Adiponectin resistance in skeletal muscle: pathophysiological implications in chronic heart failure

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Abstract

Skeletal muscle wasting is a common complication of chronic heart failure (CHF) and linked to poor patient prognosis. In recent years, adiponectin was postulated to be centrally involved in CHF-associated metabolic failure and muscle wasting. This review discusses current knowledge on the role of adiponectin in CHF. Particular emphasis will be given to the complex interaction mechanisms and the intracellular pathways underlying adiponectin resistance in skeletal muscle of CHF patients. In this review, we propose that the resistance process is multifactorial, integrating abnormalities emanating from insulin signalling, mitochondrial biogenesis, and ceramide metabolism.

Keywords Chronic heart failure; Skeletal muscle; Wasting; Adiponectin resistance

Introduction

Adiponectin, an adipocyte complement-related protein of 30 kDa, is one of the most abundant adipocytokines in the human body. Adiponectin was discovered two decades ago as an adipose tissue peptide with a prominent role in improving insulin sensitivity.[1] Further details on its physiological and pathophysiological actions have been substantiated in recent years.[2,3] To date, mounting evidence indicates a major role for adiponectin in the dysregulation of muscle metabolism among patients with chronic heart failure (CHF),[4] addressing the significance of this paper.

Adiponectin is present in the circulation of healthy individuals at very high concentrations accounting for approximately 0.05% of total plasma proteins, with levels ranging from 3 to 30 μg/mL.[5] Adiponectin circulates in a variety of isoforms, including trimers and hexamers, collectively termed low-molecular weight oligomers and high-molecular weight (HMW) multimers. The exact roles of all these different isoforms are still unclear. However, it seems that especially the HMW isoform is of more importance. HMW adiponectin is the most biologically active adiponectin form in the liver and is strongly associated with insulin resistance and several well-documented cardiovascular protective effects.[6] In particular, HMW adiponectin has been endowed with anti-diabetic, anti-atherogenic, anti-inflammatory, anti-hypertrophic, and anti-ischemic properties.[5,7–10] The functions of adiponectin across various tissues have already been extensively studied.[3,5,8,10–13] It is now appreciated that, along with leptin and resistin, adiponectin affects several metabolic processes involved in the control of energy homeostasis. Increasing fatty acid β-oxidation in skeletal muscle may be one of the most important mechanisms by which adiponectin increases insulin-sensitivity.[7,13]

CHF is associated with multiple metabolic disturbances of which many act negatively on skeletal muscle metabolism and wasting.[14–16] In CHF patients with reduced left ventricular ejection fraction (HFrEF), circulating adiponectin levels appear to increase in proportion to the severity of the disease.[17,18] In addition, these patients were shown to develop myocardial and skeletal muscle adiponectin resistance.[19,20] Over the past few years, adiponectin also emerged as an increasingly important player in the complex metabolic dysregulation related to CHF. The aim of this paper is to provide a review of current knowledge regarding the role of adiponectin in CHF, with a special focus on skeletal muscle wasting. In addition, the latest advancements in the field on mechanisms underlying CHF-associated

adiponectin resistance in skeletal and myocardial muscle will be discussed.

Adiponectin: the *Janus* molecule of chronic heart failure

The adiponectin paradox

Circulating adiponectin levels correlate inversely with multiple metabolic diseases and related disorders associated with insulin resistance. Adiponectin levels are decreased in obesity and type-2 diabetes and negatively associated with the metabolic syndrome.[21,22] Low levels of adiponectin have been linked with the presence of common cardiovascular risk factors including smoking and hypertension.[23–26] Moreover, adiponectin deficiency resulted into increased myocardial damage, cardiac hypertrophy, and heart failure.[18,23,27,28] In addition, a strong correlation between myocardial infarction and hypoadiponectinaemia was documented in a number of studies.[17,29,30] Adiponectin levels in patients with coronary artery diseases (CAD) seem to be diminished if compared with healthy subjects and are strongly predictive of incident cardiovascular events.[31]

Hence, whereas the role of adiponectin within CAD and myocardial infarction is quite straightforward, its function in CHF seems to be more complicated. Paradoxically, in patients with HFrEF, high levels of circulating adiponectin infer poor prognosis.[32–34] A recent cohort study by Baldasseroni *et al.*[35] in 107 outpatients with CAD compared circulating levels of adiponectin in patients with CAD with or without CHF. A marked elevation in plasma adiponectin levels was found in patients with overt heart failure compared with patients with CAD and no heart failure.[35] In addition, circulating adiponectin levels were higher in patients with CAD and heart failure as opposed to asymptomatic heart failure patients with normal or reduced left ventricular ejection fraction (LVEF).[35,36] Likewise, Wannamethee and colleagues[37] investigated the association of adiponectin with all-cause and cardiovascular disease (CVD) mortality in a prospective study of 4046 men aged 60–79 years, participating in the British Regional Heart Study. Adiponectin levels were the strongest positively associated with increased all cause and CVD mortality in men with diagnosed heart failure. Furthermore, the population-based Cardiovascular Health Study of 5553 older adults (≥65 years) demonstrated an increased risk of all-cause mortality for elderly heart failure subjects with adiponectin levels above 12.4 mg/L.[38]

These findings are in contrast with several *in vitro* studies supporting a beneficial role for adiponectin in CHF.[39,40] The relation of high plasma adiponectin with advanced disease state, symptomatic status and metabolic impairment in patients with CHF, however, seems to be robust.[14,33] This seemingly negative impact of adiponectin on the survival of patients with CHF has been termed '*the adiponectin paradox*'.[41] Up to now, the underlying pathophysiologic mechanisms remain unclear.

Adiponectin and disease stage

A small, but growing, literature suggests that adiponectin levels vary according to a patient's metabolic status. Low plasma concentrations of adiponectin precede the onset of type-2 diabetes.[24] Heart failure patients with diabetes have lower adiponectin levels compared with patients without diabetes.[36] This also applies to heart failure patients with or without the metabolic syndrome.[22] A distinct response in plasma adiponectin was also shown at early and late stages of the disease and according to the extent of heart failure. Increased adiponectin levels were correlated with worsening of functional capacity (i.e. New York Heart Classification; NYHA) and cardiac performance (i.e. LVEF).[28,32] However, CHF patients with preserved ejection fraction (HFpEF), previously termed diastolic heart failure, have decreased circulating adiponectin levels compared with aged matched normal controls. This lower plasma adiponectin was associated with diastolic dysfunction independently of age, BMI, and the presence of hypertension or diabetes.[42] Further, in a murine model of HFpEF, hypoadiponectinemia exacerbated hypertension, diastolic dysfunction and even heart failure.[42–45] Although, emerging evidence indicates that adiponectin plays a role in hypertension-related diseases, the pathophysiological importance of adiponectin in HFpEF remains to be investigated. Finally, several studies have demonstrated that adiponectin is a powerful independent predictor of HFrEF severity and mortality.[17,33,46] In this regard, a strong correlation between circulating N-terminal pro-brain natriuretic peptide (NT-proBNP) and adiponectin has been described.[28,47] As stated by Antonopoulos *et al.*,[48] the net effect of adiponectin in circulation likely depends on a complex interplay of the underlying disease state and the nature of the inflammatory stimuli.

Adiponectin: an undeserved bad reputation in chronic heart failure?

It is not yet clear whether increased adiponectin concentrations are beneficial, rather than detrimental to HFrEF patients.[9] For a long time, concentrations of adiponectin were believed to relate with heart failure incidence.[18,49–51] In the recent Framingham Offspring study, a community-based, prospective epidemiologic cohort study of CVD and its risk factors in 2739 participants, neither high nor low concentrations of adiponectin were related with incident heart failure.[52] Even after adjustment for body mass index (BMI), homeostasis model assessment of insulin resistance (HOMA-IR), C-reactive protein (CRP) and BNP, no apparent association was found. These

results suggest that adiponectin itself has no major role in the development of HFrEF. Instead, the increase in circulating adiponectin concentration might be induced to attenuate the overall cardiovascular risk and/or prevent heart failure progression. In this regard, Liao et al.[53] demonstrated that adiponectin deficiency in mice accelerates the transition from left ventricular hypertrophy to heart failure. Nevertheless, it also remains possible that adiponectin has a two-faced character in HFrEF. In the early stage, the increase in adiponectin production may be part of a compensatory mechanism, but for patients in the final stages of heart failure, increased adiponectin levels may worsen cardiac dysfunction, thus leading to an increased risk of mortality[54] (Figure 1).

Skeletal muscle wasting: is adiponectin the real culprit?

A key adipocytokine in skeletal muscle metabolism

It is now well-recognized that skeletal muscle energy deficiency plays a main role within the pathophysiology of heart failure. CHF patients are typified by increased protein breakdown and turnover, and a disturbed skeletal muscle glucose uptake and use.[14] In this regard, Nagoshi and colleagues[16]

Figure 1 Two faces of adiponectin in chronic heart failure. Adiponectin is a beneficial adipokine that is present at high levels in lean, healthy individuals. Low levels of adiponectin have been associated with conditions such as obesitas, hypertension, myocardial infarction, coronary artery disease, and cardiac hypertrophy. In heart failure with reduced ejection fraction, however, increases in circulating adiponectin parallel worsening of prognosis for patients. Based on clinical and experimental evidence, we hypothesize that the adipokine has two faces in heart failure with reduced ejection fraction. Elevated adiponectin likely serves as a protective and compensatory mechanism for the energy metabolic alterations that occur during the early stages of heart failure with reduced ejection fraction. In contrast, in patients with end-stage heart failure with reduced ejection fraction, high levels of adiponectin will further deteriorate cardiac function, increasing the risk of death. Green, yellow, and red colours indicate normal, low, and high levels of adiponectin, respectively. Arrows point to disease condition.
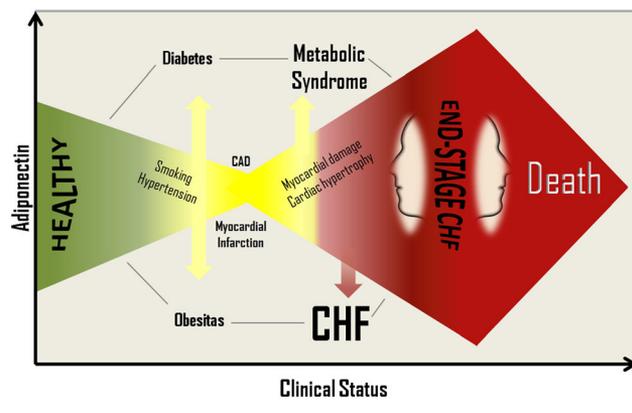

demonstrated a shift in substrate metabolism away from fatty acid β-oxidation towards glucose metabolism, marked by a decreased oxidative capacity and higher circulating levels of free fatty acids (FFA). These latter inhibit cardioprotective glycolysis and impair insulin signalling. Furthermore, patients with CHF have altered mitochondrial volume and enzyme activity in both cardiac and skeletal muscles, which argues for a generalized metabolic myopathy in heart failure[55–61] (Figure 2). In search for the molecular basis of this energy-deprived status in HFrEF patients, attention was focused on adiponectin. The skeletal muscle is a major contributor to whole-body energy expenditure, being responsible for ~80% of insulin-stimulated glucose disposal and, therefore, represents an important site of action for adiponectin. Indeed, adiponectin is involved in the regulation of skeletal muscle metabolism via its interaction with the G-protein-coupled adiponectin receptors AdipoR1 and AdipoR2.[7] The high-affinity receptor AdipoR1 is abundantly expressed in vascular endothelial cells and skeletal muscle, where it regulates glucose and lipid metabolism, whereas AdipoR2, which serves as a moderate-affinity receptor, is mainly expressed in the liver.[7] Recent characterization of mice lacking AdipoR1 and AdipoR2 confirmed important roles for these receptors in the maintenance of metabolic homeostasis.[62] In particular, simultaneous disruption of both receptors abrogates adiponectin binding resulting into glucose intolerance and insulin resistance. Adiponectin promotes glucose uptake by skeletal muscles and increases fatty acid β-oxidation by activation of multiple factors including adenosine monophosphate activated protein kinase (AMPK), peroxisome proliferator-activated receptor α (PPARα) and p38 mitogen-activated protein kinase (MAPK).[13] AMPK is the master regulator in glucose utilization and fatty acid β-oxidation, whereas the nuclear transcription factor PPARα regulates the expression of several genes involved in lipid metabolism, ultimately preventing insulin resistance. Adiponectin stimulates mitochondrial biogenesis and increases the oxidative capacity in skeletal muscle.[63,64] In this regard, Liao and his colleagues[53] reported energetic defects in a mice model of transverse aortic constriction in relation to adiponectin deficiency. More recently, increased circulating adiponectin concentrations were associated with metabolic impairments and a hyper-catabolic state in patients with HFrEF.[65,66]

All these data stress that adiponectin exercises an important impact on skeletal muscle energy metabolism in heart failure (Figure 2). The pathophysiological mechanisms underlying skeletal muscle energy alterations in CHF, however, remain to be fully established.

Adiponectin as a marker of skeletal muscle wasting

In addition to the well-described skeletal muscle energetic impairments, the majority of CHF patients develop muscle

Figure 2 Adiponectin and chronic heart failure-associated myopathy. Skeletal muscle hyperadiponectinemia contributes to energetic impairments, structural, and functional alterations of muscle fibres and hormonal imbalances, eventually culminating into a state of chronic heart failure-associated myopathy.
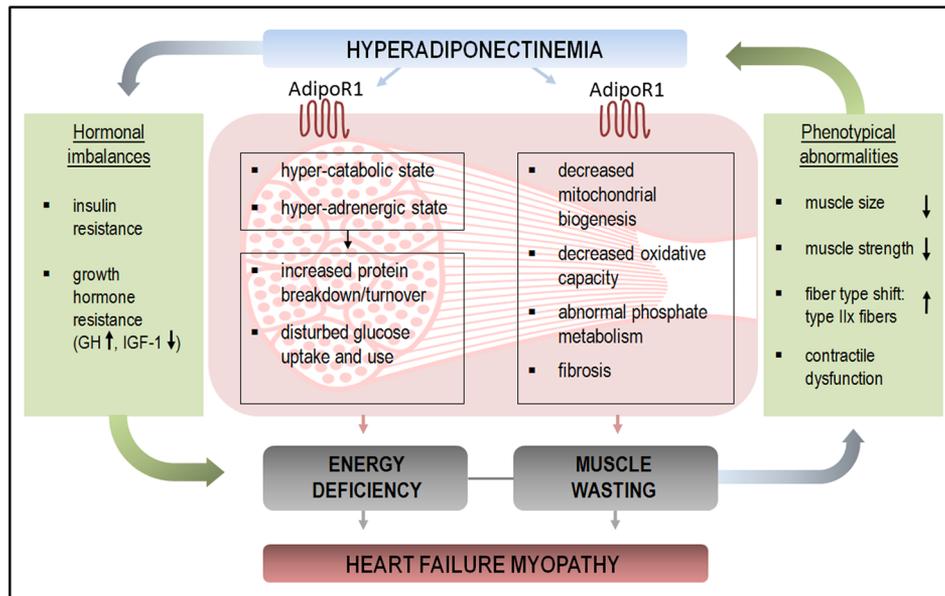
wasting.[65,67,68] Muscle wasting is recognized as a critical component of cachexia contributing to muscle weakness and fatigue.[4,67] In a recent case–control study by Mc Entegart and co-workers,[69] HFrEF patients with cachexia had remarkably higher adiponectin concentrations in comparison to patients without cachexia, irrespective of their BMI. Latest research further revealed that circulating levels of adiponectin gradually increase with decreasing muscle fibre size and muscle strength, even in non-cachectic HFrEF patients.[70,71] Furthermore, absence of adiponectin expression in the skeletal muscle of adiponectin-null knockout mice was associated with phenotypical muscle abnormalities and contractile dysfunction, including an increase in type II fibre size and a decreased peak contractile force if compared with wild-type littermates.[72] Clinical research also revealed a '*fibre type shift*' in CHF patients, characterized by a decreased proportion of slow oxidative (fatigue-resistant) type I fibres, compensated for by an increased proportion of fast glycolytic (fatiguable) type IIx fibres.[56] This shift in muscle fibre type distribution occurs similar between HFrEF and HFpEF patients.[60,73] In addition, both groups have reduced muscle oxidative capacity and abnormal intracellular phosphate metabolism[56,57,72] (*Figure* 2). These factors may all contribute to impaired functional skeletal muscle performance and, ultimately, muscle wasting.

In summary, hyperadiponectinemia seems to parallel with alterations in the structural and functional characteristics of skeletal muscle fibres in HFrEF patients, and might even act as a marker of cachexia.[4,69] Adiponectin might be centrally involved in impaired metabolic signalling, forming a potential

link between muscle wasting, disease progression and poor prognosis in HFrEF patients[65] (*Figure* 3). The exact molecular basis by which adiponectin affects skeletal (and cardiac) muscle tissue in heart failure is not completely clarified. Available data will be addressed in the next paragraphs.

Adiponectin resistance in chronic heart failure

Adiponectin resistance at the level of the skeletal muscle

Little is known about the regulation of adiponectin at the molecular level in skeletal muscle of patients with CHF. Recently, our group investigated the local adiponectin system in skeletal muscle biopsies from HFrEF patients.[19] In agreement with increased circulating adiponectin concentrations, a five-fold increase in the expression of adiponectin mRNA and protein was observed in patients with overt heart failure. Despite these increases in circulating and muscle adiponectin, the mRNA and protein expression levels of the main skeletal muscle adiponectin receptor AdipoR1 were decreased. Skeletal muscle metabolic deficiency in these patients was corroborated by results showing a deactivated PPARα/AMPK pathway and a concordant down-regulation of several target enzymes involved in the metabolism of FFA and glucose. Our results, therefore, signified the presence of a functional adiponectin resistance at the level of the skeletal muscle in

Figure 3 Hypothesis on skeletal muscle adiponectin resistance in chronic heart failure. In chronic heart failure, insulin resistance is associated with decreased levels of phosphatidylinositol 3-kinase/Akt/forkhead box protein O1 and a reduced expression of the adiponectin receptor AdipoR1. Skeletal muscle metabolic deficiency in chronic heart failure is corroborated by a deactivated adenosine monophosphate activated protein kinase/sirtuin 1/peroxisome proliferator-activated receptor α pathway and down-regulation of peroxisome proliferator-activated receptor γ coactivator1 α, ultimately progressing to a state of skeletal muscle adiponectin resistance. This adiponectin resistance contributes to lipotoxicity, and further worsens insulin resistance. All these facets infer a disease process that eventually fulminates into skeletal muscle wasting.
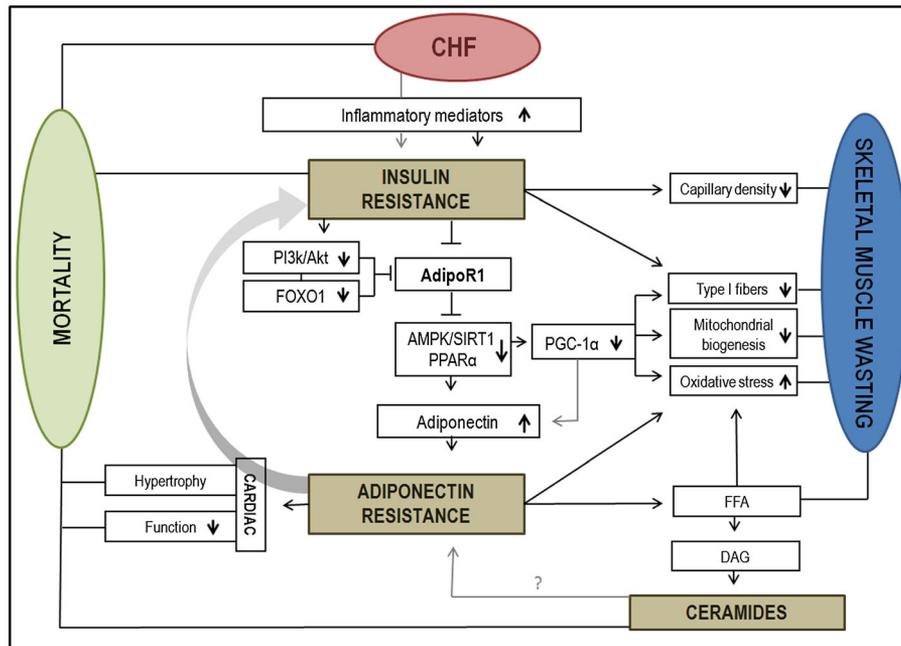
HFrEF patients.[19] Until now, data regarding skeletal muscle adiponectin signalling in HFpEF are not yet available.

CHF as a last resort, although this hypothesis needs to be tested by further investigation.

Myocardial adiponectin resistance

Adiponectin and its receptors are also expressed by myocardial tissue. In the myocardium of patients with HFrEF, adiponectin expression is increased, whereas the level of AdipoR1 is reduced.[20] Interestingly, mechanical unloading of the failing human heart through implantation of a ventricular assist device resulted into a reduction of circulating adiponectin and an increase in the level of AdipoR1 mRNA. These results suggest that myocardial adiponectin resistance in this population of CHF patients can be reversed.[20] Kreth et al.[74] recently investigated the myocardial adiponectin signalling in end-stage HFrEF patients. Besides high circulating adiponectin levels, myocardial adiponectin expression was low if compared with healthy controls, whereas myocardial AdipoR1 and AdipoR2 mRNA were increased. As such, the myocardial adiponectin pathway seems to be up-regulated in these patients with end-stage heart failure. The question remains as to what happens between early and end-stage CHF. Systemic release of cellular adiponectin might be triggered in the progression to end-stage

Adiponectin resistance and insulin resistance

Hormonal imbalances, including growth hormone resistance and insulin resistance have been previously reported as intrinsic features of CHF leading to both increased morbidity and mortality.[75] Insulin resistance progresses in parallel with the severity of heart failure and is considered instrumental in the development of skeletal muscle wasting.[76] In particular, insulin sensitivity is worse in cachectic HFrEF patients.[77] Adiponectin, by binding on its receptors, regulates insulin-sensitizing effects at the level of the skeletal muscle partly via activation of AMPK, PPAR-α, and presumably other yet unknown signalling pathways.[7,13] In a cross-sectional study of 461 men Ingelsson et al.[78] found higher circulating adiponectin concentrations with increasing skeletal muscle capillary density and in individuals with a higher proportion of slow type I muscle fibres. Taking into account that higher capillary density and more type I muscle fibres are associated with increased insulin sensitivity, adiponectin indeed mediates insulin's actions (Figure 3). In this regard, Stefan and colleagues[79] demonstrated that plasma

adiponectin concentrations in healthy humans are positively associated with *in vivo* insulin-stimulated glucose disposal, whereby low adiponectin concentrations were observed in parallel with reduced insulin sensitivity. In the case of CHF, however, adiponectin levels have not been convincingly associated with insulin sensitivity. Moreover, adiponectin concentrations are elevated in HFrEF despite a profound resistance to insulin, suggesting a disconnection between raised circulating adiponectin and improvement of insulin sensitivity in HFrEF.[80]

Impaired insulin sensitivity might also represent a causal link between adiponectin resistance and skeletal muscle wasting in heart failure.[76] As such, López Teros *et al.*[81] studied insulin levels and muscle mass in a cohort study of 147 older adults during an average follow-up period of 4.6 years and demonstrated that hyperinsulinemia is a significant risk marker of appendicular skeletal muscle wasting at older age. Still, it should be noted that Mullen *et al.*[82] found evidence for adiponectin resistance to emerge prior to the initiation of skeletal muscle insulin resistance in high-fat fed rats. The interplay between insulin and adiponectin resistance is discussed in more detail below.

Adiponectin resistance—the whys and wherefores

The hypothesis on adiponectin resistance in HFrEF patients has garnered considerable attention in the past 5 years, but has also been the subject of controversy. Adiponectin in target tissues is regulated physiologically by several circulating factors among which are hormones, chemokines, growth factors and cytokines. Crosstalk between these various signalling molecules and the adiponectin pathway constantly tunes adiponectin expression. Although primarily secreted by white adipose tissue, adiponectin is also produced by non-adipose tissues and cell types such as hepatocytes, osteoblasts, and even skeletal muscle fibres.[72,83,84] Cardiomyocytes are also capable of synthesizing adiponectin, advocating the existence of a local adiponectin system at the myocardium.[85] Whether the synthesis, expression, and release of adiponectin by non-adipose cells and tissues contribute to the increased circulating adiponectin concentrations in HFrEF is still unclear. A recent study, however, demonstrated that adiponectin production by cardiomyocytes in end-stage HFrEF patients is low, not being significantly different between CHF and healthy myocardium, making its participation to higher blood levels unlikely.[74] Further, as summarized by Yamauchi and colleagues,[86] growing evidence supports a role for adiponectin resistance in the development and progression of HFrEF, not only by promoting metabolic dysfunction, but also by affecting peripheral organs and tissues, i.e. the endothelium, liver, and skeletal muscle, and as such, contribute to the heart failure syndrome.

The state of adiponectin resistance in HFrEF fits well with the metabolic alterations in skeletal muscle, such as increased FFA availability, ultimately leading to skeletal muscle wasting (*Figure* 3). Increasing our knowledge on the regulation of adiponectin and its downstream pathways in skeletal muscle of CHF patients, and with respect to the failing heart, might therefore contribute to a greater understanding of the pathophysiology of CHF.

Pathophysiology of adiponectin resistance

Since the initial observation by Van Berendoncks *et al.*,[87] many efforts were done to identify the causes underlying adiponectin resistance in HFrEF. Although not yet completely resolved, adiponectin resistance seems to develop by multiple molecular and cellular interaction mechanisms between inflammatory and metabolic pathways. Study results will be discussed in the next paragraphs and are summarized in *Figure* 3.

New insights originating from AdipoR1

Adiponectin resistance, initially described in obesity and conditions associated with insulin resistance, is thought to be partly due to of down-regulation of the adiponectin receptor AdipoR1.[82,88] This hypothesis is supported by a number of studies in humans and rodents and laboratory work with *in vitro* cell lines.[62,88–93] Molecular cloning and knock-down of AdipoR1 have improved understanding of the physiological and pathological actions of AdipoR1 and its role in adiponectin resistance. Yamauchi *et al.*[62,63] evaluated the effects of adenovirus-mediated disruption of AdipoR1 in the skeletal muscle of $Lepr^{-/-}$ mice. AdipoR1 null mice are glucose intolerant and insulin resistant because of mitochondrial defects. Targeted disruption of AdipoR1 also resulted into the abrogation of adiponectin dependent AMPK/Sirtuin 1 (SIRT1) activation. Furthermore, adiponectin deficiency accelerated the transition from cardiac hypertrophy to heart failure and was causally related to a decreased cardiovascular function.[94] Evidence in support of a concomitant reduction in receptor protein levels came from an additional study in obese mice demonstrating a lowered AdipoR1 protein content in skeletal muscle of insulin-resistant animals.[93] Kadowaki and colleagues examined the regulation of AdipoR1 and AdipoR2 under insulin-resistant conditions.[93] mRNA levels of both receptors decreased in response to increases in insulin. In line, in a recent study by Cui *et al.*,[89] myocardial AdipoR1 expression and activation of AMPK were reduced in insulin-treated rats. In addition, an impaired AdipoR1

mediated effect was observed in insulin treated primary cultures of rat cardiomyocytes and C_2C_{12} myoblasts.[95]

Combined, these data indicate that changes in adiponectin via AdipoR1 relate to insulin signalling in CHF patients (*Figure* 3). The recently developed orally active synthetic small molecule AdipoR agonists, which specifically bind to and activate both AdipoR1 and AdipoR2, have generated promising results with regard to insulin sensitivity and mitochondrial capacity in muscles from *db/db* mice.[96] Treatment with AdipoRon increased AdipoR1/AdipoR2, AMPK, and PPARα mRNA expressions as well as the mRNA levels of genes involved in fatty acid β-oxidation, thereby ameliorating insulin resistance and glucose intolerance. Moreover, AdipoRon induced an up-regulated expression of peroxisome proliferator-activated receptor γ coactivator1 α (PGC-1α), contributing to increased mitochondrial biogenesis. It remains, however, interesting to investigate whether restoration of AdipoR1 expression will fully abrogate adiponectin resistance and lead to improvements in the metabolic and functional capacity of CHF patients.

Rethinking the role of PGC-1α

CHF is characterized by a disrupted mitochondrial function and biogenesis in which the production, transfer, and consumption of high-energy phosphatases and oxidative capacity are reduced (*Figure* 3). PGC-1α is responsible for the co-activation of transcription factors, including PPARs and ERRs (estrogen related receptors), and thereby controls mitochondrial biogenesis and several antioxidant mechanisms.[63,97,98] PGC-1α becomes up-regulated in skeletal muscle in response to Ca^{2+}/calmodulin-dependent protein kinase (CaMK). Interestingly, adiponectin stimulates CaMK via activation of AdipoR1 and AMPK/SIRT1, which would lead to the increased expression of PGC-1α. However, PGC-1α is down-regulated in the skeletal muscle of HFrEF patients.[98,99] In this respect, it has been speculated that PGC-1α might play a crucial role in the processes of skeletal muscle wasting and adiponectin resistance in CHF (*Figure* 3). Muscle-specific AdipoR1 KO mice have been able to abolish an adiponectin-related increase in CaMK and AMPK/SIRT1, but induced a decrease in the expression and deacetylation of PGC-1α. Subsequently, a decline in mitochondrial content and activity, a decrease in the number of oxidative type I fibres and ultimately, a reduction in oxidative stress-detoxifying enzymes associated with insulin resistance were found in the skeletal muscle.[63] Adiponectin over-expression led to increased muscle mitochondrial biogenesis through up-regulation of PGC-1α.[64] These findings suggest that in HFrEF, impaired AdipoR1 levels might well be causally related to a decrease in PGC-1α and a disturbed mitochondrial activity of the skeletal muscle (*Figure* 3).

The ceramide hypothesis

Increased levels of the toxic lipid intermediates, ceramide, and diacylglycerol (DAG) in the failing human myocardium and in skeletal muscle are established features of HFrEF (*Figure* 3). Recent studies have questioned the primacy of adiponectin to altered sphingolipid ceramide profiles in skeletal muscle. Data from animal studies are provided showing that ceramidase activity is affected by the actions of adiponectin.[100] Adiponectin lowers ceramide levels via activation of ceramidase, which actively metabolizes ceramides to sphingolipids in skeletal muscle with subsequent enhanced insulin sensitivity and mitochondrial biogenesis. In addition, an inverse correlation between circulating adiponectin and skeletal muscle ceramide content was demonstrated in healthy men.[101] Ceramide signalling in HFrEF further mainly depends upon adiponectin activation of both AdipoR1 and AdipoR2. Moreover, over-expression of AdipoR1 and AdipoR2 in primary neonatal ventricular cardiomyocytes reduced ceramide levels and improved insulin sensitivity.[102]

Lipotoxicity in HFrEF is a consequence of impaired fatty acid β-oxidation and storage leading to increased levels of FFA, which are in turn converted into DAG and ceramides.[103] These lipotoxic metabolites accumulate in insulin responsive tissues, including skeletal muscle. Whether this lipotoxic state contributes to adiponectin-resistance in HFrEF needs to be proven. Recently, attention was drawn to the role of fibroblast growth factor 21 (FGF21) in mediating the effect of adiponectin on energy expenditure, including ceramide metabolism. FGF21 KO mice were shown to have reduced circulating adiponectin along with increased circulating ceramide levels, thereby impairing insulin sensitivity.[104] The interaction between FGF21–adiponectin–ceramide seems a major axis for the control of energy consumption and the action of insulin.[104,105]

Nevertheless, the question remains whether a disturbed adiponectin–sphingolipid signalling is involved in the aetiology of adiponectin resistance in CHF. Very recently, Yu *et al.*[106] demonstrated a stepwise increase in plasma ceramides in HFrEF patients along with increased adiponectin levels in relation to the severity of HFrEF. In addition, an increased diaphragm ceramide content was noted in rats with CHF following coronary artery ligation and associated with disturbed energy metabolism and diaphragm dysfunction.[107] All these data highlight a main role for ceramides in the pathophysiology of HFrEF (*Figure* 3).

Connecting the chain of events: a disturbed insulin —AdipoR1 axis

The association between insulin and adiponectin was reported for the first time in an adipocyte-specific insulin KO mouse model, demonstrating that hyperadiponectinemia can result from a lack of insulin signalling in adipose tissue.[108] In addition, Lin *et al.*[68] and Kim *et al.*[90] both investigated the

interactions between adiponectin and insulin in mice with muscle-specific insulin resistance and showed that insulin resistance may lead to hyperadiponectinemia and even adiponectin resistance. Evidence was also provided that phosphatidylinositol 3-kinase (PI3K)/Akt mediates the insulin-inhibited expression of AdipoR1 in skeletal muscle.[93,109] Upon binding to its cell surface receptor, tyrosine kinase IGF-1, insulin will trigger downstream signalling cascades including PI3K/Akt.[109] Akt then regulates gene transcription through inactivation of forkhead box protein O1 (FOXO1). As such, the PI3K/Akt/FOXO1 axis has a central role in energy metabolism and signal transduction of insulin, governing insulin sensitivity (*Figure* 3). In the study of Cui *et al.*,[89] FOXO1 silencing inhibited AdipoR1 expression and the activation of AMPK, supporting the hypothesis that insulin induces a decrease in skeletal muscle AdipoR1 expression and function in a PI3K/Akt and FOXO1 dependent fashion. Moreover, the PI3K/Akt pathway is deregulated in muscle wasting, and hence, in HFrEF (*Figure* 3). As Akt is chronically activated, FOXO1 becomes inactive leading to decreased levels of AdipoR1. Interestingly, mice deficient in cardiac and skeletal muscle insulin as well as the IGF-1 receptor demonstrated FOXO1 activation, resulting from Akt inactivation, and eventually died from heart failure within the first month.[110,111] Furthermore, a study by Sun *et al.*[92] in C_2C_{12} myoblasts revealed an insulin-responsive element region in the AdipoR1 promoter, nuclear inhibitory protein, which is involved in the negative regulation of AdipoR1 by insulin. This region also seems to contain a regulatory site for FOXO1, again providing proof that FOXO1 plays a role in insulin mediated AdipoR1 expression. FOXO1 may bind directly to the AdipoR1 receptor and, ultimately, suppress AdipoR1. In this regard, overexpression of AdipoR1 will ameliorate insulin sensitivity, mediated by increased phosphorylation and expression of several intermediates in the PI3K/Akt signalling pathway.[112]

Altogether, these observations portray an important connection between insulin and AdipoR1. Consequently, elevated insulin levels may result into a decreased expression of AdipoR1, leading to diminished binding of adiponectin, a reduction in PGC-1α, and an increase in sphingolipid ceramides, ultimately progressing to adiponectin resistance (*Figure* 3). This in turn could cause a reduction of the insulin-sensitizing effects of adiponectin and start a vicious cycle.[101] In this regard, the group of Khawaja *et al.*[113] recently reported the restoration of adiponectin signalling in HFrEF patients who underwent a ventricular assist device implantation. At baseline, these patients had decreased levels of insulin together with increased expression of AdipoR1 and genes involved in fatty acid β-oxidative metabolism. The authors hypothesized that the improvements in adiponectin resistance were, at least partly, because of diminished insulin resistance.[20,113] On the contrary, Staiger *et al.*[114] demonstrated a strong positive correlation between AdipoR1 expression and insulin secretion, whereas insulin treatment did not modulate AdipoR1 in myotubes cultures

in vitro. Therefore, the insulin–AdipoR1 interaction is far from being obvious and further research is needed.

Other mechanisms potentially underlying adiponectin resistance: what one should keep in mind

The interaction between insulin, AdipoR1, and PGC-1α appears to be critical in the development of adiponectin resistance in HFrEF. However, its aetiology may be more complex and multifactorial. Adipose tissue abnormalities, oxidative stress, increased inflammatory mediators, a shift in energy metabolism in the failing heart, and deregulated natriuretic peptides might all be implicated as mediating factors in the process of adiponectin resistance in the setting of CHF.

Inflammation and oxidative stress

CHF patients have raised circulating levels of inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, and several chemokines such as IL-8. This increase in inflammatory mediators is accompanied by a corresponding increase in adiponectin levels in HFrEF patients. It has been firmly established that this increase in adiponectin renders protective effects by its anti-inflammatory actions.[28] In a study by Khan *et al.*,[20] the decrease in systemic and myocardial inflammation after ventricular assist device implantation in patients with severe heart failure and reduced ejection fraction went along with a reduction in adiponectin levels. *In vitro* experiments have shown that addition of inflammatory stimuli to cultured cardiomyocytes suppresses AdipoR1 and PPARα expression levels.[115–117] Furthermore, a significant negative correlation between plasma adiponectin and plasma oxidized low-density lipoprotein (oxLDL), a marker of oxidative stress, was found in patients with HFrEF.[118] In patients with the metabolic syndrome, lower levels of adiponectin were associated with adverse oxidative stress profiles, and thus, negatively correlated with increased production of reactive oxygen species (ROS) and measures of lipid peroxidation.[119,120] Consequently, the increase in adiponectin concentrations in HFrEF could represent a protective compensatory mechanism in an attempt to overcome the pro-inflammatory and oxidative stress conditions that characterize the disease, and as such, may have a role in the initiation and maintenance of adiponectin resistance.[32]

Natriuretic peptides

Cardiac secretion of natriuretic peptides has been associated with increased ventricular filling pressure whereas circulating

BNP levels correlated with the prognosis of CHF.[32] In addition, plasma levels of BNP have been positively associated with adiponectin levels in CHD and HFrEF patients.[18,32,47] Natriuretic peptides promoted adiponectin secretion and synthesis, mainly via their lipid mobilizing effect.[28,47] Both natriuretic peptides atrial natriuretic peptide (ANP) and BNP augmented the production of adiponectin in primary cultured human adipocytes via a cyclic GMP-dependent protein kinase (PKG) signalling pathway. Moreover, administration of recombinant ANP in patients with HFrEF increased their plasma adiponectin levels.[47] Accordingly, natriuretic peptides may add to a state of adiponectin resistance in HFrEF.

Metabolic deregulation

It is often argued that circulatory and skeletal muscle adiponectin levels increase in an attempt to compensate for the shift in energy metabolism in the failing heart.[16] Interestingly, mechanical unloading of the failing heart induced the normalization of metabolic deregulation and mitochondrial function, and went along with a decrease in circulating adiponectin.[121] For this reason, adiponectin resistance has been put forward as a counter-regulatory response to disturbed metabolic energy metabolism in patients with HFrEF.[9]

Endocytosis of AdipoR1

Surface receptor activity can be decreased because of endocytotic uptake and subsequent lysosomal degradation of proteins.[122] It has been demonstrated that exposure of high concentrations of adiponectin to hepatocytes, epithelial cells, and HeLa cells leads to the internalization of AdipoR1.[123–125] This endocytosis of AdipoR1 is probably clathrin dependent.[123] Blocking of AdipoR1 internalization enhances adiponectin-stimulated phosphorylation of AMPK. Whether the down-regulation of AdipoR1 in HFrEF is the result of receptor-mediated endocytosis needs further research.

Adipose tissue inflammation

Recent studies have questioned the primacy of abnormalities in adipose tissue in CHF as a possible mechanism of adiponectin resistance.[20,126] Adipocytes in CHF are not only decreased in number, but also much smaller in size compared with adipocytes from healthy subjects. Furthermore, patients with CHF develop adipose tissue inflammation with increased macrophage infiltration.[20] To date, it is not known if these alterations in adipose tissue contribute to adiponectin resistance in CHF.

Exercise training as a panacea for adiponectin resistance

Exercise training ameliorates exercise capacity and quality of life in HFrEF and HFpEF patients.[127–134] The mechanisms responsible for these benefits include, at least in HFrEF, anti-inflammatory effects, decreased neuro-hormonal activation, and restored insulin resistance.[127–130,135] Available data demonstrate that exercise is also able to attenuate mitochondrial, histological, and functional alterations in the skeletal muscle of patients with HFrEF and HFpEF.[136] In addition, in HFrEF, exercise was shown to down-tune the activation of the adiponectin pathway at the circulatory and at the myocyte level.[129,131] In particular, besides improvements in work efficiency and muscle strength, combined endurance and resistance exercise training lowered the circulating adiponectin concentration and normalized the muscle-specific expression of adiponectin, AdipoR1, and genes involved in lipid and glucose metabolism towards healthy control levels.[129] To date, however, no information is yet available regarding the influence of exercise on adiponectin signalling in HFpEF patients.

Future scientific perspectives

The black hole of adiponectin

The need for scientific progress in the field of metabolic alterations in CHF and the relation with skeletal muscle wasting could not be more acute. Overcoming adiponectin resistance is a major challenge and holds great opportunities to alter the progression of CHF. AdipoR1 seems to be a pivotal mediator in the process of adiponectin resistance. A major pending question is whether an abnormal expression of AdipoR1 depends on insulin and, if so, whether a disturbed insulin-AdipoR1 signalling contributes to an increased skeletal muscle adiponectin expression and, ultimately adiponectin resistance in HFrEF. In this respect, and given the evidence reported to date, it is pertinent to determine whether restoration of AdipoR1 expression will abrogate adiponectin resistance and lead to metabolic and functional improvements in HFrEF. In particular, the primary cause of muscle insulin resistance could be related to the activation of pro-inflammatory pathways and alterations in muscle lipid metabolism, and this needs further exploration. Further, heart failure trials should also be initiated to explore the role of adiponectin, especially with regard to the different stages of CHF (early vs. end-stage) and the underlying disease state.

From bench to bedside and back

Several questions about the existence of adiponectin resistance at the level of the skeletal muscle in CHF are still left

unanswered. Until now, mechanistic data have been confined to animal experiments, C_2C_{12} mouse myoblasts, and L6 myotubes cell lines. Although animal models and immortalized cell lines are convenient sources to study skeletal muscle characteristics, they are devoid of typical human traits, i.e. genotypical and developmental features associated with the '*heart failure myopathy*'. Primary cultures derived from skeletal muscle of CHF patients may represent a more suitable model. These primary myoblasts and myotubes cultures indeed retain the phenotypic and genotypic traits of the donor,[137] including morphological, metabolic, and biochemical similarities.[138–140] Specific cultured skeletal muscle cells from CHF patients may therefore represent a powerful tool that opens a path to greater insight in the role of adiponectin in CHF and may lead to important insights in the further exploration of this intriguing issues.

Conclusion

Skeletal muscle energy deficiency and adiponectin resistance are two important features of CHF. Emerging clinical and experimental evidence indicates that the initiation of adiponectin resistance is multi-factorial, integrating abnormalities emanating from insulin signalling, mitochondrial biogenesis, and ceramide metabolism. Further studies, however, are needed to fully elucidate and understand the mechanism of adiponectin resistance in CHF. Hereof, we believe that the use of primary skeletal muscle cells from CHF patients is valuable to investigate more precisely by how adiponectin resistance in CHF interrelates with muscle wasting. In the end, these new insights may open up new avenues in the treatment of patients with CHF.

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Conflict of interest

There is no actual or potential conflict of interest in relation to this article.

Abbreviations

AMPK Adenosine monophosphate activated protein kinase (PPARα) and
ANP Atrial natriuretic peptide
BMI Body mass index
BNP Brain natriuretic peptide
CAD Coronary artery disease
CaMK Calmodulin-dependent protein kinase
CHF Chronic heart failure
CRP C-reactive protein
CVD Cardiovascular disease
DAG Diaglycerol
EER Estrogen related receptors
FFA Free fatty acids
FGF21 Fibroblast growth factor 21
FOXO Forkhead box protein O
HFpEF Heart failure with preserved ejection fraction
HFrEF Heart failure with reduced ejection fraction
HMW High-molecular weight
HOMA-IR Homeostasis model assessment of insulin resistance
IL Interleukin
LMW Low-molecular weight
LVEF Left ventricular ejection fraction
MAPK Mitogen-activated protein kinase
NT-proBNP N-terminal pro-brain natriuretic peptide
NYHA New York Heart Classification
oxLDL Oxidized low-density lipoprotein
PGC-1α Peroxisome proliferator-activated receptor y coactivator1 α
PI3K Phosphatidylinositol 3-kinase
PKG cGMP-dependent protein kinase
PPARα Peroxisome proliferator-activated receptor α
ROS Reactive oxygen species
SIRT1 Sirtuin 1
TNF-α Tumour necrosis factor alpha

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