Description of a local cardiac adiponectin system and its deregulation in dilated cardiomyopathy

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Aims
Despite recent advances in medical therapy, heart failure remains a leading cause for cardiovascular mortality, and its complex pathogenesis is incompletely understood. This study was performed to identify possible new therapeutic targets in dilated cardiomyopathy (DCM).

Methods and results
Oligonucleotide microarray analysis was performed on endomyocardial biopsies (EMBs) from patients with early DCM (LVEDD ≥ 55 mm, LVEF ≤ 55%, n = 5) and control subjects (LVEDD < 55 mm, LVEF > 60%, no cardiac pathology, n = 4). Adiponectin, an adipocytokine involved in cellular metabolism, survival, and immunomodulation, was sixfold downregulated in DCM patients. Microarray data for adiponectin were confirmed by TaqMan-PCR (9.2-fold downregulation, control n = 9 vs. DCM n = 9, respectively, P < 0.05). Immunohistological analysis of EMBs showed significant downregulation of cardiac adiponectin protein expression independent of serum adiponectin (P = 0.36, ns) or serum TNFα concentrations (P = 0.46, ns). Neither the adiponectin receptor 1 (adipo-R1) nor adipo-R2 was deregulated in early DCM. Adiponectin mRNA and protein downregulation were confirmed in explanted hearts of patients with advanced DCM (LVEF < 25%, n = 8). In vitro, adiponectin incubation of neonatal rat ventricular myocytes led to activation of the pro-survival kinase PKB/Akt, increased eNOS-phosphorylation, and prevented stress-induced apoptosis of cardiomyocytes in an Akt-dependent manner. Moreover, inhibition of adiponectin secretion was accompanied by an increase in the expression of the cytokine and its receptors.

Conclusion
These data indicate the existence of a local cardiac adiponectin system regulated independent of adiponectin and TNFα serum levels and its disturbance in cardiac pathology. The study suggests a role for adiponectin in the pathogenesis of DCM and implicates the adipocytokine as a possible future therapeutic target in DCM.

Keywords
Dilated cardiomyopathy • Adiponectin • Adiponectin receptors • Heart failure

Introduction
Heart failure (HF) is a final common pathway in cardiovascular disease as a result of sustained pressure and volume overload, myocardial ischaemia and infarction, or inherited and acquired cardiomyopathies. The last decade has witnessed major advances in the understanding of the molecular mechanisms of HF in response to stress signals. A multitude of extracellular factors and signalling pathways are involved in altering transcriptional regulatory networks controlling cardiac adaptation or maladaptation, and the transition to overt HF. However, the morbidity and mortality of patients presenting with HF remains high. Therefore, new insights into the pathophysiology and the molecular signalling pathways of HF are required in order to develop novel therapeutic strategies.

Adiponectin is an adipocytokine synthesized almost exclusively by adipocytes.1 It exists in several oligomeric forms (trimeric, hexameric, and high molecular weight) in a concentration of 3–30 µg/mL plasma.2 Circulating adiponectin is present as full-length protein and proteolytic fragment (globular C-terminal domain).3 The adipocytokine binds to its receptors, adipo-R1 and adipo-R2. Adipo-R1 is expressed in skeletal muscle (SM) and other tissues, whereas adipo-R2 is predominantly expressed in

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Adiponectin plays an important role in energy homeostasis and insulin sensitivity and exerts anti-inflammatory, pro-angiogenic and anti-atherogenic properties. Plasma adiponectin levels from patients with coronary artery disease are significantly diminished when compared with normal subjects. Furthermore, adiponectin levels are negatively correlated with cardiac risk factors, such as C-reactive protein, blood pressure, diabetes, and hyperlipidaemia. Taken together, epidemiological studies in humans, correlating adiponectin serum concentrations with end-organ effects, indicate an important role for adiponectin in the pathogenesis of cardiovascular and metabolic diseases. In animal studies, adiponectin has been reported to inhibit vascular remodelling, to exert pro-angiogenic effects in vitro and in vivo, to ameliorate ischaemia–reperfusion (IR) injury, and to prevent cardiac remodelling after aortic banding as well as systolic dysfunction following IR. Furthermore, adiponectin displayed anti-inflammatory properties in an animal model of viral myocarditis. Thus, the cytokine has been shown to abate cardiac risk such as inflammation-induced cardiovascular insufficiency and direct myocardial insult. Taken together, a growing body of evidence suggests protective effects of circulating adiponectin in cardiovascular tissues.

Very recently, it has been shown that cells other than adipocytes can synthesize adiponectin, such as skeletal myocytes, liver-bound endothelial cells, and macrophages as well as cardiomyocytes. Studies to date have characterized hormone-like effects of systemic adiponectin, i.e. serum levels, upon the cardiovascular system, whereas autocrine or paracrine mechanisms of adiponectin action have not yet been investigated. Therefore, we designed a study to test our hypothesis that locally deregulated cardiac adiponectin expression is involved in the pathogenesis of dilated cardiomyopathy (DCM).

Here, we show for the first time that adiponectin is differentially expressed in the hearts of patients with DCM independent of their plasma levels and provide evidence for a local paracrine cardiac adiponectin system that appears to be involved in the pathogenesis of DCM.

### Methods

#### Characterization of study groups

Patients with moderate DCM were derived from a large series of patients initially submitted to our clinic with symptoms and signs of HF in all of whom endomyocardial biopsies (EMBs) were obtained by standard procedure following exclusion of coronary artery disease and other possible causes for cardiac dysfunction for histological, immunohistological, and molecular virological analyses. All patients had given written informed consent. After standard clinical, morphological, and functional patient characterization, their EMBs were assessed for inflammation and viral genomes. Nine DCM patients in NYHA class II without cardiac inflammation or virus, EF > 55%, LVEDD > 55 mm, comprised the ‘moderate DCM’ group (Table 1) and were compared with nine control patients submitted to our

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* *P < 0.01.
clinically to evaluate suspected cardiomyopathy, in whom the diagnostic workup finally revealed that their complaints were non-cardiac in origin. Of note, adipositas and diabetes were exclusion criteria. Explanted hearts of patients with ‘severe or end-stage’ DCM (LVEF < 25%, n = 8) presenting for heart transplantation with NYHA IV cardiac complaints were investigated in comparison with donor hearts (n = 6).

**High-density microarray studies of human cardiac biopsies**

cDNA was synthesized from 200 ng of total RNA isolated from EMBs; transcribed cRNA was processed for microarray hybridization and analysis as described previously.18

**Cell culture, adenoviral vectors, and reagents**

Neonatal rat ventricular cardiomyocytes (NRVMs) and fibroblasts (NRFBs) were isolated as described.19 Human umbilical vein endothelial cells (HUVECs) were purchased from Cambrex, and peripheral blood mononuclear cells (PBMCs) were isolated from healthy volunteers, using histopaque (Sigma). HL-1 mouse cardiomyocytes were provided by Professor Claycomb (New Orleans, LA, USA). Transduction with replication-defective adenoviral vectors encoding dominant-negative Akt (dnAkt) or β-galactosidase (β-gal) was carried out overnight. Recombinant human adiponectin produced in a mammalian expression system was purchased from R&D Systems, trypsin was acquired from Invitrogen, doxorubicin was purchased from Sigma, and monensin was bought from eBioscience.

**Quantitative real-time polymerase chain reaction**

Total RNA from human EMBs, explanted hearts, white adipose tissue (WAT), SM, or cultured cells was extracted using TRIzol® (Invitrogen); quality and quantity were checked by Agilent 2100 Bioanalyzer and the following primer sets from Applied Biosystems were used for quantitative real-time polymerase chain reaction (qRT-PCR): human APN (Hs00605917_m1), APN-R1, APN-R2, TNFa, BNP, GAPDH (Hs99999905_m1), and HPRT1 (Hs99999999_m1). Rat genes for APN, APN-R1, APN-R2, GAPDH, and HPRT1. Evaluation was performed by DDCt method.20

**Immunoblot analysis and immunohistochemistry**

Protein extraction from tissues and cultured cells and western blotting was performed by standard procedures. Anti-AMPK (AMP-activated protein kinase) antibody (1:250 dilution, Cell Signaling), anti-phospho-Akt antibody (1:500, Cell Signaling), anti-eNOS antibody (1:1000, Cell Signaling), anti-phospho-eNOS antibody (1:1000, Cell Signaling), anti-Akt1 (1:1000, BD Transduction Laboratories), anti-eNOS (1:1000, Cell Signaling), anti-AMPK (1:1000, Cell Signaling), and anti-adiponectin (1:500, R&D Systems) were used as primary antibodies and anti-rabbit IgG/HRP, anti-goat IgG/HRP, or anti-mouse IgG/HRP conjugate (1:2500 dilution, Promega) as secondary antibodies. Immunoblots were quantified using NIH-Image® software. Immunostaining for adiponectin and its receptors in EMBs and explanted hearts was performed on frozen sections as described previously.17 The positive area fraction was calculated by digital image analysis.17

**Cell apoptosis assays**

NRVMs were grown on 24-well slides, transduced with β-gal or dnAkt, and cultured under mitogen-deprived conditions ± adiponectin/BSA for 72 h. Adiponectin was changed every 24 h. Cells were then fixed with 4% paraformaldehyde and stained with Hoechst dye according to the manufacturer’s instructions. In total, 100 cells were counted from three independent experiments. In another set of experiments, HL-1 cells were grown to subconfluence, serum-starved for 12 h, and incubated with doxorubicin ± adiponectin for 24 h. Apoptotic cell death was determined by Hoechst staining.

**Inhibition of adiponectin secretion**

NRVMs were grown in six-well chambers. Medium was changed every 24 h. On day 3 in culture, cells were incubated with increasing concentrations of monensin (2, 5, 10 μM). Cells were lysed and total mRNA was extracted for qRTs-PCR 12 and 24 h after monensin incubation.

**Statistical analysis**

Microarray data were analysed using the Significance Analysis of Micro-arrays. For the analysis of qRT-PCR, Student’s t-test was applied to evaluate statistical significance. A value of P < 0.05 was considered statistically significant.

**Results**

**Cardiac adiponectin mRNA abundance in dilated cardiomyopathy**

Microarray analysis of EMBs from patients with moderate DCM exhibited several deregulated HF-associated genes such as natriuretic peptide precursors A and B, tropomyosin 3, collagen type IV and VIII, frizzled-related protein, actinin, and FGF2. Surprisingly, one of the strongest downregulated genes in the moderate DCM group was adiponectin (−6.0-fold by microarray, Figure 1A). In order to validate these results, mRNA abundance was quantified by qRT-PCR. Cardiac adiponectin was 9.2-fold downregulated in patients with moderate HF (P < 0.05, DCM vs. control, Figure 1B). Those data were corroborated in explanted hearts from patients with end-stage DCM (−6.7-fold, P < 0.05, Figure 1B).

**Cardiac adiponectin protein expression in dilated cardiomyopathy**

Adiponectin protein expression in EMBs was evaluated by immunohistochemistry (Figure 2A–C). Adiponectin could be readily detected around cardiomyocytes in control EMBs (Figure 2A). Interstitial cells exhibited positive staining as well. In contrast, adiponectin staining was generally diminished in DCM-EMBs (Figure 2B). Digital image analysis showed significant downregulation of adiponectin immunostaining in EMBs from patients with moderate DCM (P < 0.05) (Figure 2C). Those results were corroborated by western blotting of explanted hearts from patients with end-stage DCM (Figure 2D). Adiponectin was significantly downregulated in these patients when compared with controls (100 ± 11 vs. 71 ± 16%, P < 0.05). The observed downregulation of adiponectin protein in cardiac tissue could be related to serum concentrations of the cytokine. Serum levels were determined by ELISA but did not differ between controls and patients with moderate DCM (Figure 3A). Moreover, adiponectin downregulation in the heart could be secondary to increased serum concentrations of TNFα, a known negative regulator of adiponectin expression,21 but
TNFα serum levels were also not different between moderate DCM and healthy controls (Figure 3B). In summary, these data indicate the existence of a local cardiac adiponectin system regulated independent of systemic adiponectin and TNFα serum levels.

**Adiponectin receptors in dilated cardiomyopathy**

Next, we investigated the expression of the adiponectin receptors adipo-R1 and -R2 in human hearts. Receptor mRNA abundance was neither regulated in moderate nor in end-stage DCM (Figures 1A and 4A; data not shown). A trend to higher expression levels of adipo-R1 and -R2 in the cardiomyopathy groups did not reach statistical significance. By immunohistochemistry, both receptors were prominently expressed on endothelial cells (Figure 4B) but cardiomyocytes expressed both receptor types as well. Particularly, strong immunostaining was displayed by damaged cardiomyocytes. Adipo-R1 and -R2 were also found on interstitial cells. In accordance with the qRT-PCR data, adipo-R1 was the predominantly expressed adiponectin receptor in human heart. Importantly, the overall distribution pattern of the receptors differed significantly from that of its ligand adiponectin, suggesting
the possible existence of additional ligands in the heart. Quantitation showed a trend to upregulation of both receptors in DCM that did not reach statistical significance (Figure 4C).

Signalling pathway activation by adiponectin in cardiomyocytes and possible functional relevance

To determine whether local adiponectin downregulation may be functionally relevant, the activation of signal transduction pathways in NRVMs following adiponectin incubation was studied in vitro. As described previously, adiponectin led to the activation of AMPK. An increase of AMPK phosphorylation was detected as early as 5 min following adiponectin application (data not shown). Moreover, the survival kinase Akt was phosphorylated following adiponectin incubation (Figure 5A). The activation of Akt was followed by a transient increase of eNOS-phosphorylation (Figure 5B). Apoptosis is one mechanism involved in the molecular pathogenesis of DCM. The protein kinase Akt exerts anti-apoptotic and pro-survival effects in NRVMs, and activation of Akt by adiponectin could prevent apoptosis in this setting. When HL-1 cells were serum-starved for 72 h in the presence or absence of adiponectin and stained with HOECHST dye (Figure 6A and B), adiponectin prevented the apoptosis induced by prolonged serum starvation (29 ± 5 vs. 9 ± 3%, \( P \), 0.01). Moreover, this effect could be reversed by transduction with an adenoviral vector encoding a dominant-negative form of Akt (dn-Akt), indicating that adiponectin prevents apoptosis by phosphorylating and activating Akt (Figure 6A). As a second model of Akt-dependent apoptosis, doxorubicin-induced toxicity was employed. HL-1 cells were incubated with doxorubicin for 24 h. Doxorubicin induced a pronounced apoptotic response (Figure 6B) which was significantly inhibited by adiponectin (40 ± 9 vs. 27 ± 6%, \( P \), 0.05). Again, transduction with dnAkt partly abolished the cytoprotective
Local adiponectin regulation in the heart

The dissociation between systemic and cardiac adiponectin expression (Figures 1–3) indicates that adiponectin mRNA and protein expression is locally regulated within human hearts in vivo. Next, we determined mRNA levels in cultured rat cardiomyocytes and cardiac fibroblasts (Figure 7A), as well as in human tissues (Figure 7B). Cardiomyocytes were the main source of cardiac adiponectin, whereas the receptors adipo-R1 and -R2 were expressed by cardiomyocytes, cardiac fibroblasts, endothelial and inflammatory cells as well. Notably, the expression levels of adiponectin and its receptors in cardiac muscle (CM), although much lower than in adipose tissue (WAT), were rather higher than in SM (Figure 7B). Endothelial cells and PBMCs exclusively expressed adipo-R1 and -R2 but not adiponectin. In order to better characterize cardiac adiponectin expression, blood was simultaneously drawn from aortic root and coronary sinus vein of 10 additional control patients. Adiponectin serum levels were 9 ± 4% higher in the coronary sinus when compared with the aortic root (P < 0.05), indicating adiponectin synthesis within the heart. In summary, adiponectin system components in the heart show relevant expression levels in comparison with other human tissues.

To investigate a possible auto/paracrine regulation of the system, we used a simple cell culture model. Cardiac myocytes synthesize and secrete adiponectin.16 We determined a mean adiponectin concentration of 1.8 ± 0.5 ng/mL in cell supernatant after 24 h of in vitro culture (n = 6). If an auto/paracrine negative feedback loop with suppression of adiponectin synthesis via adiponectin receptor-dependent signalling would exist, blockade of the secretion of adiponectin and other possible cardiomyocyte-derived factors with monensin, an inhibitor of protein transport from the endoplasmic reticulum to the Golgi complex, should lead to increased adiponectin synthesis and possibly also upregulation of adiponectin receptors. Indeed, we observed a dose-dependent increase of adipo-R1 and -R2 as well as adiponectin mRNA expression by qRT-PCR following monensin incubation (Figure 7C) (160 and 170% for adipo-R1 and -R2, respectively, P < 0.05; 770% for adiponectin, P < 0.01, at 10 μM monensin). These data are consistent with the existence of a local cardiac adiponectin system responding to an auto/paracrine regulatory mechanism.

Discussion

This study delineates a local adiponectin system in the human heart and its deregulation in DCM. Until very recently, adiponectin has been thought to be exclusively synthesized and secreted by adipocytes and to exert its hormonal effects in distant tissues. Here, we propose an additional auto/paracrine mode of adiponectin action within the heart. We show adiponectin and adiponectin receptor expression in healthy human hearts and downregulation of intra-myocardial adiponectin mRNA and protein in patients with moderate and severe DCM independent of TNFα or adiponectin serum concentrations, implicating adiponectin in the pathophysiology of the disease. Blockade of protein secretion from cardiomyocyte cultures induced strong upregulation of adiponectin and moderately increased expression of its receptors consistent with the existence of auto/paracrine adiponectin regulation within the heart. Furthermore, adiponectin inhibited stress-induced apoptosis commonly associated with DCM. Taken together, our data demonstrate deregulated local cardiac expression of adiponectin in DCM that might play an important role in the pathogenesis of HF.
Figure 4 Adiponectin receptors in dilated cardiomyopathy. (A) Bar graph illustrates mRNA expression of adipo-R1 in control subjects and patients with moderate dilated cardiomyopathy. Data are expressed as mean ± SD (NS, not significant). (B) Frozen sections of heart tissue (endomyocardial biopsies) were incubated with a human anti-adipo-R1 or -R2 antibody, and distribution in moderate cardiomyopathy was determined employing a horseradish peroxidase-conjugated secondary antibody by immunohistochemistry. Upper left panel shows adipo-R1 distribution, and lower left panel illustrates expression of adipo-R2. Right panels show higher magnification images. Both receptors were predominantly expressed on endothelial cells (EC, black arrow) but cardiomyocytes stained positive as well (CM, yellow arrow). There is not a typical sarcolemmal staining pattern as observed for adiponectin. (C) Quantitative computerized analysis of adiponectin receptor immunoreactivity in endomyocardial biopsies of controls and patients with moderate dilated cardiomyopathy. Bar graphs indicate positive area fractions (AF) for adipo-R1 and -R2, respectively. Data are expressed as mean ± SD (NS, not significant).
humans have investigated the correlation between adiponectin serum concentrations and cardiac disease.\textsuperscript{7,25} Low adiponectin serum levels are correlated with coronary artery disease\textsuperscript{5,26,27} and were also found in hypertensive patients.\textsuperscript{28} Studies of adiponectin expression in DCM patients have been inconclusive, partly due to a survival benefit of a high body mass index (BMI) that is inversely correlated with adiponectin expression.\textsuperscript{29,30}

Only very recently, adiponectin has been shown to be also expressed in cultured skeletal\textsuperscript{31} and cardiac myocytes\textsuperscript{16} as well as in the hearts of rats and mice.\textsuperscript{32,33} Moreover, expression of adipo-R1 and adipo-R2 in cardiomyocytes has been demonstrated.\textsuperscript{32,34} However, the current study is the first to characterize local expression of adiponectin and its receptors in normal and diseased human hearts and its possible functional effects. Importantly, the diminished expression of adiponectin in DCM hearts investigated here was not correlated with TNF\textsubscript{a} serum levels, a known negative regulator of the cytokine, indicating TNF\textsubscript{a}-independent regulation of adiponectin in HF. In a previous study, we have described diminished cardiac expression of adiponectin in inflammatory cardiomyopathy, but no circulating cytokine levels were measured in that study.\textsuperscript{18} We further show that cardiac adiponectin downregulation is also independent of adiponectin serum levels in DCM. This is the first evidence of distinct adiponectin regulatory systems in humans and has major implications for the development of novel therapeutic strategies for heart failure.

![Figure 5](image1.png)

**Figure 5** Adiponectin activates AMPK and Akt-dependent signalling pathways in neonatal rat ventricular cardiomyocytes in vitro. Time course of Akt (A) as well as eNOS (B) activation after incubation of NRVMs with adiponectin (10 \( \mu \text{g/mL} \)). Whole cell lysates were used for western blotting. Adiponectin leads to activation of Akt and eNOS, thereby increasing nitric oxide production. Representative western blots are shown, and data are expressed as mean \( \pm \) SD for three experiments in each group.

![Figure 6](image2.png)

**Figure 6** Adiponectin inhibits stress-induced apoptosis of cardiomyocytes. HL-1 cells were cultured to subconfluence, transduced with adenoviral vectors encoding \( \beta \)-gal or dnAkt, and incubated with doxorubicin (5 \( \mu \text{M} \)) for 24 h. NRVMs were cultured, transduced with \( \beta \)-gal or dn-Akt, and mitogen-deprived for 72 h in the presence of adiponectin (10 \( \mu \text{g/mL} \)) or BSA (10 \( \mu \text{g/mL} \)). Apoptotic cells were quantified by counting Hoechst-positive nuclei. Data are presented as mean \( \pm \) SD for three independent experiments performed in triplicate [\( *P < 0.05, \,**P < 0.01 \) vs. doxorubicin (5 \( \mu \text{M} \)) and serum (–), respectively].
implications for studies of adiponectin in human cardiovascular disorders. The amount of locally synthesized cardiac adiponectin is low when compared with adipose tissue, but comparable with SM, whereas the protein is abundantly present in serum. The nature of its isoforms which act upon the heart has not yet been clarified. Of note, globular adiponectin, a cleaved form of adiponectin that is present in extremely low concentrations in serum, has a higher potency than the full length form.36,37 Furthermore, Pajvani et al. demonstrated that mutated trimeric forms of adiponectin that are unable to associate to oligomers and are therefore subject to proteolytic cleavage show an ~30-fold increase in biological activity. Thus, high-molecular weight forms of adiponectin possibly represent precursors for the biologically active lower molecular weight forms generated by membrane-bound and possibly tissue-specific proteases. If so, the specific adiponectin form generated locally in small quantities and not the overall adiponectin protein concentration determines the biological effects on its target cells. In addition, local remodeling and changes in extracellular matrix composition resulting in increased wall tension, together with rarification of the vessel structure and reduced collateralization in DCM, may impede systemic delivery of the cytokine to its target cells. Future studies employing transgenic models of local cardiac adiponectin overexpression or knockout (KO) are needed to clarify the relative contribution of local and systemic adiponectin expression in cardioprotection.

Figure 7 Local adiponectin regulation in cardiomyocytes in vitro. Primary neonatal rat ventricular cardiomyocytes (NRVMs) and rat cardiac fibroblasts as well as human peripheral blood mononuclear cells and human umbilical vein endothelial cells were isolated and cultured in vitro. Human white adipose tissue (WAT) and skeletal muscle (SM) were taken from subcutis or major pectoralis muscle. RNA was extracted and qRT-PCR was carried out. The main source of cardiac adiponectin are cardiomyocytes (NRVMs) and neonatal rat fibroblasts (NRFB), whereas endothelial cells (EC) and inflammatory cells (peripheral blood mononuclear cells, PBMCs) do not synthesize the cytokine (A and B). Those cells express considerable amounts of adiponectin receptors, hence serving as effector cells for adiponectin action. The amount of RNA expression in human cardiac muscle (CM) is ~300× lower than in white adipose tissue (WAT), but higher than in skeletal muscle (B). Data are presented as mean ± SD for three independent experiments. (C) Neonatal rat ventricular myocytes were cultured in vitro. Secretion of adiponectin into the medium was blocked by monensin, an inhibitor of glycoprotein secretion. Cells were then collected and mRNA was extracted. Adiponectin receptor 1 and 2 mRNA and adiponectin mRNA abundance were determined by qRT-PCR. Data are presented as mean ± SD (*P < 0.05, **P < 0.01 vs. control).
Adiponectin was locally downregulated in DCM patients. The mechanisms underlying this downregulation are as yet unknown. Furukawa et al. demonstrated that oxidative stress inhibits adiponectin expression and suggested that this may contribute to decreased adiponectin levels in obesity. Cross-talk between inflammatory cells and cardiomyocytes, resulting in adiponectin downregulation, as shown for monocytes in adipose tissue, is another potential mechanism. Several lines of evidence suggest that the downregulation of adiponectin in failing human hearts is functionally significant. First, inhibition of protein secretion in cardiomyocyte cultures led to upregulation of adiponectin and its receptors providing first evidence of a local regulatory feedback loop. Although we do not identify any specific cardiomyocyte-derived secreted factor (e.g. adiponectin itself) which exerts a negative feedback upon adiponectin expression, it is evidence for the existence of such a factor which needs to be further defined. Secondly, adiponectin induced the activation of intracellular signalling pathways known to be protective in cardiomyocytes and other cell types. Thirdly, adiponectin ameliorated stress-induced apoptosis by activation of the serine threonine kinase Akt.

Several lines of evidence support the notion that cardiac apoptosis plays a causal role in the development of HF and that inhibition of apoptosis improves contractile dysfunction. Administration of IGF-1, an upstream regulator of Akt, reduces myocardial apoptosis in response to ischaemia and reperfusion in rats and acts as a survival factor for cultured cardiomyocytes. The cytoprotective effect of IGF-1 can be abolished by a dominant-negative form of Akt, whereas constitutively active Akt protects cardiomyocytes from apoptotic cell death in the absence of IGF-1. Gene transfer of constitutively active Akt inhibits cardiomyocyte apoptosis and ameliorates doxorubicin-induced contractile dysfunction. Therefore, Akt activation as shown here in vitro may be one of the mechanisms by which adiponectin attenuates apoptosis and contractile dysfunction in failing human myocardium.

Secondly, adiponectin incubation caused an increase in eNOS phosphorylation and NO production in cardiomyocytes. Enhanced NO generation by adiponectin via activation of the AMPK-Akt-eNOS signalling pathway has been demonstrated in endothelial cells. Protein expression of eNOS is downregulated in human DCM, and cardiomyocyte-specific overexpression of eNOS increased left ventricular (LV) performance, limited LV remodelling, and reduced hypertrophy in a murine model of HF. Moreover, in a recent study by Ichinose et al., chronic pressure overload in eNOS−/− mice resulted in eccentric LV hypertrophy and impaired systolic and diastolic functions. Furthermore, the African American Heart Failure Trial demonstrated a significantly reduced mortality rate in patients with class III and IV HF receiving an NO donor. Finally, NO is known to preserve endothelial function that is impaired in DCM. Therefore, activation of eNOS as shown here and increased generation of NO by adiponectin might ameliorate hypertrophy, LV remodelling, and endothelial dysfunction in DCM.

DCM is also associated with cardiac hypertrophy on a cellular level, and recent studies have demonstrated a role of adiponectin in the pathogenesis of hypertrophy. Fujikawa et al. determined the important role of adip-R1 and adip-R2 in the adiponectin-induced suppression of endothelin 1-induced cellular hypertrophy in vitro. Shibata et al. demonstrated that adiponectin suppresses cardiac hypertrophy in response to pressure overload in vivo. Adiponectin might thus exert cardioprotective effects in cardiomyopathy by preventing cardiac hypertrophy. The hypertrophic response in adiponectin KO mice is mediated by AMPK. The activation of AMPK by adiponectin improves myocardial glucose and lipid metabolism. Derangements in glucose and lipid utilization within the myocardium are hallmarks of DCM. Recently, it has been shown that AMPK prevents post-ischaemic cardiac dysfunction. Moreover, 3 weeks after transverse aortic constriction, pulmonary congestion and LV dimensions were significantly greater in adiponectin KO mice than WT mice. The development of DCM in adiponectin KO mice was accompanied by a decrease in AMPK expression and increased insulin resistance. Taken together, several lines of evidence indicate a role of the adiponectin-AMPK pathway.
signalling axis in cardioprotection, in addition to Akt and eNOS activation as discussed earlier.

There are several other mechanisms that might contribute to cardioprotection by adiponectin. The adipocytokine is known to suppress TNFα expression in cardiomyocytes1 which evokes a number of important effects on cardiomyocytes (hypertrophy, apoptosis), cardiac matrix (collagen degradation), as well as alterations in LV chamber geometry commonly altered in DCM. Furthermore, the anti-inflammatory effect of adiponectin might be beneficial.55 In this regard, adiponectin induced the expression of anti-inflammatory cytokines, such as IL-10 and IL-1 receptor antagonist, by human monocytes, macrophages, and dendritic cells, reduced the phagocytic capacity in macrophages,54 and decreased the ability to evoke an alloreactive T-cell response. Adiponectin application was protective in a mouse model of viral myocarditis.14 Moreover, hypoadiponectinemia is closely related to impaired vasoactivity in humans. Adiponectin exerts pro-angiogenic effects.8,9 The imbalance of anti- and pro-angiogenic factors in the progression of DCM has recently been demonstrated.55 In conclusion, several lines of evidence implicate adiponectin upstream of different important biological processes involved in the complex pathophysiology of HF. The local expression and regulation of the cytokine in the heart are diminished in DCM. Clarification of its actions in cardiovascular disease as well as the individual contributions of systemic and locally expressed adiponectin is of clinical importance and thus warrants further investigation.

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