Increased Levels of Retinol Binding Protein 4 in Patients With Advanced Heart Failure Correct After Hemodynamic Improvement Through Ventricular Assist Device Placement

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Background: Chronic heart failure is associated with higher risk for developing diabetes mellitus. Secretory products from adipocytes may contribute to the deterioration in glycemic control and increased insulin resistance (IR). Retinol binding protein 4 (RBP4) is an adipose tissue-derived protein with pro-diabetogenic effects. The aim of the present study was to investigate the relationship of RBP4 in patients with heart failure.

Methods and Results: Serum levels of RBP4, insulin, and fasting glucose were assessed in 58 patients with severe heart failure at the time of left ventricular assist device (LVAD) implantation and in 44 patients at the time of explantation, as well as in 10 normal control subjects. Serum RBP4 levels were measured by specific enzyme-linked immunosorbent assay, and IR was assessed using the homeostatic model of IR (HOMA-IR). Fasting glucose, insulin and HOMA-IR were significantly higher in patients at the time of LVAD implantation compared to controls (all P<0.01). RBP-4 and HOMA-IR significantly decreased after LVAD implantation (21.7±8.8 mg/dl to 16.0±3.8 mg/dl, P<0.05; 4.2±2.7 to 2.5±2.0, P<0.01).

Conclusions: Patients with advanced heart failure have increased levels of RBP4, and LVAD implantation reduces RBP4. These findings implicate RBP4 in the cascade of reversible metabolic derangements in advanced heart failure. (Circ J 2012; 76: 2148–2152)

Key Words: Heart failure; Insulin resistance; Retinol binding protein 4; Ventricular assist device

Advanced chronic heart failure has been associated with hyperinsulinemia and insulin resistance (IR) and increased risk for the development of impaired glucose tolerance and diabetes mellitus. It has been proposed that the alteration of glucose metabolism leads to progressive deterioration of skeletal muscle and myocardial function and is responsible for the clinical features of fatigue and reduced exercise tolerance in patients with heart failure. The development of IR in heart failure is likely multi-factorial, and the result of various hemodynamic and hormonal changes influencing differential expression of pro-inflammatory and anti-inflammatory mediators and adipocytokines, including tumor necrosis factor (TNF)-α, leptin, resistin and adiponectin.

Retinol binding protein 4 (RBP4) is an adipocytokine that mediates the transport of retinol from the liver to various target tissues. It is synthesized and stored in both hepatocytes and adipocytes and its expression is increased in adipose tissue that has reduced expression of the glucose transporter-4.

Several mechanisms have been proposed to explain the relationship between RBP4 and IR, namely that RBP4 is associated with an increase in hepatic gluconeogenesis by enhancing the expression of phosphoenolpyruvate carboxykinase, and attenuates insulin signaling in skeletal muscle via phosphatidylinositol 3-kinase (PI3K). Although studies in humans have demonstrated conflicting results between RBP4 and parameters of the metabolic syndrome, RBP4 remains a protein with diabetogenic potential and may contribute to the IR described in advanced heart failure.
In a recent study by Bobbert et al., RBP4 levels and the homeostasis model assessment as an index of IR (HOMA-IR) were significantly increased in patients with dilated inflammatory cardiomyopathy. That study found that the expression of RBP 4 mRNA is partially regulated by serum interleukin (IL)-8, which is an inflammatory cytokine known to be elevated in advanced heart failure.

In the present study, we examined serum RBP4 levels and IR in patients with advanced heart failure requiring left ventricular assist device (LVAD) implantation. Hemodynamic unloading of the heart in patients with LVAD has been demonstrated to improve myocardial contractility, remodeling and intercellular signaling. We hypothesized that these hemodynamics improvements decreased IR and RBP4 levels.

**Methods**

**Study Design**
The study cohort consisted of 58 patients with advanced heart failure undergoing LVAD implantation at Columbia University Medical Center between January 2002 and June 2010. Smoking behavior, medical history and cardiovascular medications of all patients at the time of LVAD implantation were assessed by a questionnaire. Blood samples were obtained immediately prior to LVAD implantation (n=58) and at explantation (n=44). Diabetic profiles including serum RBP4 concentration before and after LVAD implantation were evaluated. Blood samples were also obtained from 10 healthy individuals without renal, hepatic, metabolic or cardiovascular disease who never smoked, as controls.

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

**Serum Analysis**
Blood samples were collected from all patients and controls and stored after adequate centrifugation at −80°C prior to assay. Serum levels of RBP4 and TNF-α were determined using a specific enzyme-linked immunosorbent assay following the manufacturer’s instructions (R&D Systems). HOMA-IR was used to calculate IR. All samples were examined in duplicate and mean individual serum concentrations were used for statistical analysis.

**Statistical Analysis**
Mean ± SD are reported for all continuous variables. Normality was evaluated for each variable from normal distribution plots and histograms. Comparisons for continuous variables between patients and controls were analyzed using Student’s unpaired t-test. Categorical data between patients and controls were compared using chi-square test. Data at LVAD implantation and explantation were analyzed using Student’s paired t-test. Correlational analysis was performed using Pearson’s correlation coefficients. P<0.05 was considered significant. Statistical analysis was performed using SPSS version 18.

**Results**

**Patient Characteristics**
Clinical characteristics of all subjects at the time of LVAD implantation are listed in Table 1. Mean left ventricular ejection fraction derived from echocardiography at the time of LVAD implantation was 17.6±5.9%. Analysis for concomitant diseases and medications showed that 34.5% (n=20) of patients had a prior diagnosis of diabetes mellitus at the time of LVAD implantation. Fifteen of the patients were either on insulin (n=10) or oral anti-diabetic agents (n=5). The anti-diabetic treatment for patients with known diabetes was not changed except for the dose of medication during LVAD support. The average duration of LVAD implantation was 146±106 days.

**Effect of LVAD Implantation on Laboratory Results**
Table 2 lists the laboratory results before and after LVAD surgery. Platelet counts and sodium concentration increased, and both renal function and hepatic function as reflected by serum creatinine, blood urea nitrogen, total bilirubin, direct bilirubin and alanine aminotransferase levels. Significantly improved after LVAD implantation. Brain natriuretic peptide levels significantly decreased on LVAD support.

**Diabetic Profile Serum RBP4 and TNF-α Concentrations**
Table 3 summarizes the comparison of diabetic profiles and serum RBP4 concentrations among controls and the entire cohort of patients before and after LVAD placement, as well as a subgroup of patients without diabetes mellitus. Fasting glucose, insulin levels, HOMA-IR, RBP4 and HbA1c levels were significantly higher in patients at the time of LVAD implantation compared to controls, even in the subgroup of patients without diabetes. These parameters significantly decreased after LVAD implantation. Laboratory markers at the time of LVAD explantation remained higher than in controls except for RBP4 (Table 3; Figure 1B). Serum TNF-α, which is a marker for systemic pro-inflammatory state, was higher in patients before LVAD implantation compared to controls. Serum TNF-α level decreased after LVAD implantation, but was still higher than in controls (Table 3).

Subsequent analysis did not demonstrate significant correlations between RBP4 and HOMA-IR (Figure 2), fasting glu-
### Table 2. Laboratory Results Before and After LVAD Surgery

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients undergoing LVAD implantation</th>
<th></th>
<th></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before LVAD</td>
<td>After LVAD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (×10^3/μl)</td>
<td>7.3±2.4</td>
<td>9.4±3.7</td>
<td>9.9±4.7</td>
<td>0.375</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte subset (%)</td>
<td>34.2±7.2</td>
<td>14.4±8.5†</td>
<td>14.2±7.8**</td>
<td>0.900</td>
<td></td>
</tr>
<tr>
<td>Hct (%)</td>
<td>41.6±3.8</td>
<td>34.5±5.7†</td>
<td>33.7±3.8**</td>
<td>0.337</td>
<td></td>
</tr>
<tr>
<td>Platelets (×10^3/μl)</td>
<td>258.6±38.7</td>
<td>175.5±31.6**</td>
<td>208.1±62.4*</td>
<td>0.0004</td>
<td></td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>143.6±3.2</td>
<td>132.9±6.3**</td>
<td>136.7±3.6**</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>4.3±0.2</td>
<td>4.2±0.6</td>
<td>4.2±0.4</td>
<td>0.262</td>
<td></td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>13.1±3.4</td>
<td>37.1±20.8**</td>
<td>23.6±13.7*</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Crea (mg/dl)</td>
<td>1.1±0.3</td>
<td>1.6±0.6*</td>
<td>1.3±0.6</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Alb (mg/dl)</td>
<td>4.3±0.3</td>
<td>3.5±0.5†</td>
<td>3.5±0.5†</td>
<td>0.398</td>
<td></td>
</tr>
<tr>
<td>T-Bil (mg/dl)</td>
<td>0.6±0.4</td>
<td>1.5±0.9*</td>
<td>1.0±0.7</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>D-Bil (mg/dl)</td>
<td>0.3±0.3</td>
<td>0.5±0.5</td>
<td>0.4±0.4</td>
<td>0.0088</td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>19.9±7.4</td>
<td>35.3±39.9</td>
<td>29.3±19.4</td>
<td>0.285</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>20.4±12.3</td>
<td>37.2±34.3</td>
<td>24.6±16.3</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>BNP (pg/μl)</td>
<td>13.2±4.6</td>
<td>1,493.5±1,286.5†</td>
<td>740.4±649.3†</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Data given as mean±SD. *P<0.05, **P<0.01, †P<0.001 vs. controls (unpaired t-test).

LVAD, left ventricular assist device; WBC, white blood cell count; Hct, hematocrit; BUN, blood urea nitrogen; Crea, creatinine; Alb, albumin; T-Bil, total bilirubin; D-Bil, direct bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BNP, brain natriuretic peptide.

### Table 3. Diabetic Profiles, Serum RBP4 and TNF-α

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>All patients</th>
<th>Patients without DM</th>
<th></th>
<th></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before LVAD</td>
<td>After LVAD</td>
<td>Before LVAD</td>
<td>After LVAD</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>93.0±15.8</td>
<td>133.5±38.9**</td>
<td>114.3±40.8</td>
<td>118.0±34.9**</td>
<td>94.4±15.3</td>
<td>0.043</td>
</tr>
<tr>
<td>Fasting insulin (μU/ml)</td>
<td>8.5±10.3</td>
<td>36.3±30.0**</td>
<td>26.7±25.9*</td>
<td>28.5±22.5**</td>
<td>22.2±19.0**</td>
<td>0.172</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.1±1.2</td>
<td>4.2±2.7**</td>
<td>2.5±2.0*</td>
<td>2.8±2.3**</td>
<td>2.1±1.8*</td>
<td>0.022</td>
</tr>
<tr>
<td>RBP4 (mg/dl)</td>
<td>16.0±3.9</td>
<td>21.7±8.8*</td>
<td>16.0±3.8</td>
<td>20.8±6.6*</td>
<td>15.6±4.2</td>
<td>0.008</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.8±0.6</td>
<td>7.1±1.5**</td>
<td>6.2±1.0*</td>
<td>6.2±0.7**</td>
<td>5.5±0.5*</td>
<td>0.018</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>1.0±0.3</td>
<td>4.6±0.9**</td>
<td>2.3±0.4**</td>
<td>4.2±0.6**</td>
<td>2.3±0.6*</td>
<td>0.053</td>
</tr>
</tbody>
</table>

Data given as mean±SD. *P<0.05, **P<0.01 vs. control (unpaired Student’s t-test).

RBP4, retinol binding protein 4; TNF, tumor necrosis factor; DM, diabetes mellitus; LVAD, left ventricular assist device; HOMA-IR, homeostatic model of insulin resistance.

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**Figure 1.** Comparison of (A) homeostatic model of insulin resistance (HOMA-IR) and (B) retinol binding protein 4 (RBP4) between controls and patients at the time of left ventricular assist device implantation, and among patients between implantation and explantation.
Retinol Binding Protein 4 in HF

cose, insulin or serum creatinine concentration. The changes in RBP4 and HOMA-IR before and after LVAD surgery, which were calculated as (pre-LVAD value)–(post-LVAD value), did not show a significant correlation.

**Discussion**

Heart failure is characterized by an increase in the production of cytokines that mediate both compensatory and pathological responses, and is associated with the development of metabolic derangements including impaired glucose tolerance and IR. An increased understanding of the molecular mechanisms underlying severe heart failure has led to several potential areas of investigation to elucidate the mechanisms underlying IR and the predisposition for developing diabetes mellitus. Prior studies have identified cytokine-induced IR with leptin, TNF-α and adiponectin as regulatory elements of energy metabolism in patients with moderate heart failure.2–4 More recently, RBP4 has been implicated in the regulation of energy expenditure and IR. Bobbert et al reported increased plasma levels of RBP4 in patients with dilated cardiomyopathy and its association with IR.11 RBP4 elevation and its reversibility after mechanical support in patients with advanced heart failure, however, have not yet fully elucidated.

The present data demonstrate that RBP4 levels are significantly increased in patients with advanced heart failure, and decrease in response to mechanical unloading and hemodynamic correction following the implantation of an LVAD. Fasting glucose and insulin as well as HOMA-IR are markedly elevated in patients with heart failure before LVAD implantation compared to controls, and show tendencies to decrease after LVAD implantation. IR accompanied by elevation of RBP4 and its normalization after LVAD implantation were also seen in patients without diabetes mellitus. In the present patient cohort, no association was found between RBP4 and renal function.

In a previous study, diabetic patients with advanced heart failure showed a marked improvement in glucose control after LVAD implantation.12 That study, however, was limited to routine laboratory markers of glucose homeostasis and did not assess IR or sensitivity according to circulating levels of insulin. Further, the diagnosis of diabetes mellitus was based on medical records and did not reflect the dynamic disease process of patients in the end-stage of heart failure with a high degree of cytokine activation. In contrast, the present study evaluated the diabetic profile of patients with and without a known history of diabetes.

We speculate that the reason for the remarkable dynamics and reduction in RBP4 after LVAD implantation, irrespective of changes in HOMA-IR, fasting insulin or glucose level, is that RBP4 is also partially controlled by systemic inflammation and the detrimental effects of impaired hemodynamics in addition to glucose tolerance. RBP4 may reflect the improvement of inflammation derived from adequate circulation by the use of LVAD more significantly than other parameters associated with glucose intolerance. It has been reported that circulating IL-8 induces RBP4 mRNA expression in patients with inflammatory cardiomyopathy.11 Several studies demonstrated a reduction in IL-8 following LVAD implantation.13–15 Therefore, the present finding of a reduction in serum RBP4 after LVAD implantation could be influenced by the reduction of IL-8 signaling associated with mechanical unloading. In the present study, we did not measure IL-8 but measured TNF-α, and found a reduction of TNF-α after LVAD surgery. The present results suggest that the systemic inflammatory condition in advanced heart failure is associated with increased circulating levels of cytokines. Mechanical unloading of the failing heart by LVAD decreases molecular markers of inflammation. Although we did not measure changes in wall stress or workload in the present patients, the changes in these parameters are anticipated based on prior reports.16–18 A similar target of investigation would be the influence of tissue ischemia, hypoxia and increased generation of reactive oxygen species on RBP4 expression. A recent study by Takebayashi et al analyzing the effects of RBP4 on vascular endothelial cells, demonstrated that RBP4 enhances endothelial nitric
oxide synthase (eNOS) production via stimulation of PI3K/Akt/eNOS pathways leading to vasodilation.\textsuperscript{19} Hypoxia has also been implicated in the promotion of vasodilation through these pathways in ischemia stress models of porcine coronary artery endothelium.\textsuperscript{30} In line with these observations, we suspect that the observed reductions in serum RBP4 levels following hemodynamic correction result from a multi-factorial pedigree of improved neurohumoral signaling, improved oxygenation and marked reduction in systemic inflammation following enhanced perfusion of peripheral tissues with LVAD placement. Further studies are required to provide insights into candidate mechanisms.

The present study was limited by the small cohort, retrospective nature and the absence of serial measurements of neurohumoral and inflammatory markers. We measured RBP4 levels only twice in each patient, at the time of LVAD implant and at explant, therefore, we could not investigate how long it would take for a reduction of RBP4, as well as normalization of IR after LVAD implantation. Another limitation of this study was the absence of an analysis of the effects of renin-angiotensin system inhibition on glucose tolerance during LVAD support. We also did not measure serum levels of neurohormones in the present cohort. Because the described reductions in IR and RBP4 would implicate improved glucose control in heart failure patients, it would be beneficial to correlate these findings with parameters of subsequent glucose control and measurements of insulin requirements. Finally, due to risk of subsequent infection and other complications, we did not perform prolonged and repeated pressure monitoring postoperatively in the majority of patients. Therefore, we could not include a detailed postoperative hemodynamic assessment. Prospective studies to investigate the systemic benefits from LVAD support on glucose metabolism are needed.

In conclusion, serum RBP4 levels in patients with advanced heart failure may provide novel insights into metabolic derangements and the potential reversibility of these abnormalities. The clinical benefit of mechanical unloading on IR and RBP4 levels needs to be further characterized to improve understanding of impaired glucose metabolism and its impact on clinical outcome parameters in advanced heart failure.

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Disclosures
The authors declare no conflicts of interest.

References