi-
ocytes. Our current study showed only a weak correla-
tion between serum adiponectin and circulating BNP levels.
These findings suggest the existence of a functional cardio-
metabolic signaling axis linking increased cardiac filling
pressures and impaired cardiac metabolism to a specific
response of adipose tissue with increased expression levels of
adiponectin (Figure 4). However, reductions in circulating
adiponectin levels and improvement of adiponectin resistance
may also be due to a combined effect on renal function and β-adrenergic signaling.

Our study has several limitations. First, the histological
analysis performed in our current study is restricted to apical

Figure 4. Proposed model for adipose tissue inflammation and adiponectin reg-
ulation in myocardial and adipose tissue in heart failure (HF). Under normal condi-
tions, adiponectin activates AMPK signal-
ing through its receptors AdipoR1 and
AdipoR2 to maintain tissue normal ox-
dative metabolism and insulin sensitivity.
In HF, insulin resistance is associated
with elevated levels of circulating adi-
ponectin but reduced myocardial
expression of AdipoR1 and AdipoR2.
Increased levels of natriuretic peptides
(brain natriuretic peptide, BNP) and tis-
sue hypoxia have been linked to
increased production of adiponectin by
adipocytes. Hypoxia has also been sug-
gested to increase adipose tissue mac-
rophage infiltration in conditions such as
obesity and result in the release of proin-
fiammatory cytokines that contribute to
the development of insulin resistance.
These metabolic derangements are par-
tially reversible after hemodynamic cor-
rection through left ventricular assist
device (LVAD) implantation in advanced
HF, leading to improved adiponectin
sensitivity and reduced adipose tissue
macrophage infiltration. The response to
hemodynamic correction after LVAD
placement is highlighted by red arrows.
TNF indicates tumor necrosis factor.
areas of the myocardium due to the location of the VAD inflow cannula in the implantation sample. One-third of our HF study cohort had ischemic heart disease as the primary reason for VAD implantation. Therefore, myocardium from these hearts would also contain scar tissue, depending on the infarct area extension. Histological investigation of the right ventricle, which remained to be less influenced by myocardial infarction in such patients, was not performed. Second, we did not stratify our groups in regard to device type because of the small number of patients. We recently reported more profound effects of mechanical unloading by pulsatile flow devices compared with continuous flow devices.\(^{19}\) Device-specific effects on adipokine signaling and adiponectin resistance should be investigated in a larger cohort. Third, we could not subdivide our study cohort in regard to a history of diabetes because of the relatively small number of patients. Finally, increased levels of myocardial phospho-AMP-kinase (pAMPK) after VAD implantation as presented in this study may not be the ideal marker of increased cardiac adiponectin sensitivity. Other factors also affect pAMPK levels, most notably catecholamines.\(^{18}\) From this perspective, the reduced pAMPK levels observed in pre-VAD tissues and the subsequent increase post-VAD implantation might also reflect \(\beta\)-adrenergic signaling defects and improvements. The adiponectin sensitivity should be assessed with a dynamic in vitro assay. However, because the available tissues used for the analysis in this study were obtained over a period of several years, the tissue samples were too inconsistent for the performance of an enzymatic activity assay.

In conclusion, patients with advanced HF show reduced adipocyte size, adipose tissue inflammation, and insulin resistance accompanied by derangements in adipokine levels and signaling. Mechanical unloading of the failing myocardium by VAD implantation was associated with a resolution of both adipose tissue inflammation and myocardial adiponectin resistance. These findings suggest a novel role for adipose tissue in the pathogenesis of systemic metabolic derangements of patients with advanced HF and underline the reversibility of metabolic changes after hemodynamic correction.

### Sources of Funding
This work was supported by grants from the National Heart, Lung, and Blood Institute to Dr Schulze (K23 HL095742-01, P30 HL101272-01, and UL1 RR 024156). Dr Khan was supported by a T32 Training Grant from the National Institutes of Health. Dr Chokshi was supported by the Doris Duke Fellowship Program.

### Disclosures
None.

### References
Patients with advanced heart failure develop metabolic derangements that affect a multitude of organ systems including the lungs, kidneys, liver, and skeletal muscle. The current study investigated adipose tissue inflammation and the regulation of the adipose tissue–derived molecule adiponectin. Adipose tissue of patients with advanced heart failure showed increased infiltration of macrophages and reduced adipocyte cell size. Patients with advanced heart failure had increased circulating levels of adiponectin with reduced expression of myocardial adiponectin receptors AdipoR1 and AdipoR2 as well as reduced activation of AMP kinase, a known downstream signaling molecule. These findings are suggestive of a functional adiponectin resistance in advanced heart failure. Mechanical unloading of the failing myocardium with a concomitant increase in cardiac output after implantation of a left ventricular assist device resulted in reduced adipose tissue macrophage infiltration and reduction of circulating levels of adiponectin. Further, myocardial levels of AdipoR1 and AdipoR2 increased and activation of AMPK was enhanced after left ventricular assist device implantation. These novel findings suggest a potential role of adipose tissue inflammation and adiponectin resistance in the pathogenesis of advanced heart failure.
Adipose Tissue Inflammation and Adiponectin Resistance in Patients With Advanced Heart Failure: Correction After Ventricular Assist Device Implantation
Raffay S. Khan, Tomoko S. Kato, Aalap Choksi, Michael Chew, Shuiqing Yu, Christina Wu, Parvati Singh, Faisal H. Cheema, Hiroo Takayama, Collette Harris, Gisette Reyes-Soffer, Ralph Knöll, Hendrik Milting, Yoshifumi Naka, Donna Mancini and P. Christian Schulze

*Circ Heart Fail.* 2012;5:340-348; originally published online February 29, 2012; doi: 10.1161/CIRCHEARTFAILURE.111.964031
*Circulation: Heart Failure* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/5/3/340

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation: Heart Failure* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation: Heart Failure* is online at: http://circheartfailure.ahajournals.org//subscriptions/