Unravelling the adiponectin paradox: novel roles of adiponectin in the regulation of cardiovascular disease

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Abstract

Adipose tissue (AT) has recently been identified as a dynamic endocrine organ secreting a wide range of adipokines. Adiponectin is one such hormone, exerting endocrine and paracrine effects on the cardiovascular system. At a cellular and molecular level, adiponectin has anti-inflammatory, anti-oxidant and anti-apoptotic roles, thereby mitigating key mechanisms underlying cardiovascular disease (CVD) pathogenesis. However, adiponectin expression in the human AT as well as its circulating levels are increased in advanced CVD states, and it is actually considered by many as a "rescue hormone". Due to the complex mechanisms regulating adiponectin's biosynthesis in the human AT, measurement of its levels as a biomarker in CVD is highly controversial, given that it exerts protective effects on the cardiovascular system but at the same time its increased levels flag advanced CVD. In this review article we present the role of adiponectin in CVD pathogenesis and we discuss its role as a clinical biomarker.

Key words: Adiponectin; adipose tissue; redox signalling; NADPH-oxidases; eNOS

Table of Links

TARGETS	LIGANDS
Enzymes[a]	Adiponectin
AMP kinase	BNP
DPP4	Empagliflozin
eNOS	IL6
iNOS	IL10
Nuclear hormone receptors[b]	Infliximab
PPARα	Leptin
PPARγ	Liraglutide
Other protein targets[c]	MCP1
Adipo1 receptor	Metformin
Adipo2 receptor	Neuregulin 4
CRP	Pitavastatin
TNFα	S1P

These Tables of Links list key protein targets and ligands in this article that are hyperlinked* to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in The Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c]Alexander *et al.*, 2015a,b,c).

Abbreviations

AT, adipose tissue; CVD, cardiovascular disease; WAT, white adipose tissue; BAT, brown adipose tissue; TNF, tumour necrosis factor; MCP1, monocyte chemoattractant protein 1; IL6, interleukin 6; CAD, coronary artery disease; Nrg4, neuregulin 4; SNP, single-nucleotide polymorphism; PPAR: peroxisome proliferator-activated receptor; CRP, C reactive protein; IL10, interleukin 10; 4-HNE, 4-hydroxynonenal; Adipo1 receptor, adiponectin receptor 1; Adipo2 receptor, adiponectin receptor 2; AMPK, AMP kinase; FoxO1, forkhead box protein O1; S1P, sphingosine 1-phosphate; NFkB, nuclear factor kappa B; AP1, activator protein 1; VSMC, vascular smooth muscle cell; CAD, coronary artery disease; eNOS, endothelial nitric oxide synthase; PVAT, perivascular adipose tissue; EpAT, epicardial adipose tissue; iNOS, inducible nitric oxide synthase; CHD, coronary heart disease; HF, heart failure; RAAS, renin angiotensin aldosterone system; IRS1, insulin response substrate 1; T2DM, type 2 diabetes mellitus; BNP, brain natriuretic peptide; GLP-1, glucagon-like peptide 1; DPP4, dipeptidyl peptidase 4

The nomenclature of all drugs and molecular targets mentioned is consistent with the Concise Guide to Pharmacology as published by the *British Journal of Pharmacology* (Alexander *et al.*, 2015)

Introduction

It has been increasingly recognised that adipose tissue (AT) is not merely an energy storage depot, but rather a dynamic endocrine organ, secreting numerous adipokines with wide-reaching effects on human homeostasis (Ouchi *et al.*, 2011). These adipokines originate from the adipocytes and/or the stromal cells within the AT, including macrophages, T-cells, fibroblasts and other cell populations, and they exert endocrine or paracrine effects on the cardiovascular system, playing a critical role in cardiovascular disease (CVD) pathogenesis. The adipocytokines may roughly be classified as pro- or anti- inflammatory, depending on their net effect (Table 1). The "secretome" of AT appears to have large regional variability, as it is highly dependent on the anatomical depot, with visceral being the type of AT with net pro-inflammatory secretome profile and the gluteal being the depot with net anti-inflammatory (or cardioprotective) secretome (Lee *et al.*, 2013).

Obesity is characterised by an increase in predominantly visceral AT mass, which in turn is associated with CVD and a range of metabolic conditions (Ouchi *et al.*, 2011). The "apple shape" obesity (male-type accumulation of abdominal/visceral fat) is associated with increased cardiovascular risk while the "pear shape obesity" (female-type accumulation of gluteal fat) is considered to be neutral or even protective for the cardiovascular system. Importantly, the circulating levels of many adipokines are reportedly dysregulated in abdominal obesity which is typically linked with insulin resistance, shifting towards a pro-inflammatory phenotype, while in gluteal obesity the adipokine balance is shifted towards an inti-inflammatory profile (Berg *et al.*, 2005).

Adiponectin is secreted almost exclusively from AT (Scherer *et al.*, 1995), and its circulating levels are reduced in obesity and insulin resistance (Kadowaki *et al.*, 2006). It exerts a wide range of beneficial effects on the cardiovascular system, having anti-inflammatory, anti-apoptotic,

antioxidant and vasorelaxant properties (Kadowaki *et al.*, 2005). Reduced adiponectin levels may contribute to the increased risk for cardiovascular complications in obesity, insulin resistance and diabetes (Hung *et al.*, 2008). However, adiponectin levels are markedly increased in advanced CVD states such as heart failure (HF) (Berg *et al.*, 2005). Therefore, the clinical significance of adiponectin as a biomarker in CVD remains controversial. Nonetheless, recent studies have further elucidated the direct and indirect roles of adiponectin in CVD, providing novel perspectives for future clinical implications.

In this review, we discuss the cardiovascular effects of adiponectin, specifically its anti-atherogenic actions and its role in the dynamic cross-talk between AT and the cardiovascular system. We next examine the interpretation of circulating adiponectin levels as a biomarker of CVD progression. Finally, we evaluate its potential role as a therapeutic target in CVD pathogenesis.

Brief overview of adipose tissue structure and biology

AT contains adipocytes as well as a variety of non-adipose cells (including endothelial cells, pericytes and immune cells), which collectively constitute its vascular-stromal fraction (Coelho *et al.*, 2013). Due to their ability to store or hydrolyse lipids depending on whole-body energy requirements, adipocytes comprise important energy-storing sources able to influence systemic energy expenditure, while they can also secrete a wide range of hormones called adipokines, with important local and systemic effects on human homeostasis (Stern *et al.*, 2015). Stromal cells, on the other hand, have mainly supportive functions, although immune stromal cells such as macrophages and lymphocytes (mainly T cells) orchestrate local inflammatory responses and

secrete cytokines with potential systemic effects under a variety of pathophysiological stimuli (Stern *et al.*, 2015).

AT can broadly be divided in two types: white AT (WAT) and brown AT (BAT) (Coelho *et al.*, 2013). WAT comprises the vast majority of AT mass in adults and is responsible for lipid storage, energy expenditure regulation and secretion of adipocytokines (Coelho *et al.*, 2013). Compared to WAT, BAT contains much smaller lipid droplets and a larger amount of blood vessels, hence its darker, macroscopically brown appearance (Kiess *et al.*, 2008). From a functional point of view, BAT is also able to store lipids, but it more frequently oxidises such lipids within the adipocytes for heat production rather than supplying lipids for utilisation by other tissues (Coelho *et al.*, 2013). Its rich vasculature facilitates its thermo-regulatory properties. It is unclear if BAT secretes adipocytokines of biological importance, although recent evidence suggests that it may indeed secrete circulating factors with biological implications such as neuregulin 4 (Nrg4) (Wang *et al.*, 2014).

WAT can be broadly divided into subcutaneous and visceral, based on anatomical criteria. These AT depots also have distinct biological characteristics including adipocyte size, vascularity, inflammatory infiltration, receptor expression and adipocytokine secretion profile (Ibrahim, 2010). Indeed, visceral AT is expanded in obesity (Lee *et al.*, 2013), and is associated with greater infiltration by inflammatory cells (Alexopoulos *et al.*, 2014), greater intracellular lipid accumulation and lipid peroxidation (Ibrahim, 2010), while it drives systemic insulin resistance and has a mainly pro-atherogenic secretome (i.e., less adiponectin, more tumour necrosis factor-α (TNFα)) (Lee *et al.*, 2013) compared to subcutaneous AT. Increased visceral AT volume has also been consistently associated with increased cardiovascular risk (Kuk *et al.*, 2006), suggesting that dysregulation of this particular depot is significant for cardiovascular disease pathogenesis.

Epicardial AT comprises a small, visceral AT depot that produces various secreted molecules, while being also able to "communicate" with the heart and the coronary arteries due to its unique anatomical proximity with these organs (Iacobellis, 2015). EpAT is partly regarded as perivascular AT (PVAT), as it surrounds the coronary arteries, while it may also interact with the underlying heart muscle in a paracrine way. EpAT is able to secrete both pro-inflammatory (e.g. monocyte chemoattractant protein (MCP1), TNFα etc.) and anti-inflammatory (e.g. adiponectin) adipocytokines, which are able to enter the systemic as well as the coronary circulation (Iacobellis, 2015). EpAT surrounding the coronary arteries may promote the onset of coronary artery disease (CAD), as confirmed by its increased M1:M2 infiltrating macrophage ratio and elevated secretion of detrimental adipocytokines such as resistin, TNFα and Interleukin 6 (IL6) (Hirata *et al.*, 2011). EpAT may also propagate myocardial disease such as atrial fibrillation via the secretion of adipofibrokines and the subsequent establishment of atrial fibrosis (Venteclef *et al.*, 2015). Importantly, epicardial AT may act as a recipient of local signals originating from its underlying structures such as the myocardium, and alter its secretome accordingly to exert paracrine effects (Antonopoulos *et al.*, 2016). At a clinical level, EpAT thickness and volume are increased in obesity and diabetes mellitus (Iacobellis, 2015), and this increase has been associated with CVD, cardiac hypertrophy and extent of CAD as evaluated by calcium score (Djaberi *et al.*, 2008; Hirata *et al.*, 2015).

Although it is now widely accepted that AT secretes a variety of adipocytokines, the roles of the individual components of its secretome remain unclear. Leptin, for example, is an adipokine with controversial roles in CVD, but the majority of studies agree that hyperleptinaemia and leptin resistance are possibly associated with insulin resistance and CVD (Patel *et al.*, 2008). That is obviously not equivalent to leptin being a pro-atherogenic or otherwise detrimental hormone for

the cardiovascular system per se. Data on this topic once again highlight our minimal understanding of the direct roles of leptin under physiological and pathophysiological conditions as opposed to the sum of cofounding factors that collectively determine circulating leptin levels and leptin resistance. Interleukin 10 (IL10), on the other hand, while being an anti-inflammatory cytokine (Pinderski *et al.*, 2002), has been positively associated with CVD (Welsh *et al.*, 2011). No mechanistic interpretation for this finding exists, while it is plausible that this positive association may comprise a compensatory mechanism. Many more studies are required to unravel the specific roles of the individual components of AT secretome.

Adiponectin: structure and biosynthesis

Adiponectin, produced almost exclusively by adipocytes, is the most abundant adipokine (Arita *et al.*, 1999). Circulating adiponectin forms three major oligomeric multimers with poorly known biological roles: a low-molecular weight (LMW) trimer, a middle-molecular weight (MMW) hexamer, and a high-molecular weight (HMW) 12- to 18-multimer (Pajvani *et al.*, 2003). WAT seems to be the dominant source of circulating adiponectin levels (Zhang *et al.*, 2002). Although subcutaneous and visceral WAT are both able to produce adiponectin to a significant extent, the expression of adiponectin in visceral AT may be of greater clinical significance, since it displays striking variation under certain pathophysiological states such as obesity (Wajchenberg, 2000). Furthermore, in small AT depots proximal to the cardiovascular system (i.e., epicardial AT, PVAT) the expression of adiponectin may interestingly be regulated mainly by local stimuli (Antonopoulos *et al.*, 2016; Margaritis *et al.*, 2013).

Adiponectin's coding gene, *ADIPOQ*, has been mapped to chromosome 3q27 (Saito *et al.*, 1999), and at least 10 single-nucleotide polymorphisms (SNPs) have been described in it. Such SNPs may

be non-functional, or they can influence the basal adiponectin mRNA expression or protein structure, interfering with its signalling. At present, the functional implications of SNPs in the *ADIPOQ* locus are not well understood. Two common *ADIPOQ* SNPs (+45T>G and +276G>T, located in exon 2 and intro, 2 respectively) have been proposed as regulators of adiponectin circulating levels (Melistas *et al.*, 2009; Shin *et al.*, 2006). These SNPs are in linkage disequilibrium with polymorphisms in the 3' untranslated region of the adiponectin mRNA and they may affect mRNA stability and slicing (Mackevics *et al.*, 2006). Importantly, the +45T>G SNP is associated with reduced circulating adiponectin levels and elevated cardiovascular risk in healthy individuals (Antonopoulos *et al.*, 2013). Moreover, a genome-wide association study that included 382 early-onset hypertensive subjects has revealed a quantitative trait locus of adiponectin that affects adiponectin levels and cardiometabolic risk (Chung *et al.*, 2011). A variety of novel adiponectin SNPs have been confirmed to increase risk for diabetes and cardiovascular disease in recent meta-analyses (Dastani *et al.*, 2012).

The biosynthesis of adiponectin is regulated by various factors. In particular, peroxisome proliferator-activated receptor-γ (PPARγ), a master regulator of adipocyte differentiation as well as an insulin-sensitising mediator, has been shown to upregulate adiponectin expression and secretion in humans (Margaritis *et al.*, 2013). Additionally, pro-inflammatory molecules such as TNFα (Wang *et al.*, 2005a) and C reactive protein (CRP) (Yuan *et al.*, 2012) downregulate the expression of adiponectin, while *in vivo* inflammation has been confirmed as a negative regulator of adiponectin expression and secretion in humans (Antonopoulos *et al.*, 2014). Importantly, *ADIPOQ* gene expression is triggered by brain natriuretic peptide (BNP) post-receptor signalling in adipocytes, as demonstrated in cell culture (Tsukamoto *et al.*, 2009) and human AT explants (Antonopoulos *et al.*, 2014). Oxidative stress is also a regulator of adiponectin biosynthesis, albeit

in more complex ways than previously believed, depending on the nature of the oxidant stimulus. Exposure of adipocytes to reactive oxygen species such as hydrogen peroxide (H_2O_2) results in reduction of *ADIPOQ* expression (Kamigaki *et al.*, 2006), an effect recently reproduced in human epicardial AT explants (Antonopoulos *et al.*, 2016), and is independent of PPARγ signalling. On the contrary, more stable end-oxidation products such 4-hydroxynonenal (4-HNE) are able to upregulate the expression of *ADIPOQ* in human AT in a PPARγ-dependent manner (Antonopoulos *et al.*, 2015; Antonopoulos *et al.*, 2016).

Interestingly, insulin also decreases circulating adiponectin levels, suggesting that hyperinsulinaemia observed in cases of insulin resistance could reduce plasma adiponectin (Motoshima *et al.*, 2002). On the other hand, the co-existence of impaired insulin post-receptor signalling in these cases makes extrapolation of conclusions difficult regarding the association of insulin with adiponectin (Yadav *et al.*, 2013). On this basis, it may be presumed that adiponectin reflects insulin resistance status rather than directly regulating insulin signalling per se. However, based on the large number of studies in both cell and animall models that have replicated the direct insulin-sensitizing effects of adiponectin (Ruan *et al.*, 2016), it seems that adiponectin has insulin-sensitizing properties.

At a clinical level, adiponectin levels are lower in males and in subjects with obesity as well as insulin resistance and type 2 diabetes (Kadowaki *et al.*, 2006). However, the whole spectrum of the mechanisms controlling adiponectin biosynthesis *in vivo* remains unclear.

Established actions of adiponectin

Post-receptor signalling of adiponectin

Adiponectin exerts its pleiotropic biological effects through its integral membrane receptors, Adipo1 and Adipo2 receptors, as well as possibly via T cadherin. AMP kinase (AMPK) and PPAR signalling are two major downstream axes of adiponectin signalling (Yamauchi *et al.*, 2014). Adipo1 and Adipo2 receptors are transmembrane receptors that are expressed to variable extent in most cell types. Adiponectin acting via Adipo1 receptor promotes intracellular calcium influx to activate Ca^{2+}/calmodulin-dependent kinase kinase and AMPK, which exerts insulin-sensitizing properties while regulating fatty acid oxidation; adiponectin acting via Adipo2 receptor results in an increased production of PPARα ligands (Yamauchi *et al.*, 2014). Insulin signalling downregulates the expression of Adipo1 and Adipo2 receptors, whereas insulin resistance is also associated with reduced expression of these receptors, potentially due to the accompanying hyperinsulinaemia; the underlying mechanism appears to be mediated by FoxO1(Tsuchida *et al.*, 2004).

T cadherin is a surface molecule reportedly binding adiponectin. Since no functional intracellular domain has been described for T cadherin, it is unclear whether it can actually behave as an adiponectin receptor or it merely acts as an adiponectin-binding protein, similarly to a decoy receptor (Yamauchi *et al.*, 2014). On the other hand, T cadherin is expressed in cardiomyocytes, vascular smooth muscle cells (VSMCs) and endothelial cells, regulating proliferation, migration and survival (Resink *et al.*, 2009). Interestingly, the reportedly cardioprotective effects of adiponectin in mice require the presence of T cadherin (Denzel *et al.*, 2010). Consequently, T cadherin may be a significant functional adiponectin receptor, although currently being understudied.

Relatively recently, adiponectin signalling has been associated with increased ceramidase activity, thereby degrading ceramides and producing sphingosine 1-phosphate (S1P) (Holland *et al.*, 2011),

a function that is adiponectin receptor-mediated, as highlighted in adiponectin receptor-deficient mouse models, but AMPK-independent. Stimulation of ceramidase activity has multiple beneficial metabolic and insulin-sensitising effects, whereas S1P itself is a diverse second messenger with reportedly anti-inflammatory and anti-apoptotic functions (Maceyka *et al.*, 2012). The interchangeable balance in the cellular levels of ceramides and S1P has been called the "sphingolipid rheostat", and has been proposed as a regulator of cell fate, with ceramides inducing apoptosis and S1P favouring proliferation (Van Brocklyn *et al.*, 2012). The physiological importance of the sphingosine rheostat has recently been recognised in the prevention of diabetes and diseases involving biological senescence in general (Haass *et al.*, 2015), and the potential ability of adiponectin to regulate this parameter may be of crucial importance in the regulation of cardiovascular responses to a variety of stressful and pathogenic stimuli (Holland *et al.*, 2011). However, more mechanistic studies are required to address the ability of adiponectin to regulate the sphingolipid rheostat as well as the biological significance of this hypothetical mechanism. The ceramidase- and/or S1P-mediated effects of adiponectin may constitute a step forward to understand the pleiotropic effects of adiponectin, and introduce an exciting new perspective in the study of adiponectin signalling in general. However, their significance in CVD is still unknown.

Roles in atherosclerosis

Adiponectin is a key modulator of vascular homeostasis. Indeed, it exerts a plethora of anti-inflammatory properties of potential significance for CVD, including reduced recruitment of lymphocytes in atherosclerotic lesions, downregulation of CRP and inhibition of TNFα- and nuclear factor kappa B (NFκB)-mediated pr-inflammatory signalling (Ebrahimi-Mamaeghani *et al.*, 2015). Adiponectin also reduces the expression of adhesion molecules in both endothelial cells

and monocytes (Ebrahimi-Mamaeghani *et al.*, 2015) while inhibiting macrophage lipid accumulation and foam cell formation (Ouchi *et al.*, 2001). Moreover, adiponectin reduces macrophage production of pro-inflammatory cytokines while increasing anti-inflammatory cytokine production (Kumada *et al.*, 2004; Yamaguchi *et al.*, 2005); accordingly, macrophages from adiponectin-deficient mice display increased expression of inflammatory M1-type markers and reduced anti-inflammatory M2-type markers (Ohashi *et al.*, 2010).

Adiponectin also has potent anti-oxidant effects which inhibit pre-inflammatory redox-sensitive signalling such as NFkB and activator protein 1 (AP1) (Ebrahimi-Mamaeghani *et al.*, 2015) while improving endothelial function. Indeed, adiponectin increases phosphorylation of Akt and endothelial nitric oxide synthase (eNOS) at Ser1177 (Ouchi *et al.*, 2000), whereas it also decreases the Rac1-mediated activation of NADPH-oxidases, key pre-oxidant enzymes that are actively involved in atherogenesis (Antonopoulos *et al.*, 2015). Adiponectin also inhibits endothelial cell apoptosis via AMPK-mediated downregulation of caspase-3 (Ebrahimi-Mamaeghani *et al.*, 2015), while it reduces the proliferation of VSMCs, an important mechanism of atherosclerotic lesion progression (Wang *et al.*, 2005b); consistently, adiponectin was shown to impair angiotensin II-mediated VSMC remodelling via nitric oxide and the RhoA pathway (Nour-Eldine *et al.*, 2016). Finally, adiponectin has antithrombotic properties (Kato *et al.*, 2006), potentially via its ability to increase NO production, that may be able to reduce the incidence of acute atherosclerotic complications.

The aforementioned actions of adiponectin may be relevant *in vivo*. Many animal studies have confirmed the ability of adiponectin to inhibit atherogenesis *in vivo* (Li *et al.*, 2007). On the contrary, other studies have failed to document an association between adiponectin levels and atherosclerotic plaque development in mice. Human studies, on the other hand, have identified a

close association between low adiponectin and early atherosclerosis evaluated by carotid intima-media thickness (Iglseder *et al.*, 2005), but this association is not consistent across all studies (Matsuda *et al.*, 2004). A recent meta-analysis inconclusively suggests that there may be an inverse association between circulating adiponectin levels and carotid intima-media thickness (Gasbarrino *et al.*, 2016). Further studies have described an association between adiponectin levels and CAD (Schautz *et al.*, 2012), whereas adiponectin expression is decreased in the epicardial AT of patients with CAD (Iacobellis *et al.*, 2005). However, the association between low adiponectin levels and the development of CAD has failed to be shown in several prospective studies (Lawlor *et al.*, 2005; Lindsay *et al.*, 2005). Consequently, the clinical implications of adiponectin are more obscure in humans as explained in following paragraphs.

Adiponectin and the cross-talk between AT and vascular wall

Vascular oxidative stress is a key feature of atherogenesis (Antoniades *et al.*, 2009), and studies suggest that uncoupled eNOS is a salient mechanism linking impaired endothelial function with elevated vascular superoxide anion (O_2^-) production in humans (Guzik *et al.*, 2002). Atherosclerosis is associated with increased vascular oxidative stress and eNOS uncoupling due to oxidation of the critical eNOS co-factor tetrahydrobiopterin (BH_4) (Antoniades *et al.*, 2007). Data from endothelial cell cultures suggests that adiponectin stimulates NO production (Hattori *et al.*, 2003) in part, through eNOS activation via a PI3 kinase/Akt-mediated phosphorylation (Cao *et al.*, 2009). As such, under conditions of increased vascular oxidative stress, this action could actually impair endothelial function as activation of uncoupled eNOS would result into more O_2^-/peroxynitrite (ONOO-) generation.

In our recent studies using human PVAT and human vessels, we observed that adiponectin, released from PVAT and diffused to the wall of the adjacent vessel or reaching the vascular wall through the circulation, has the ability to improve eNOS coupling by restoring endothelial BH4 bioavailability, while at the same time activating eNOS via Akt-mediated phosphorylation at its activatory site Ser1177 (Margaritis *et al.*, 2013). This results into improved redox balance in the vascular endothelium with lower O_2^- and higher NO bioavailability. In addition to these effects, we also observed that adiponectin supresses O_2^- generation in human vessels by reducing NADPH-oxidase activity via Akt-mediated suppression of Rac1 activation and p47phox phosphorylation, thus resulting into reduced activity of NOX2 and NOX1 isoforms of NADPH-oxidases (Antonopoulos *et al.*, 2015).

In an attempt to explain the paradoxically positive correlation between *ADIPOQ* gene expression in PVAT and O_2^- generation in the adjacent human vessel, we discovered that under conditions of increased oxidative stress, the vascular wall releases oxidation products (i.e. 4-HNE) which are then diffused to the PVAT activating local PPARγ signalling that results into increased *ADIPOQ* gene expression and adiponectin release. This feedback loop comprises a paracrine defence mechanism of the vascular wall to oxidative damage, hosted within its PVAT. The concept of an inside-to-outside signal from the vessel to its PVAT introduced the hypothesis that the communication between the human PVAT and the vascular tissue is bi-directional, in a way that PVAT behaves as a "sensor" of vascular oxidation replying back by secreting products regulated by PPARγ signalling in the adipocytes and possibly other cell types within this AT depot. The concept of this bi-directional communication between PVAT and the vascular wall is described in Figure 1A.

Adiponectin and the cross-talk between AT and the myocardium

Adiponectin may have cardioprotective effects, suppressing pathological cardiac remodelling and reducing myocardial oxidative stress in experimental models (Essick *et al.*, 2011). Adiponectin-deficient mice have enhanced concentric cardiac hypertrophy and increased mortality in response to pressure overload and angiotensin II infusion compared to wild-type mice, which were reversed following adiponectin supplementation (Shibata *et al.*, 2004). Although clinical studies are limited, the beneficial role of adiponectin in cardiac hypertrophy may translate to humans. In a study of 933 middle-aged subjects, low plasma adiponectin levels were independently associated with left ventricular mass index, a marker of left ventricular hypertrophy (Paakko *et al.*, 2010).

In addition to mitigating pathological cardiac remodelling, adiponectin reduces myocardial oxidative stress, protecting against ischaemia-reperfusion injury. Adiponectin accumulates in myocardial tissue subjected to ischaemia-reperfusion injury (Shibata *et al.*, 2007), where it reduces oxidative stress through downregulation of inducible nitric oxide synthase (iNOS) and the gp91[phox] subunit of NOX2 isoform of NADPH-oxidases (Tao *et al.*, 2007). Work from our group has recently revealed novel roles for adiponectin in the cross-talk between epicardial AT (EpAT) and human myocardial redox state (Antonopoulos *et al.*, 2016). In an *ex vivo* model of human myocardium, adiponectin was found to reduce the activity of myocardial NADPH-oxidases by inhibiting the translocation of Rac1 and p47[phox], important subunits of the functional enzyme, to the cell membrane in an AMPK-mediated manner.

Obesity and insulin resistance are characterised by impaired fatty acid oxidation in the heart in response to increased fatty acid bioavailability, thus leading to lipotoxicity (Young *et al.*, 2002), whereas adiponectin signalling can ameliorate these effects (Turer *et al.*, 2012). Furthermore, diabetes-associated hypoadiponectinaemia is associated with impaired mitochondrial biogenesis,

making the diabetic heart more sensitive to myocardial dysfunction and acute myocardial infarction injury (Yan *et al.*, 2013). Additionally, adiponectin is able to ameliorate myocardial dysfunction and increase cardiac contractility in obese, diabetic mice, potentially via a mechanism involving c-jun and IRS1 (Dong *et al.*, 2009).

Work from our group has identified a paradoxical positive association between myocardial oxidative stress and *ADIPOQ* expression in EpAT (Antonopoulos *et al.*, 2016). Investigating this finding, we showed that end oxidation products such as 4-HNE originating from myocardial tissue upregulate *ADIPOQ* expression in EpAT in a PPARγ-dependent manner. This constitutes, again, a bi-directional regulatory defence loop similar to the cross-talk between PVAT and vessel wall, whereby EpAT acts as a dynamic recipient of oxidative signals from the adjacent myocardium and is able to respond appropriately by increasing local adiponectin production as a rescue mechanism against oxidative stress (figure 1B). The study population of this particular work was quite homogeneous in terms of cardiac function and had a narrow age window, making the interaction of these parameters with the described findings highly unlikely.

Adiponectin as a clinical biomarker in cardiovascular disease

A range of clinical studies have produced conflicting results as to the utility of adiponectin as a biomarker of cardiovascular risk and CAD progression. The Rancho Bernardo study (Laughlin *et al.*, 2007) was the first long-term study to corroborate the usefulness of adiponectin as a biomarker in coronary heart disease (CHD), demonstrating that higher adiponectin levels were associated with a favourable cardiovascular risk profile in both sexes from the same population. However, other prospective studies have failed to show an association between adiponectin and the incidence of CAD (Lawlor *et al.*, 2005). Contrary to expectation, these studies associated higher circulating

adiponectin levels with increased CVD risk and total mortality (Sook Lee *et al.*, 2013), and with higher risk of stroke (Hao *et al.*, 2013). In contrast, adiponectin levels comprise an independent inverse predictor of cardiovascular outcome in patients with CAD (Nakamura *et al.*, 2004), recent myocardial infarction (Kojima *et al.*, 2007), or with T2DM (Schulze *et al.*, 2005). Age also seems to be an important factor influencing circulating adiponectin. Indeed, plasma adiponectin levels are inversely, albeit weakly, associated with CAD risk in relatively young patients (Sattar *et al.*, 2006), while also being positively associated with CVD mortality in elderly patients even without pre-existing CVD (Wannamethee *et al.*, 2007). Although the aforementioned studies may differ regarding their population characteristics, warranting further investigation, they certainly indicate that circulating adiponectin levels are determined by a multitude of factors; even more so, they potentially reflect a combination of both detrimental and beneficial mechanisms, as suggested by recent meta-analyses with null overall findings regarding adiponectin's predictive value (Hao *et al.*, 2013; Sook Lee *et al.*, 2013).

The value of adiponectin as a clinical biomarker in HF is also controversial. Whilst both the anti-inflammatory actions of adiponectin and the role of pro-inflammatory cytokines in HF are well-established, there is an apparently paradoxical increase in adiponectin levels in chronic HF (Behre, 2007), which may be predictive of morbidity and mortality (McEntegart *et al.*, 2007). Several theories have been proposed to explain this apparent paradox. Adiponectin levels may be raised because of the hyper-catabolic state in severe HF (Behre, 2007). Indeed, adiponectin levels are apparently raised in HF patients only in the presence of cachexia (McEntegart *et al.*, 2007), and it has been suggested that elevated plasma adiponectin reflects disease mechanisms leading to hypercatabolic states, although it is unclear if adiponectin contributes to such states or whether its increase is part of a compensatory mechanism.

Recently a cross-sectional study of 575 patients with ischaemic heart disease revealed that plasma BNP, not low-grade inflammation, was the main driver of adiponectin in CHD (Antonopoulos *et al.*, 2014), a finding that is consistent with the ability of BNP to stimulate *ADIPOQ* expression in adipocytes (Tsukamoto *et al.*, 2009). In contrast, low-grade inflammation reduced adiponectin levels in populations without significant CVD and low plasma BNP, consistent with low adiponectin levels being predictive of the onset of CVD. Other studies have also demonstrated that plasma levels of BNP may be able to explain the controversy between circulating adiponectin levels and prediction of CVD (Wannamethee *et al.*, 2011), and this finding may explain the age-related discrepancies amongst studies previously mentioned. Therefore plasma adiponectin levels are highly influenced by circulating BNP levels and flag severe CHD. However, patients with genetically-determined reduction of adiponectin levels have higher myocardial oxidative stress as they abolish the antioxidant benefits of adiponectin, therefore the interpretation of plasma adiponectin in clinical practice is challenging, and its value as a biomarker is not clear.

In conclusion, the usefulness of plasma adiponectin as a biomarker in CVD is controversial. Discrepancies in the literature may mirror the disparity in disease stage and differences in the populations included in the studies. Data so far appear to show that decreased plasma adiponectin in healthy individuals is predictive of the onset of atherosclerosis. However, this association is weaker at later stages of atheromatous disease, while heart and renal failure (hyper-catabolic states) are associated with increased adiponectin levels. Consequently, interpretation of plasma adiponectin concentration must take into account the underlying CVD state, background inflammation, and BNP levels.

Adiponectin as a therapeutic target

Given its established antiatherogenic and insulin-sensitising actions, adiponectin comprises an attractive therapeutic target. However, the inherent difficulties in the production of recombinant adiponectin, the high plasma concentration of the endogenous adipokine, and its predicted brief circulating half-life *in vivo*, introduce technical difficulties to direct regulation of its bioavailability (Halberg *et al.*, 2009). In fact, animal studies have revealed recombinant adiponectin to be unable to improve glycaemic control (Tullin *et al.*, 2012), despite the protein being correctly folded and biologically active. This may be due to species-specific parameters or technical problems regarding the pharmacokinetic & pharmacodynamic profile of the protein. Further studies are required to elucidate the therapeutic potential of recombinant adiponectin.

Several drug classes used in the treatment of T2DM and in CVD have been found to increase circulating adiponectin levels. Many of these agents presumably act via PPARγ, which is known to upregulate HMW adiponectin (Yamauchi *et al.*, 2008). Thiazolidinediones (TZDs), PPARγ agonists used widely in the treatment of T2DM, increase plasma adiponectin levels in obese and diabetic patients, and in healthy controls (Yu *et al.*, 2002). Given that TZDs have a range of undesirable biological effects apart from increasing adiponectin levels, a non-TZD selective PPARγ agonist, such as INT131, may be advantageous (Higgins *et al.*, 2008). Interestingly, targeting the renin-angiotensin-aldosterone system (RAAS) also increases plasma adiponectin levels (Clasen *et al.*, 2005), possibly secondarily to enhancing insulin sensitivity or increasing adipogenesis (Furuhashi *et al.*, 2003). Some Angiotensin Receptor blockers (ARBs) have PPAR ligand activity; consistently, ARBs induce adiponectin gene transcription in humans (Watanabe *et al.*, 2006).

Incretins such as glucagon-like peptide 1 (GLP-1) and GLP-1 analogues (e.g. liraglutide) as well as dipeptidyl dipeptidase 4 (DPP4) inhibitors, which act by increasing the bioavailability of

incretins, have variably displayed the ability to elevate circulating adiponectin levels (Kim Chung le *et al.*, 2009; Li *et al.*, 2011; Sahebkar *et al.*, 2016). Similarly, empagliflozin, a sodium-glucose cotransporter 2 inhibitor used in the treatment of T2DM, reportedly increased adiponectin levels (Tahara *et al.*, 2016). It is unclear whether such effects can be attributed to the improvement of systemic insulin sensitivity or are due to unidentified direct effects on AT. Infliximab, an anti-TNFα monoclonal antibody clinically used in rheumatoid arthritis, is able to upregulate adiponectin (Nishida *et al.*, 2008), but further studies are needed to address the extent of adiponectin regulation by anti-inflammatory drugs. Interestingly, some statins such as pitavastatin are able to increase circulating adiponectin levels, and this may be a mechanism involved in the pleiotropic effects of these drugs, despite being conflicting with most statins' established ability to impair insulin sensitivity (Arnaboldi *et al.*, 2015). Finally, lcz696, a combined inhibitor of angiotensin receptor and neprilysin, may be able to increase adiponectin levels due to its ability to improve the bioavailability of BNP (Gradman, 2015), although this potential effect needs to be validated in large-scale clinical studies.

A variety of pharmacological agents frequently used in clinical practise directly or indirectly modulate the activation of AMPK, an important downstream mediator of adiponectin signalling. Indeed, both TZDs and metformin are able to activate AMPK (Fryer *et al.*, 2002), and this provides a potential cross-talk with adiponectin signalling. In fact, although adiponectin stimulates AMPK activation, pharmacological induction of AMPK activity by metformin has been shown to downregulate adiponectin expression and release by 3T3-L1 adipocytes, in contrast with TZDs (Huypens *et al.*, 2005). Concordantly, troglitazone treatment has been associated with almost three-fold increase of plasma adiponectin, whereas metformin has been shown not to affect circulating adiponectin levels in humans *in vivo* (Phillips *et al.*, 2003). This suggests that the effect

of TZDs such as troglitazone on adiponectin is mainly determined by their potent stimulation of PPARγ activity; on the contrary, metformin, which predominantly acts via AMPK activation, may be able to stimulate downstream AMPK-dependent components of adiponectin signalling, but it is not able to induce adiponectin production from AT. Furthermore, some studies have demonstrated that pioglitazone and metformin treatment may be able to upregulate the expression of AdipoR1 and AdipoR2 (Coletta *et al.*, 2009; Metais *et al.*, 2008), implying that these drugs may also improve adiponectin downstream signalling. Further research on the cross-talk between AMPK and adiponectin signalling is required to allow the development of new pharmacological interventions targeting the AMPK/adiponectin axis.

Targeting the adiponectin receptors would be an alternative way of regulating the actions of adiponectin. One strategy to achieve that may be to target PPARγ, which at least in mice, upregulates Adipo1 and Adipo2 receptors (Yamauchi *et al.*, 2013). A recent study identified an orally active small molecule named AdipoRon, which binds to Adipo1 and Adipo2 receptors, activating PPARγ and AMPK pathways (Okada-Iwabu *et al.*, 2013). In hope of optimizing the agonist, the group went on to establish the crystal structure of the Adiponectin receptors using X-ray crystallography (Okada-Iwabu *et al.*, 2015). Additional validation of such novel AdipoR-targeting drugs is now required at the level of animal and human models.

As mentioned above, plasma adiponectin is already endogenously increased in advanced CVD, and this can be regarded as a systemic compensatory response to the underlying disease state. As such, it may be questioned whether and to what extent targeting of adiponectin can be of further benefit in patients with advanced disease. Indeed, it is unknown whether elevated adiponectin is able to counterbalance the underlying disease mechanisms that often drive hyperadiponectinaemia. It is also uncertain whether pharmacological targeting of systemic adiponectin levels in advanced

CVD can improve cardiovascular disease progression. Furthermore, it is unknown if adiponectin signalling remains intact in cases of severe CVD, or if adiponectin resistance, namely the reduced responsiveness of downstream signalling molecules to adiponectin, is also present. If such adiponectin resistance is documented at a cardiac or vascular level, then strategies to "sensitize" the target tissues to adiponectin may prove to be a rational therapeutic strategy against cardiovascular disease (Van Berendoncks *et al.*, 2011). More studies are needed to address the therapeutic potential of adiponectin in patients with advanced CVD.

Conclusions

Adiponectin is a key adipokine with multitudinous biological effects. At the cellular level, adiponectin has anti-inflammatory and anti-apoptotic effects, reducing cardiovascular oxidative stress and improving eNOS coupling. Despite these apparently beneficial effects, high circulating adiponectin levels do not appear to necessarily translate into better clinical outcomes in patients with CVD. Indeed, hyperadiponectinemia is paradoxically a negative prognostic indicator in patients with advanced HF, though this likely reflects the underlying disease state, BNP levels and systemic inflammation. These interactions compromise the value of adiponectin as a reliable biomarker in coronary atherosclerosis. Clearly, the role of adiponectin in cardiovascular homeostasis is incompletely understood and further research is warranted. Nonetheless, the anti-inflammatory and cardioprotective effects of the adipokine still make it a promising therapeutic target.

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Table 1: Established adipocytokines and their biological roles

Adipokine	Biological roles				
	IR	*Inflammation*	*Oxidative stress*	*Atherogenesis*	*CVD risk*
Adiponectin	Insulin-sensitizing (Ruan *et al.*, 2016)	Anti-inflammatory (Turer *et al.*, 2012)	Anti-oxidant (Antonopoulos *et al.*, 2015; Margaritis *et al.*, 2013)	Anti-atherogenic (Okamoto *et al.*, 2002)	Variable context-dependent relationship (Ebrahimi-Mamaeghani *et al.*, 2015; Sook Lee *et al.*, 2013)
Leptin	Possibly direct insulin-sensitizing effect (Yadav *et al.*, 2013) Hyperleptinaemia is associated with IR (Patel *et al.*, 2008)	modulates T-cell mediated & innate immunity (Scotece *et al.*, 2014)	Presumably pro-oxidant roles (Jay *et al.*, 2006)	Controversial (Chiba *et al.*, 2008; Hoffmann *et al.*, 2016)	Positive association with CVD risk (Kajikawa *et al.*, 2011)
Resistin	Induces IR (Steppan *et al.*, 2001)	Pro-inflammatory (Bokarewa *et al.*, 2005)	Pro-oxidant (Chen *et al.*, 2010)	Pro-atherogenic (Jung *et al.*, 2006)	Positive association with CVD risk (Fontana *et al.*, 2015)
TNFα	Induces IR (Johnson *et al.*, 2013)	Pro-inflammatory (Zelova *et al.*, 2013)	Pro-oxidant (Mittal *et al.*, 2014)	Pro-atherogenic (Battes *et al.*, 2014)	Positive association with CVD risk (Ridker *et al.*, 2000)
IL6	Induces IR (Rotter *et al.*, 2003)	Pro-inflammatory (Libby *et al.*, 2002)	Pro-oxidant (Mittal *et al.*, 2014)	Pro-atherogenic (Huber *et al.*, 1999)	Positive association with CVD risk (Bermudez *et al.*, 2002)
IL10	Ameliorates IR (Hong *et al.*, 2009)	Immuno-regulatory & anti-inflammatory (Ouyang *et al.*, 2011)	Potentially anti-oxidant (Haddad *et al.*, 2002)	Anti-atherogenic (Han *et al.*, 2015)	Positive association with CVD risk, potentially as a compensatory mechanism (Welsh *et al.*, 2011)
Omentin	Insulin-sensitizing (Lis *et al.*, 2015)	Anti-inflammatory (Yamawaki *et al.*, 2011)	Anti-oxidant (Kazama *et al.*, 2012)	Anti-atherogenic (Hiramatsu-Ito *et al.*, 2016)	Inverse association with CVD risk (Shibata *et al.*, 2011)
Apelin	Potentially insulin-sensitizing (Yue *et al.*, 2010)	Controversial (Hashimoto *et al.*, 2007; Leeper *et al.*, 2009)	Controversial (Hashimoto *et al.*, 2007; Than *et al.*, 2014)	Controversial (Chun *et al.*, 2008; Hashimoto *et al.*, 2007)	Inverse association in small-scale clinical studies (Kadoglou *et al.*, 2010; Zhou *et al.*, 2014)

IR: Insulin resistance; TNFα: Tumour necrosis factor alpha; IL6: Interleukin 6; IL10: Interleukin 10; CVD: Cardiovascular disease.

Legends to the figures

Figure 1: Overview of the role of adiponectin in the cross-talk between adipose tissue (AT) and the cardiovascular system for the regulation of cardiovascular redox state. Adiponectin, originating from adjacent perivascular fat or from "remote" AT depots via systemic circulation, has direct anti-oxidant and anti-atherogenic effects on the human vascular wall (panel A). In particular, it is able to reduce the enzymatic activity of NOX1 and NOX2 isoforms of NADPH-oxidases via phospho-Akt mediated inhibition of Rac1 and p47[phox] translocation to the cell membrane, resulting in reduced generation of superoxide (O_2^-) and increased bioavailability of tetrahydropterin (BH4) (panel A). Adiponectin also phosphorylates endothelial nitric oxide synthase (eNOS) at its activation site Ser1177 via activation of the PI3K/Akt pathway; these effects result in reduced oxidative stress, improved eNOS coupling and increased nitric oxide (NO) bioavailability (panel A). Moreover, end-oxidation products such as 4-hydroxynonenal (4-HNE) that are generated in the vascular wall because of oxidative stress are able to diffuse towards the adjacent PVAT, where they upregulate the expression of PPARγ, a potent upstream inducer of *ADIPOQ* expression (panel A). Similarly, adiponectin exerts anti-oxidant effects on the human myocardium via phospho-AMPK mediated inhibition of Rac1 and p47[phox] membrane translocation and consequent reduction of NADPH-oxidase enzymatic activity, while generation of oxidation products such as 4HNE is able to upregulate *ADIPOQ* expression in epicardial AT (EpAT) via PPARγ signalling (panel B). In parallel, adiponectin expression is under the opposing regulation of the stimulatory BNP as well as the inhibitory local and systemic inflammatory stimuli (panel B). These loops constitute novel bi-directional circuits that allow PVAT and EpAT to act as recipients of vascular or myocardial redox-driven signals and subsequently respond appropriately as local defence mechanisms against oxidative stress. The responsiveness of such loops might

however be dependent on anatomical parameters as well as systemic factors such as insulin

resistance.