Functional Adiponectin Resistance at the Level of the Skeletal Muscle in Mild to Moderate Chronic Heart Failure

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Background—Adiponectin is an antiinflammatory, insulin-sensitizing, and antiatherogenic adipocytokine that plays a fundamental role in energy homeostasis. In patients with chronic heart failure (CHF), high circulating adiponectin levels are associated with inverse outcome. Recently, adiponectin expression has been identified in human skeletal muscle fibers. We investigated the expression of adiponectin, the adiponectin receptors, and genes involved in the downstream lipid and glucose metabolism in the skeletal muscle of patients with CHF.

Methods and Results—Muscle biopsies (vastus lateralis muscle) were obtained from 13 patients with CHF and 10 healthy subjects. mRNA transcript levels of adiponectin, adiponectin receptors (AdipoR1 and AdipoR2), and downstream adiponectin-related enzymes were quantified by real-time reverse transcriptase polymerase chain reaction. Adiponectin expression in the skeletal muscle of patients with CHF was 5-fold higher than in healthy subjects ($P<0.001$), whereas AdipoR1 was downregulated ($P=0.005$). In addition, the expression of the main genes involved in downstream pathway (peroxisome proliferator-activated receptor-α [PPAR-α] and both AMP-activated protein kinase-α1 and -α2 subunits) as well as their target genes in lipid (acyl-coenzyme A dehydrogenase C-14 to C-12 straight chain) and glucose metabolism (hexokinase-2) were significantly reduced in CHF. The strong positive correlation found between AdipoR1 and PPAR-α/AMP-activated protein kinase gene expression was confirmed in PPAR-α null mice, suggesting a cause-and-effect relationship. Immunohistochemical staining confirmed the presence of adiponectin in the skeletal muscle.

Conclusions—Despite increased adiponectin expression in the skeletal muscle, patients with CHF are characterized by downregulation of AdipoR1 that is most probably linked to deactivation of the PPAR-α/AMP-activated protein kinase pathway. These facts suggest functional adiponectin resistance at the level of the skeletal muscle in CHF. (Circ Heart Fail. 2010;3:185-194.)

Key Words: adiponectin ■ energy metabolism ■ heart failure

Chronic heart failure (CHF) is a complex syndrome of hemodynamic, neurohormonal, and metabolic abnormalities. The classical hallmark of CHF is exercise intolerance. This phenomenon is increasingly recognized as a consequence of peripheral maladaptive responses, such as endothelial dysfunction and skeletal muscle abnormalities. At the level of the peripheral skeletal muscle, ultrastructural mitochondrial changes, fiber shift, and muscle atrophy contribute to impaired functional performance.1-2 In addition, profound metabolic changes involving mitochondrial bioenergetics have been put forward to explain the energetic deficit that characterizes skeletal muscles of patients with CHF.3 Recently, the impact of insulin resistance (IR) on the progression of CHF4 and the existence of a metabolic vicious cycle in heart failure5 have been highlighted.

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Adiponectin is an antiinflammatory, insulin-sensitizing, and antiatherogenic adipocytokine that plays a fundamental role in energy homeostasis.6 Observational studies in healthy subjects and in those at cardiovascular risk have confirmed the expected protective role of adiponectin.7 Increased levels of circulating adiponectin have been observed repeatedly in patients with CHF.8-12 The exact source of these increased levels remains unclear. Adiponectin secretion is not exclusive
to adipose tissue, and recent data show that other cells, including skeletal muscle myocytes and cardiomyocytes, are capable of its synthesis. Increased circulating levels of adiponectin in CHF are associated with inverse outcome. This fact remains unexplained and difficult to reconcile with the IR state that characterizes the CHF syndrome. Although speculative, increased adiponectin concentrations have been suggested as a compensatory mechanism to overcome IR and inflammatory changes. On the other hand, a hitherto unrecognized detrimental effect of adiponectin in this specific population remains to be excluded.

Through its receptors (ie, adiponectin receptor [AdipoR] 1 mainly expressed by skeletal muscle and AdipoR2 mainly expressed by liver tissue), adiponectin is intricately related to skeletal muscle substrate use. Adiponectin promotes glucose uptake by skeletal muscles, inhibits gluconeogenesis, and stimulates β-oxidation of fatty acids. Downstream effectors of the AdipoRs include AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-α (PPAR-α). AMPK is considered an energy-sensing enzyme that on stimulation enhances glucose use and fatty acid oxidation, whereas PPAR-α is a key nuclear transcription factor, regulating expression of genes involved in fatty acid uptake and use.

The regulation of the local adiponectin system at the level of the skeletal muscle and its metabolic relevance in patients with CHF have not been explored yet. Therefore, the aim of this study was to examine the expression of adiponectin and its receptors at the level of the skeletal muscle in patients with CHF compared with healthy subjects. In addition, signaling pathways (ie, AMPK and PPAR-α) and their downstream targets involved in glucose and fatty acid metabolism were assessed. To further unravel the interaction between the adiponectin system and these pathways, expression was assessed in the skeletal muscle of PPAR-α knockout mice.

Methods

Subjects

Thirteen patients with CHF and systolic dysfunction due to ischemic or dilated cardiomyopathy were recruited from the Cardiac Rehabilitation Centre of the Antwerp University Hospital (Edegem, Belgium). Patients in New York Heart Association class II to III were included if left ventricular ejection fraction was <45% (assessed by radio nucleotide ventriculography) and if they had been stable on medical treatment and symptoms for at least 1 month before inclusion. Exclusion criteria were recent acute coronary syndrome or revascularization (<3 months), valvular disease requiring surgery, exercise-induced myocardial ischemia or malignant ventricular arrhythmia, or acute myocarditis or pericarditis and cerebrovascular or musculoskeletal disease preventing exercise testing or training. Patients with acute or chronic infections, allergies, cancer, or inflammatory diseases; diabetes mellitus treated with thiazolidinediones; fibrates treatment; and serum creatinine values ≥2 mg/dL also were excluded to avoid possible metabolic interference. Collected data consisted of clinical evaluation by a cardiologist (medical history, including medications used; New York Heart Association classification based on patient information; and physical examination), echocardiography, radio nucleotide angiography, collection of fasting blood samples, and cardiopulmonary exercise testing. Patients with CHF were compared with 10 healthy subjects of similar age, gender, and body mass index and with a normal lipid profile. The study was approved by the local ethics committee, and a written informed consent was obtained from all patients and healthy subjects.

Cardiopulmonary Exercise Testing

Symptom-limited cardiopulmonary exercise testing was performed on a treadmill or cycle ergometer in a nonfasting condition and under medication. Two protocols were used, aiming at an optimal exercise duration of 8 to 12 minutes. The ramp protocol started with an equivalent of 20 or 40 W, proceeding with incremental steps of 10 or 20 W/min, respectively. Breath-by-breath gas exchange measurements permitted online determination of ventilation, oxygen uptake, and carbon dioxide production. Patients were continuously monitored with 12-lead ECG, whereas automatic blood pressure measurement was performed every 2 minutes. Peak oxygen consumption (VO_{2\text{peak}}) was expressed as the highest attained oxygen uptake.

Biochemical Analyses

Fasting blood samples were collected between 8 AM and 9 AM. Serum creatinine, total cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein cholesterol levels were assessed immediately on Vitros Fusion 5.1 instruments, using reagents from Ortho Clinical Diagnostics. Plasma was separated by centrifugation, and aliquots were stored at −80°C.

Circulating adiponectin, tumor necrosis factor-α, and interleukin-6 concentrations were measured using commercially available enzyme-linked immunosorbent assays. Details are available in the online-only Data Supplement. Standards and samples were run in duplicate according to the manufacturer’s recommendations. Internal controls (low, medium, and high) were implemented to value assay performance (Quantikine enzyme-linked immunosorbent assays kit controls). N-terminal prohormone brain natriuretic peptide (NT-proBNP) was determined using a sandwich immunoassay on an Elecsys 2010. The homeostasis model assessment-IR index was calculated as (fasting plasma insulin [µU/mL] × fasting plasma glucose [mmol/L])/22.5.

Muscle Biopsies

Muscle biopsies were obtained from the vastus lateralis muscle under aseptic conditions and under local anesthesia (2% lidocaine) using the Bergstrom needle technique. The procedure was separated by at least 1 day from exercise testing or training. The biopsies were immediately frozen in liquid nitrogen and stored at −80°C. A small part of the tissue was transferred in formaldehyde and embedded in paraffin.

RNA Isolation and Quantitative Real-Time Reverse Transcriptase Polymerase Chain Reaction

Total RNA was extracted from frozen skeletal muscle samples (10 to 20 mg) using the Trizol reagent technique. RNA concentrations were determined by absorbance at 260 nm (A_{260}), and purity was estimated by A_{260}/A_{280} ratio determination. Oligo-dT first-strand cDNA was synthesized from 2-µg total RNA using Superscript II reverse transcriptase. Real-time reverse transcriptase polymerase chain reaction was performed for mRNA relative quantification as previously described.

The gene-specific primers for each gene are listed in Table 1. mRNA levels for all target genes, given as mean±SEM in arbitrary units (AU), were normalized to β2-microglobulin and TATA box-binding protein levels using GeNorm software.

PPAR-α Knockout and Control Mice

To further unravel downstream signaling pathways of the adiponectin system, an experimental model using PPAR-α knockout mice was used. The potential differential influence of fiber type was explored, and expression profiles of both slow-twitch oxidative
(soleus) and fast-twitch (gastrocnemius) skeletal muscle were assessed. The generation of PPAR-α-deficient mice with a C57BL/6J genetic background has been described elsewhere.22,23 Adult male PPAR-α−/− mice (n=10) were compared with their appropriate control or wild-type C57Bl6 mice (n=8). Gene expression profiles of the knockout and wild-type mice were determined by reverse transcriptase polymerase chain reaction with primer pairs for mouse adiponectin (forward, GATGGCACCTCTGGGAGAGA; reverse, CCAGTCGTGCCGTCATAAT), AdipoR1, AdipoR2, PPAR-α, AMPK-α1, and AMPK-α2 (Table 1). Animals were housed under temperature-controlled conditions (21°C) and had free access to water and a standard mouse chow. In vivo studies were conducted under European Union guidelines for the use and care of laboratory animals.

**Immunohistochemistry**

Immunohistochemical detection of adiponectin was performed on skeletal muscle sections of 4 patients with CHF and 4 healthy controls as described in the Data Supplement.

**Western Blot**

Immunoblot protein levels of phosphorylated AMPK and total AMPK were determined for 8 patients with CHF and 8 healthy subjects by using the following primary antibodies: antipan-antibody AMPK cell signaling. The protocol is described in detail in the Data Supplement.

**Statistical Analyses**

All results are presented as mean±SEM. Considering the limited number of subjects in all groups and the large spread within certain variables, nonparametric tests were used. Baseline characteristics of the groups were compared using the Mann-Whitney U or Kruskal-Wallis test for continuous variables. The χ² test was used for categorical variables. Spearman rank correlation coefficients between variables were calculated to examine cross-sectional associations of adiponectin and its receptors with other clinical and biochemical variables. A P<0.05 was considered statistically significant.

**Results**

**Clinical Characteristics**

Thirteen patients with CHF (8 men) with a mean age of 60.1±2.2 years were included in the study. Heart failure was of ischemic etiology in 61.5%; the remaining patients suffered from idiopathic cardiomyopathy. Mean left ventricular ejection fraction was 25.2±2.3%. The majority (61.5%) of patients were in New York Heart Association functional class II, and 5 (38.5%) were in class III. All patients were treated with an angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, or both; 77% were treated with a β-blocker; and 92% received a diuretic agent. Statins were prescribed in 39% of the patients. Complementary clinical characteristics are listed in Table 2. Ten healthy subjects were included and submitted to the same assessments as patients. Whereas the healthy subjects and patients with CHF were comparable with respect to age, gender, and anthropometric parameters, patients with CHF exhibited decreased exercise capacity, dyslipidemia, a proinflammatory cytokine profile, and IR (Table 2).

**Plasma Adiponectin Concentrations**

Circulating adiponectin levels were significantly higher in patients with CHF than in healthy subjects (Table 2). Moreover, disease severity, as determined by NT-proBNP levels, was associated with higher adiponectin concentration (7.58±1.35 mg/L for patients with NT-proBNP levels below the median versus 14.36±1.72 mg/L for those with NT-proBNP above the median, P=0.026) (Figure 1).

**Adiponectin and AdipoR1 and AdipoR2 mRNA Expression**

Skeletal muscle mRNA content of adiponectin was 5-fold higher in patients with CHF than in healthy subjects (348±64 AU versus 64±17 AU; P<0.001) (Figure 2). AdipoR1
mRNA transcription was significantly downregulated in patients with CHF (P=0.005), whereas AdipoR2 mRNA expression did not differ between the groups (Figure 2). There was no relationship between circulating levels of adiponectin and adiponectin mRNA expression (r=0.301, P=0.173). Local adiponectin mRNA transcription tended to correlate negatively with the expression of AdipoR1 (r=-0.355, P=0.096), but a strong negative correlation was found with the expression of PPAR-α (r=-0.526, P=0.010) and AMPKα1 subunit (r=-0.545, P=0.007).

### AdipoR1 and Signaling Pathways

The mRNA content of PPAR-α (P<0.001) and one of its target genes in lipid metabolism, acyl-coenzyme A dehydrogenase C-14 to C-12 straight chain (ACADM) (P=0.036), was significantly downregulated in patients with CHF versus healthy subjects (Figure 3). Similarly, mRNA content of both catalytic AMPK subunits (AMPK-α1, P=0.004; AMPK-α2, P=0.018) and the AMPK target gene in glucose metabolism, hexokinase-2 (HK2) (P<0.001), were downregulated (Figure 3). No significant difference in mRNA expression was observed for

### Table 2. Comparison of Healthy Subjects and Patients With CHF at Baseline

<table>
<thead>
<tr>
<th></th>
<th>Healthy Subjects (n=10)</th>
<th>Patients With CHF (n=13)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58.0±2.5</td>
<td>60.1±2.2</td>
<td>0.693</td>
</tr>
<tr>
<td>Gender, % male</td>
<td>70</td>
<td>62</td>
<td>0.673</td>
</tr>
<tr>
<td>BMI, kg/m</td>
<td>26.0±1.2</td>
<td>27.5±1.5</td>
<td>0.522</td>
</tr>
<tr>
<td>Peak oxygen consumption, ml · kg⁻¹ · min⁻¹</td>
<td>37.7±3.1</td>
<td>20.1±1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximal workload, W</td>
<td>217±21</td>
<td>117±11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L [*]</td>
<td>4.80±0.24</td>
<td>5.09±0.34</td>
<td>0.456</td>
</tr>
<tr>
<td>HDL, mmol/L [*]</td>
<td>1.66±0.15</td>
<td>1.19±0.11</td>
<td>0.009</td>
</tr>
<tr>
<td>LDL, mmol/L [*]</td>
<td>2.68±0.14</td>
<td>3.41±0.35</td>
<td>0.203</td>
</tr>
<tr>
<td>TG, mmol/L [*]</td>
<td>0.92±0.16</td>
<td>1.56±0.20</td>
<td>0.017</td>
</tr>
<tr>
<td>CRP, mg/L [*]</td>
<td>1.1±0.3</td>
<td>3.2±0.9</td>
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</tr>
<tr>
<td>Sedimentation, mm/h [*]</td>
<td>3.70±0.54</td>
<td>17.27±3.32</td>
<td>&lt;0.001</td>
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<tr>
<td>Glucose, mmol/L</td>
<td>4.71±0.11</td>
<td>6.25±0.73</td>
<td>0.172</td>
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<tr>
<td>Insulin, μU/mL [*]†</td>
<td>6.84±1.08</td>
<td>15.92±3.85</td>
<td>0.009</td>
</tr>
<tr>
<td>HOMA-IR [*]†</td>
<td>1.40±0.20</td>
<td>4.24±1.11</td>
<td>0.017</td>
</tr>
<tr>
<td>IL-6, pg/mL [*]†</td>
<td>1.24±0.17</td>
<td>4.18±1.09</td>
<td>0.011</td>
</tr>
<tr>
<td>TNF-α, pg/mL [*]</td>
<td>2.58±0.37</td>
<td>4.03±0.46</td>
<td>0.059</td>
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<tr>
<td>Adiponectin, mg/L [*]</td>
<td>6.66±1.33</td>
<td>10.97±1.46</td>
<td>0.043</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; CRP, C-reactive protein; HOMA-IR, homeostasis model assessment estimated insulin resistance; IL-6, interleukin-6; and TNF-α, tumor necrosis factor-α.

*Data available for 12 patients with CHF.
†Data available for 8 healthy subjects.

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**Figure 1.** A, Circulating adiponectin levels in healthy controls (n=10) versus patients with CHF (n=12) with NT-proBNP levels below and above the median NT-proBNP of the CHF group. Data are presented as mean±SEM. *P<0.05; ***P<0.001. Overall Kruskal-Wallis test, P=0.014. B, Scatterplot of the association between circulating adiponectin levels and maximal workload. C, Scatterplot of the association between circulating adiponectin levels and peak oxygen consumption (VO₂peak).
PPAR-γ coactivator-1α ($P = 0.088$) and for carnitine palmitoyltransferase 1B ($P = 0.186$). Strong positive correlations were found between mRNA expression of AdipoR1 on the one hand and PPAR-α ($r = 0.761$, $P < 0.001$), AMPK-α1 and -α2 subunits ($r = 0.466$, $P = 0.025$, and $r = 0.455$, $P = 0.029$, respectively), and the investigated target genes ACADM ($r = 0.510$, $P = 0.013$), carnitine palmitoyltransferase 1B ($r = 0.629$, $P < 0.001$), and HK2 ($r = 0.689$, $P < 0.001$) on the other hand, suggesting a link between AdipoR1 expression and the PPAR-α/AMPK pathway (Figure 4).

The phosphorylation state of AMPK was 3 times lower in patients with CHF than in healthy subjects (554 ± 154 AU versus 1951 ± 467 AU; $P < 0.05$), with no significant change in total AMPK, indicating a decreased AMPK activation (Figure 5).

**PPAR-α Knockout Mice**

To obtain a better insight into the regulation of gene expression of the adiponectin system, PPAR-α knockout mice were used (Figure 6). In wild-type mice, PPAR-α expression was 20-fold times higher in the oxidative soleus muscle than in the glycolytic superficial gastrocnemius muscle ($P < 0.0001$). In the soleus muscle of PPAR-α$^{-/-}$ mice only, mRNA transcription of AdipoR1 ($P = 0.003$) and both AMPK-α1 and -α2 subunits ($P = 0.001$ and $P = 0.016$, respectively) was downregulated. AdipoR2 expression was unaffected by PPAR-α deficiency, whereas adiponectin tended to decrease ($P = 0.088$).

**Adiponectin Expression in the Skeletal Muscle**

Immunohistochemistry confirmed adiponectin protein expression (Figure 7) in the skeletal muscle fibers. Muscle fibers of patients with CHF stained abundantly for adiponectin, whereas almost no adiponectin was present in the muscle fibers of the healthy subjects. Computerized quantitative analysis of adiponectin protein expression in patients with CHF versus healthy subjects revealed a significantly higher protein expression level in the muscle fibers of the patients with CHF ($P = 0.021$) (Figure 7).

**Discussion**

The independent prognostic value of high circulating adiponectin levels has been reported repeatedly in several chronic diseases, including CHF. However, this study focuses for the first time on the deregulation of the local adiponectin system in the skeletal muscle of patients with CHF. The following novel and interesting findings emerge from this study.

1. Adiponectin mRNA expression is 5-fold higher in skeletal muscle of patients with CHF than in healthy controls, whereas the AdipoR1 is significantly downregulated.
2. Disruption of the local adiponectin system is reflected in changes of key regulators of skeletal muscle substrate use. A significant reduction in mRNA expression of PPAR-α and fatty acid oxidation target genes is demonstrated. In addition, AMPK mRNA and the phosphorylated AMPK protein expression are decreased.
3. Expression of AdipoR1, PPAR-α, AMPK, and their downstream targets are strongly correlated.
4. The decreased expression of AdipoR1 and both AMPK catalytic subunits at the level of the soleus muscle in PPAR-α null mice suggests that AdipoR1 and AMPK subunit expression are at least in part under the control of PPAR-α.

**Increased Adiponectin in CHF**

Until now, several mechanisms have been called on to explain the presence of increased circulating adiponectin levels in CHF. First, because adiponectin has been attributed an antiinflammatory and insulin-sensitizing role, the observed increased levels might be interpreted as a compensatory increase to restore metabolic homeostasis. Second, several studies have reported
strong correlations between natriuretic peptides and adiponectin levels. Although both biomarkers might simply reflect disease severity without any causal relation, recent investigations have shown that natriuretic peptides promote adiponectin secretion.\textsuperscript{9,24} Third, another interesting hypothesis is the existence of adiponectin resistance in CHF.\textsuperscript{25}

Adiponectin mainly originates from adipose tissue. However, it has been shown recently that adiponectin also can be produced by other cells, including skeletal muscle fibers and cardiac myocytes.\textsuperscript{26,27} Skeletal muscle is an important target for adiponectin mainly through binding to the muscle-specific AdipoR1.\textsuperscript{17} We show for the first time that adiponectin mRNA and protein contents are increased in skeletal muscle of patients with CHF, suggesting that skeletal muscle might also participate in the overall increase in circulating adiponectin. However, the absence of a relation between circulating adiponectin levels and skeletal muscle expression in patients with CHF suggests that other adiponectin sources are involved. In CHF, the existence of a local cardiac adiponectin system has recently been shown,\textsuperscript{15} but the potential role of adipose and hepatic tissue needs further exploration.

Deactivation of the PPAR-\(\alpha\)/AMPK Pathway in Skeletal Muscle in CHF

We next investigated whether the signaling pathways controlling lipid and glucose metabolism could be involved in the deregulation of the adiponectin system in CHF. The transcriptional factor PPAR-\(\alpha\) has been shown to control fatty acid oxidation by binding to specific nucleotide sequences known as peroxisome proliferator

![Figure 4. Scatterplot of the association between mRNA expression levels of AdipoR1 and PPAR-\(\alpha\) (A), ACADM (B), AMPK-\(\alpha\)1 subunit (C), and HK2 (D).](image)

![Figure 5. AMPK phosphorylation within human skeletal muscle. Western blots of pAMPK (phosphor-treonine-172) and total AMPK in biopsy samples of the vastus lateralis of patients with CHF (n=8) and healthy subjects (n=8). Top, representative western blots. Bottom shows mean values expressed as mean±SEM. *P<0.05.](image)
responsive elements, resulting in transcription and activation of genes involved in fatty acid uptake and β-oxidation.\textsuperscript{28} The present results show for the first time that downregulation of PPAR-α in skeletal muscle of patients with CHF coincides with decreased expression of its downstream target gene ACADM, thereby confirming a deactivation of the PPAR-α pathway in heart failure. Moreover, in skeletal muscle from patients with CHF, the AMPK-α1 and -α2 catalytic subunits also were downregulated and correlated strongly with PPAR-α expression ($r=0.674$, $P<0.001$, and $r=0.699$, $P<0.001$, respectively). Besides affecting the abundance of both catalytic subunits of AMPK, its phosphorylated status also was decreased. This posttranslational change might explain the downregulation of the target gene HK\textsuperscript{29,30} responsible for the first step in glucose metabolism pathways. These results sup-

Figure 6. mRNA transcript levels of gene products involved in local adiponectin system (adiponectin, AdipoR1, and AdipoR2) and AMPK-α1 and -α2 subunits in soleus muscles of wild-type (WT) (n=8) and PPAR-α$^{-/-}$ mice (n=10). Results are given as mean±SEM. (\textdagger)$P<0.1; $*P<0.05; $**P<0.01; $***P<0.001 versus WT mice.

Figure 7. Adiponectin protein expression in biopsy samples of the vastus lateralis muscle of patients with CHF (n=4) and healthy subjects (n=4). Expression of adiponectin was determined by immunohistochemical staining. A, Adiponectin protein expression in healthy subjects (left panel) versus patients with CHF (right panel), magnification of $\times 20$. B, Computerized quantitative analysis of protein expression by immunohistochemistry in healthy subjects versus CHF. Bar graphs show mean±SEM of mean positive stained area fraction of adiponectin. $*P<0.05$. 
port the theory of a skeletal muscle metabolic deficiency in CHF.

Link Between Adiponectin System and Disturbed Energy Metabolism in CHF

Interestingly, AdipoR1 expression, but not AdipoR2, was downregulated in patients with CHF and strongly correlated with expression of PPAR-α and the 2 catalytic subunits of AMPK, thereby indicating a cause-and-effect relationship. These correlations were present in both healthy subjects and patients with CHF, suggesting a disease-independent regulation. Indeed, confirmatory data are provided in the PPAR-α null mice in which deletion of PPAR-α also led to downregulation of AdipoR1 and AMPK-α subunits. In addition, the latter findings suggest that downregulation of PPAR-α in skeletal muscles of patients with CHF could be the primum movens leading to decreased AdipoR1 expression. This is in line with other studies showing that activation of PPAR-α increases adiponectin receptor expression in human macrophages and in adipose tissue and that pharmacological intervention with PPAR-α agonists upregulates adiponectin receptors, at least in adipose tissue. The observed inverse relation between PPAR-α and local adiponectin expression in human skeletal biopsies, however, differs from our findings in PPAR-α−/− mice and implies a regulatory mechanism for adiponectin expression that is independent of PPAR-α in the setting of CHF.

Adiponectin has been shown to activate both AMPK and PPAR-α pathways and to increase the expression of AdipoR1 in myotubes derived from lean subjects. In patients with CHF, however, despite increased muscle and circulating adiponectin levels, the PPAR-α/AMPK pathway is deactivated, resulting in decreased AdipoR1 and fatty acid and glucose metabolism enzymes. All these observations argue for a state of adiponectin resistance in this disease. The results of this study show that the cardiac “functional adiponectin resistance” in CHF recently postulated to explain the decreased mRNA and protein expression of AdipoR1 in the left ventricle of infarcted mouse hearts can be extended to the skeletal muscle. This adiponectin resistance seems to arise from deactivation of the PPAR-α pathway. A proinflammatory cytokine profile, which typifies CHF, has been shown to inhibit PPAR-α. One possible explanation is that increased levels of tumor necrosis factor-α observed in patients with CHF could be the trigger, leading to deactivation of the PPAR-α pathway. The negative correlation between PPAR-α expression and circulating levels of tumor necrosis factor-α (r = −0.531, P < 0.011) supports this notion. IR is another interesting possibility. In our study, however, we did not detect a significant correlation between the mRNA expression profiles and the homeostasis model assessment IR.

It is tempting to speculate that overexpression of adiponectin by skeletal muscle and increased circulating adiponectin represent a compensatory mechanism to counteract compromised energy metabolism as a result of downregulation of the PPAR-α/AMPK/AdipoR1 pathway.

We observed a strong and inverse relationship between exercise parameters and circulating adiponectin in patients with CHF. This finding supports the assertion that the more the skeletal muscle metabolism is disturbed, the higher the adiponectin compensatory mechanism is activated.

Study Limitations

First, our study population consists of a relatively small number of patients with CHF with mild to moderate functional impairment. Nonetheless, the described differences in terms of mRNA expression profile of adiponectin and genes involved in glucose and lipid metabolism were statistically significant and large. Although speculative, one could assume that in patients who are more severely ill, the established differences would have been even more pronounced as suggested by the strong correlations between adiponectin level and maximal workload or peak oxygen consumption. Moreover, it has been shown that already in patients with CHF with mild impairment, strength per unit muscle is reduced. Because our study number was limited by the relative invasive procedure involved and the restricted selection criteria for study participation, our findings need confirmation in larger study populations. Because we included patients with CHF with different disease etiologies, we performed a subanalysis comparing patients with CHF with ischemic versus idiopathic etiology of their disease. Although obviously limited by the small number of patients in each subgroup, we could not detect any differences in mRNA expression profile of genes involved in the PPAR-α/AMPK pathway.

Second, it should be noted that our patients are on optimal medical treatment and, therefore, are administered a number of pharmacological agents that theoretically could affect metabolism. Angiotensin-converted enzyme inhibitors, angiotensin II receptor blockers, statins, or their combination have been reported to increase circulating adiponectin levels, whereas carvedilol recently has been shown to decrease plasma adiponectin in CHF. However, results are inconsistent, and because these drugs are considered the standard treatment with proven benefits in terms of morbidity and mortality, they cannot be withdrawn.

Third, the hypothesis of functional adiponectin resistance needs to be confirmed with functional tests in isolated human skeletal muscle cells. This involves invasive procedures to allow sampling of large muscle biopsies under general anesthesia. In this study, however, we showed that deactivation of adiponectin target genes, which together with increased levels of circulating adiponectin levels, support the presence of a functional adiponectin resistance.

Conclusion

Parallel to increased circulating adiponectin levels, adiponectin expression in skeletal muscle is significantly increased in patients with CHF compared with healthy controls. Disturbances in skeletal muscle metabolism are supported by deactivation of the PPAR-α/AMPK pathway and downregulation of several target genes involved in both fatty acid oxidation and glucose metabolism. The fact that circulating levels and local adiponectin expression are increased together with downregulation of AdipoR1 expression and PPAR-α/
AMPK deactivation makes a strong case for the existence of a functional adiponectin resistance. Hence, increased adiponectin concentrations probably represent a protective mechanism to counteract adiponectin resistance and the compromised energy metabolism.

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**Disclosures**
None.

**References**
Skeletal muscle dysfunction is an important determinant of exercise intolerance in patients with chronic heart failure (CHF). In addition to structural and functional abnormalities, changes involving mitochondrial bioenergetics explain the energetic deficit that characterizes skeletal muscles of these patients. Recently, insulin resistance has gained attention as a relevant pathophysiological mechanism in CHF. Adiponectin is an insulin-sensitizing cytokine, promoting the uptake and oxidation of glucose and fatty acids at the level of the skeletal muscle. High circulating adiponectin levels infer poor prognosis in patients with CHF. However, the regulation of the local adiponectin system at the level of the skeletal muscle and its metabolic consequences in this population have not been explored previously. In this study, comparison of skeletal muscle biopsies from healthy subjects and patients with CHF showed that in the latter, mRNA expression for adiponectin was significantly increased, whereas it was reduced for the main adiponectin receptor. In addition to these local changes, deactivation of the peroxisome proliferator-activated receptor-α/AMP-activated protein kinase pathway and downregulation of several target genes involved in both fatty acid oxidation and glucose metabolism were shown. Taken together, these findings suggest the existence of a functional adiponectin resistance. Hence, increased circulating adiponectin concentrations probably represent a protective mechanism to counteract adiponectin resistance and the compromised energy metabolism. The inverse relationship between exercise parameters and circulating adiponectin in patients with CHF supports the assertion that the more the skeletal muscle metabolism is disturbed, the higher the adiponectin compensatory mechanism is activated.
Functional Adiponectin Resistance at the Level of the Skeletal Muscle in Mild to Moderate Chronic Heart Failure
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SUPPLEMENTAL MATERIAL

Expanded methods

Biochemical analysis

Human Adiponectin Quantikine ELISA: minimal detection limit of 0.246 ng/L, intra-assay precision of 2.5% for mean 19.8 mg/L, SD 0.50 (n=20) and inter-assay precision of 3.2% for mean 12.5 mg/L, SD 0.41 (n=40); Human TNFα/TNFSF1A Quantikine HS ELISA: minimal detection limit of 0.106 pg/mL, intra-assay precision of 4.3% for mean 11.5 pg/mL, SD 0.49 (n=20) and inter-assay precision of 7.3% for mean 10.5 pg/mL, SD 0.76 (n=41); Human IL-6 Quantine HS ELISA; minimal detection limit of 0.039 pg/mL, intra-assay precision of 7.8% for mean 2.45 mg/L, SD 0.19 (n=20) and inter-assay precision of 7.2% for mean 2.78 mg/L, SD 0.20 (n=36).

The coefficient of variation for NT-proBNP was 1.3% (n=10) at a level of 221 pg/mL and 1.2% (n=10) at a level of 4091 pg/mL.

Immunohistochemistry (IHC)

The 5 µm paraffin tissue sections were deparaffinized and hydrated. A dextran-based method (Dako REAL EnVision Detection System; DakoCytomation A/S, Glostrup, Denmark) was used to detect the antigen. The primary antibody used was the mouse anti-human adiponectin [19F1] (1:100; Abcam, Cambridge, UK). Horseradish peroxidase activity was visualized with 3,3’- diaminobenzidine tetrahydrochloride, and hematoxylin was used for nuclear staining. Negative controls were performed by replacing the primary antibody with normal mouse serum at the same concentration of the primary antibody. Negative controls displayed an absence of signal. The images were analyzed using a color image analysis system (Image-Pro Plus 4.1; Media Cybernetics, Inc., Silver Spring, MD).

Western blot

Protein extracts (50 µg) of vastus lateralis muscles from CHF patients and healthy subjects were loaded onto SDS-polyacrylamide gels and separated for 120 min at 120 V. After electrophoresis, the proteins were transferred to Hybond nitrocellulose membranes (Amersham) using a Bio-Rad blot system for 90
min at 150 V. Thereafter, the blots were blocked with 5% milk in PBS for 60 min at room temperature, followed by incubation with a primary antibody at 4°C overnight. Specific antibodies were used to measure the protein content of phospho- and total αAMPK (#2535 and #2532, Cell Signaling, Ozyme France). After washing, the membranes were incubated with horseradish peroxidase secondary antibody (#7074 Cell Signaling) for 60 min and revealed with enhanced chemiluminescent substrate (PIERCE dura, Fischer, France). Light emission was detected by autoradiography and quantified using an image-analysis system (Chemidoc XRS, Biorad).