Vascular effects of adiponectin: molecular mechanisms and potential therapeutic intervention


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ABSTRACT

Adiponectin is a major adipocyte-secreted adipokine abundantly present in the circulation as three distinct oligomeric complexes. In addition to its role as an insulin sensitizer, mounting evidence suggests that adiponectin is an important player in maintaining vascular homoeostasis. Numerous epidemiological studies based on different ethnic groups have identified adiponectin deficiency (hypoadiponectinaemia) as an independent risk factor for endothelial dysfunction, hypertension, coronary heart disease, myocardial infarction and other cardiovascular complications. Conversely, elevation of circulating adiponectin concentrations by either genetic or pharmacological approaches can alleviate various vascular dysfunctions in animal models. Adiponectin exerts its vasculoprotective effects through its direct actions in the vascular system, such as increasing endothelial NO production, inhibiting endothelial cell activation and endothelium–leucocyte interaction, enhancing phagocytosis, and suppressing macrophage activation, macrophage-to-foam cell transformation and platelet aggregation. In addition, adiponectin reduces neointima formation through an oligomerization-dependent inhibition of smooth muscle proliferation. The present review highlights recent research advances in unveiling the molecular mechanisms that underpin the vascular actions of adiponectin and discusses the potential strategies of using adiponectin or its signalling pathways as therapeutic targets to combat obesity-related metabolic and vascular diseases.

Key words: adipokine, adiponectin, adiponectin receptor, atherosclerosis, cardiovascular disease, diabetic complication, endothelial dysfunction.

Abbreviations: ACE, angiotensin-converting enzyme; ACTA, acyl-CoA:cholesterol acyltransferase; AMPK, AMP-activated protein kinase; AngII, angiotensin II; apoE, apolipoprotein E; APPL1, adaptor protein containing pleckstrin homology domain, phosphotyrosine-binding domain and leucine zipper motif 1; ARB, AngII-receptor blocker; BMI, body mass index; (hs-)CRP, (high-sensitivity) C-reactive protein; C-reactive protein; eNOS, endothelial NO synthase; Erk-1, early growth response protein-1; ERK1/2, extracellular-signal-regulated kinase 1/2; GPCR, G-protein-coupled receptor; HMW, high-molecular-weight ('mass'); HSP90, heat-shock protein 90; HUVEC, human umbilical vein endothelial cell; IL, interleukin; ICAM-1, intercellular adhesion molecule-1; IMT, intima-media thickness; LDL, low-density lipoprotein; LMW, low-molecular-weight ('mass'); LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; MMW, middle-molecular-weight ('mass'); NF-κB, nuclear factor κB; ox-LDL, oxidized LDL; PDGF-BB, platelet-derived growth factor-BB; PKA, cAMP-dependent protein kinase; PPAR, peroxisome-proliferator-activated receptor; ROS, reactive oxygen species; SMC, smooth muscle cell; T2DM, Type 2 diabetes mellitus; TIMP-1, tissue inhibitor of metalloproteinases-1; TLR, Toll-like receptor; TNF-α, tumour necrosis factor-α; TZD, thiazolidinedione; VCAM-1, vascular cell adhesion molecule-1; VSMC, vascular SMC.

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INTRODUCTION

Obesity, which is the accumulation of excessive adipose tissue, is closely associated with an increased risk of cardiovascular morbidities, including hypertension, atherosclerosis and other vascular complications. Previously regarded as just a storage depot of excess energy, the adipose tissue is now recognized to be a highly versatile endocrine gland, secreting a large number of bioactive molecules (collectively called adipokines) [1]. Adipokines are actively involved in diverse biological processes, including energy metabolism, insulin sensitivity, immune responses and vascular homeostasis. As obesity develops, adipose tissue is infiltrated with numerous activated macrophages, leading to the augmented production of various proinflammatory factors, such as TNF-α (tumour necrosis factor-α), IL (interleukin)-6, MCP-1 (monocyte chemoattractant protein-1), resistin, leptin, serum amyloid A3, lipocalin-2 and PAI-1 (plasminogen activator inhibitor-1) [2]. These adipose-tissue-derived factors act either in a paracrine manner to perpetuate local inflammation in adipose tissue or in an endocrine manner to induce insulin resistance and vascular dysfunction. Aberrant production of adipokines has recently been proposed to be a key mechanism that links obesity to increased risk of vascular complications [3].

Although most adipokines impair insulin sensitivity and promote vascular diseases, adiponectin appears to possess antidiabetic, anti-atherogenic and anti-inflammatory activities [4]. In recent years, adiponectin has attracted much attention due to its pleiotrophic salutary effects on obesity-related cardiometabolic complications. The pathophysiological roles of adiponectin and adiponectin receptors in insulin resistance, T2DM (Type 2 diabetes mellitus) and the metabolic syndrome have been extensively reviewed elsewhere [4,5]. In this review, we will focus on the recent research related to the vascular actions of adiponectin. In addition, the potential of using adiponectin or its receptors as targets for therapeutic intervention of vascular diseases will also be discussed.

Full-length adiponectin is composed of 247 amino acid residues, including the N-terminal hypervariable region, followed by a conserved collagenous domain comprising 22 Gly-Xaa-Yaa repeats and a C-terminal C1q-like globular domain. In both mouse and human plasma, adiponectin is present predominantly as three major oligomeric forms [12,13]. The monomeric form of adiponectin has never been detected under native conditions. The basic unit of the oligomeric adiponectin is the homotrimer, also called MMW [low-molecular-weight (‘mass’)] adiponectin [14,15]. Two subunits of the adiponectin trimer are linked by a disulfide bond mediated through a cysteine residue in the collagen-like domain to form a hexamer, also termed MMW [middle-molecular-weight (‘mass’)] adiponectin. The hexamer provides the building block for the formation of a bouquet-like HMW [high-molecular-weight (‘mass’)] adiponectin comprising 12–18 protomers. Post-translational modifications are required for the intracellular assembly of the HMW oligomeric complex in adipocytes [16]. Different oligomeric forms of adiponectin act on different targets and possess distinct biological functions. Adiponectin is abundantly present in the circulation, accounting for approx. 0.01% of the total human plasma protein [17]. Unlike most other adipokines, circulating levels of adiponectin are decreased significantly in obesity and its co-morbidities, including insulin resistance, T2DM, coronary heart disease, stroke, non-alcoholic fatty liver disease and steatohepatitis, and several types of cancers (breast, colon and prostate) [18–20]. Prospective studies have demonstrated that adiponectin deficiency (hypoadiponectinaemia) is associated causally with increased prevalence and/or poor prognosis of these diseases, independent of other classical risk factors. On the other hand, elevation of circulating adiponectin by either genetic or pharmacological approaches in animal models has been shown to be effective in preventing most obesity-related medical complications, by its direct actions on multiple target tissues [21–25].

ADIPONECTIN RECEPTORS

Two adiponectin receptors (AdipoR1 and AdipoR2) have been identified [26]. Both receptors contain seven transmembrane domains, but are structurally and functionally distinct from classical GPCRs (G-protein-coupled receptors). Both AdipoR1 and AdipoR2 have an inverted membrane topology with a cytoplasmic N-terminus and a short extracellular C-terminus of approx. 25 amino acids. In C2C12 myotubes, AdipoR1 and AdipoR2 mediate increased AMPK (AMP-activated protein kinase), PPARY (peroxisome-proliferator-activated receptor α) ligand activities, and glucose uptake and fatty acid oxidation by adiponectin [26]. Recent studies have identified APPL1, an adaptor protein containing a PH (pleckstrin

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comparing with normotensive healthy subjects, even significantly lower concentrations of plasma adiponectin [35,36]. Subjects with essential hypertension had adiponectin as an independent risk factor for hypertension. Investigations have demonstrated low levels of adiponectin independent of the insulin resistance index, BMI (body mass index), and dyslipidemia [33,34]. In addition, results obtained from both cross-sectional and prospective investigations have demonstrated low levels of adiponectin as an independent risk factor for hypertension [35,36]. Subjects with essential hypertension had significantly lower concentrations of plasma adiponectin compared with normotensive healthy subjects, even after adjustment for confounding factors by multiple regression analysis [37]. In a recent 5-year follow-up study including 577 non-diabetic Chinese subjects [36], we found that normotensive subjects with baseline serum adiponectin levels in the lowest sex-specific tertile had a significantly increased risk of becoming hypertensive, suggesting that hypoadiponectinaemia may contribute to the pathogenesis of hypertension in humans.

The inverse correlation between serum adiponectin concentrations and carotid IMT (intima-media thickness), a well-established measure of subclinical atherosclerosis, has been reported in a number of studies in both healthy subjects and patients with T2DM [38,39]. In a study of 140 obese juveniles (mean age, 13.5 years), serum levels of adiponectin were correlated negatively with carotid IMT, even after controlling for BMI, the insulin resistance index, cholesterol, triacylglycerols (triglycerides), blood pressure, hs-CRP (high-sensitivity C-reactive protein), gender and age [40]. On the other hand, in another recent study involving 887 middle-aged individuals, a significant inverse age-adjusted association of serum adiponectin with carotid IMT was only observed in men, but not in women [41]. This association was attenuated after adjustments for other risk factors.

Numerous epidemiological studies have identified hypoadiponectinaemia as a predictor for coronary artery disease, acute coronary syndrome, myocardial infarction and ischaemic cerebrovascular disease, independent of classical cardiovascular risk factors [42]. A prospective study of patients with renal failure demonstrated that the risk of adverse cardiovascular events decreased by 3% for each 1 mg/ml increase in serum adiponectin levels, and the relative risk of adverse cardiovascular events was 1.56 times higher among patients in the first adiponectin tertile compared with those in the third tertile [43]. In another prospective nested case-control study, high plasma levels of adiponectin were found to be associated with a significantly decreased risk of myocardial infarction over a follow-up period of 6 years among 18225 male participants without a previous history of cardiovascular disease [44]. This association was independent of hypertension, diabetes or inflammation, and was only partly explained by changes in lipid profiles.

Plasma adiponectin levels have been shown to be closely associated with several surrogate markers of vascular diseases. A significant inverse correlation was observed between adiponectin and hs-CRP, a well-established inflammatory marker closely associated with atherosclerosis [45,46]. Recent studies have also demonstrated a negative association of plasma adiponectin with other markers of inflammation and atherosclerosis, including A-FABP (adipocyte fatty-acid-binding protein) [47] and lipocalin-2 [48]. On the other hand, plasma adiponectin correlated positively with the number of endothelial progenitor cells, which have recently been identified as significant contributors to vascular repair [49]. These

PROTECTION BY ADIPONECTIN AGAINST VASCULAR DISEASES

Clinical data

Over the past several years, numerous epidemiological investigations based on different ethnic groups have repeatedly documented a close association of adiponectin deficiency with the development of almost every stage of vascular disease [18,19]. Hypoadiponectinaemia was found to be a significant predictor of endothelial dysfunction in both periphery and coronary arteries, independent of the insulin resistance index, BMI (body mass index) and dyslipidemia [33,34]. In addition, results obtained from both cross-sectional and prospective investigations have demonstrated low levels of adiponectin as an independent risk factor for hypertension [35,36]. Subjects with essential hypertension had significantly lower concentrations of plasma adiponectin compared with normotensive healthy subjects, even...
Adiponectin exerts its vasculoprotective activities through multiple mechanisms. SM cells, SMCs.

Clinical findings collectively support an aetiological role of adiponectin deficiency in the development of various vascular complications in humans.

**Animal-based investigations**

Consistent with the clinical observations described above, adiponectin-deficient mice have been shown to be more susceptible to develop vascular disorders. Adiponectin-knockout mice had a significantly increased neointimal hyperplasia after acute vascular injury [50,51], an impaired endothelium-dependent vasodilation on an atherogenic diet [52] and an elevated blood pressure compared with their wild-type littermates [53]. On the other hand, both adenovirus-mediated overexpression of full-length adiponectin [54] and transgenic overexpression of globular adiponectin [50] resulted in a marked alleviation of atherosclerotic lesions in apoE (apolipoprotein E)-deficient mice, and also caused a significant improvement in endothelial dysfunction and hypertension in several mouse models of obesity [52,53].

The protective effect of adiponectin against vascular disorders has also been observed in a recent study in a rabbit model with spontaneous atherosclerosis [55]. Intravascular ultrasonography analysis revealed a significantly reduced atherosclerotic plaque area in abdominal aortas of rabbits after local treatment with adiponectin through injection of recombinant adenoviruses compared with those treated with adenovirus expressing β-galactosidase as a control. Adiponectin-mediated attenuation of atherosclerosis in this model was associated with the decreased expression of adhesion molecules such as VCAM-1 (vascular cell adhesion molecule-1) and ICAM-1 (intercellular adhesion molecule-1).

**Pleiotropic effects of adiponectin on the vascular system**

In addition to its beneficial effects on insulin sensitivity and lipid metabolism, adiponectin exerts its multiple vasculoprotective effects through its direct actions on the vascular system, including endothelial cells, monocytes and macrophages, leucocytes, platelets and SMCs (smooth muscle cells) (Figure 1). Adiponectin is effective in preventing almost every pathogenic event involved in atherosclerotic plaque formation, as summarized below.

**Augmentation of endothelial NO production**

Endothelium-derived NO protects the vascular system by enhancing vasodilation and inhibiting platelet aggregation, monocyte adhesion and SMC proliferation [56]. Decreased bioavailability and/or impaired production of NO cause endothelial dysfunction, which is now recognized as one of the earliest changes in
Atherosclerosis. Both the full-length and globular domain of adiponectin have been shown to increase endothelial NO production in several types of endothelial cells, through activation of eNOS (endothelial NO synthase) [57–60]. Adiponectin stimulates eNOS phosphorylation at Ser\textsuperscript{1177} and also enhances the interaction between eNOS and HSP90 (heat-shock protein 90), a complex required for the maximal activation of eNOS (Figure 2).

A more recent study from our laboratory has shown that both AdipoR1 and AdipoR2 are required for mediating adiponectin-induced NO production [27]. Adiponectin stimulation facilitates the interaction between its two receptors with APPL1, a novel adaptor protein involved in signal transduction of multiple extracellular stimuli. Suppression of APPL1 expression by RNAi (RNA interference) significantly attenuated adiponectin-induced phosphorylation of AMPK at Thr\textsuperscript{172} and eNOS at Ser\textsuperscript{1177}, as well as the complex formation between eNOS and HSP90, resulting in a marked reduction of NO production. In \textit{db/db} diabetic mice, APPL1 expression in small mesenteric arteries was decreased markedly compared with their lean controls. Notably, decreased APPL1 expression was associated with impaired vasodilation in response to adiponectin [27]. These findings support the key role of APPL1 as a signalling relay that mediates the adiponectin-induced cellular signalling cascade leading to NO production. On the other hand, overexpression of a constitutively active form of AMPK alone was sufficient to stimulate eNOS activation and NO production, even when APPL1 expression was suppressed [27], suggesting that AMPK acts downstream of APPL1 and is directly responsible for both eNOS phosphorylation at Ser\textsuperscript{1177} and its interaction with HSP90 (Figure 2). However, the detailed signalling events that link APPL1 with AMPK activation remain to be elucidated. There is also evidence suggesting the involvement of PI3K (phosphoinositide 3-kinase) in adiponectin-induced endothelial NO production, possibly through activation of AMPK [59–61].

**Antioxidant activities of adiponectin in the endothelium**

Increased oxidative stress underlies the pathogenesis of vascular dysfunction in obesity and diabetes [62]. The key feature of oxidative stress is the increased production of vascular ROS (reactive oxygen species), resulting in the quenching of NO and activation of pro-inflammatory signalling pathways, such as PKC (protein kinase C) and NF-\kappa B (nuclear factor \kappa B) [62]. Globular adiponectin inhibits both basal and ox-LDL (oxidized LDL (low-density lipoprotein))-induced ROS release in bovine endothelial cells, possibly through suppression of NADPH oxidase [63]. A more recent study has demonstrated that both full-length and globular adiponectin inhibited high-glucose-induced ROS generation in cultured HUVECs (human umbilical vein endothelial cells) [64]. In addition, the cardioprotection of adiponectin against myocardial ischaemia/reperfusion might also be attributed, at least in part, to the reduction of oxidative stress by adiponectin [65]. Consistent with these in \textit{vitro} findings, clinical studies have observed a negative association between plasma adiponectin and markers of oxidative stress (such as urinary 8-epi-prostaglandin-F\textsubscript{2a}) [66,67].

The effect of adiponectin on the suppression of excessive ROS production under hyperglycaemic conditions was abolished by pretreatment of cells with the PKA (cAMP-dependent protein kinase) inhibitor H-89, but not by the AMPK inhibitor compound C [64]. Although the two adiponectin receptors are structurally distinct from classical GPCRs, both full-length and globular adiponectin have been shown to elevate intracellular cAMP levels in endothelial cells. Furthermore, activation of cAMP signalling by treatment with forskolin or dibutyryl-cAMP mimicked the effects of adiponectin in decreasing glucose-induced ROS generation, whereas activation of AMPK by AICAR (5-amino-4-imidazolecarboxamide riboside) only had a modest effect. Together, these results suggest that the
antioxidant activities of adiponectin are mediated by the cAMP/PKA pathway (Figure 2). Nevertheless, whether or not AdipoR1 and/or AdipoR2 are involved in the adiponectin-induced increase in cAMP and suppression of ROS production remains to be clarified.

Effects of adiponectin on endothelial cell activation
Endothelial cell activation, characterized by increased expression of adhesion molecules (such as ICAM-1, VCAM-1 and E-selectin), is an early event in atherogenesis. Adiponectin exerts its anti-inflammatory effects on the endothelium by suppressing both TNF-α- and resistin-induced expression of adhesion molecules and IL-8, which, in turn, results in the attenuation of monocyte attachment to endothelial cells [68]. In addition, adenovirus-mediated expression of adiponectin decreases the expression of adhesion molecules in the aortic tissue of apoE-deficient mice [54] and a rabbit model with atherosclerosis [60]. The anti-inflammatory effects of adiponectin in endothelial cells appear to be mediated by PKA-dependent suppression of NF-κB activation [69] (Figure 2).

By contrast, a more recent study has demonstrated that acute treatment with globular adiponectin activates NF-κB and enhances the expression of adhesion molecules and MCP-1 in endothelial cells, through activation of the sphingosine kinase signalling pathway [70]. The discrepancy between these reports can be attributed to different forms of adiponectin or different incubation times in each study. Indeed, a previous study has shown that different oligomeric forms of adiponectin may have opposite functions in modulating NF-κB activity in C2C12 myotubes [71].

Suppression of the leucocyte–endothelium interactions
Recruitment of circulating leucocytes into the endothelium is now recognized as an important step in the pathophysiology of both macrovascular and microvascular diseases [72]. The pathological leucocyte–endothelium interaction triggers the exposure of the vascular wall and surrounding tissues to the damaging action of activated leucocytes. A recent study in adiponectin-knockout mice found that adiponectin deficiency caused a 2-fold increase in leucocyte rolling and a 5-fold increase in leucocyte adhesion in the microcirculation [73]. These changes were associated with a significantly decreased NO level, but an increased expression of E-selectin and VCAM-1 in the vascular endothelium. On the other hand, systemic administration of recombinant adiponectin to adiponectin-deficient mice significantly restored endothelial NO to a physiological level and suppressed the expression of adhesion molecules, and attenuated the leucocyte–endothelium interactions. In addition, pre-treatment with adiponectin also protected wild-type mice against TNF-α-induced leucocyte–endothelium interactions [73]. The inhibitory effects of adiponectin on leucocyte adhesion and adhesion molecule expression were abolished by the eNOS inhibitor L-NAME (N\textsuperscript{G}-nitro-L-arginine methyl ester), suggesting that eNOS/NO signalling is required for the anti-inflammatory activities of adiponectin in endothelial cells (Figure 2).

Protection of the endothelium from apoptosis
Endothelial cell injury is considered a critical event in the pathogenesis of atherosclerosis, plaque erosion and thrombus formation [74]. In atherosclerotic lesions, the turnover rate of endothelial cells is accelerated and local apoptosis of these cells is implicated in this process. Endothelial cell injury can be induced by high glucose, AngII (angiotensin II) and ox-LDL. The HMW oligomeric form of adiponectin, but not its trimeric or hexameric complexes, inhibits apoptosis and caspase 3 activity in HUVECs, through the activation of the AMPK signalling pathway [75]. On the other hand, the globular domain of adiponectin dose-dependently inhibited AngII-induced apoptosis in HUVECs, possibly through restoring the eNOS–HSP90 interaction and eNOS activation [76]. Whether or not the two adiponectin receptors and/or APPL1 is obligatory for the anti-apoptotic activity of this adipokine in endothelial cells needs to be clarified further in future studies.

Inhibition of macrophage activation and foam cell formation
A critical step in the development of atherosclerotic plaques is the infiltration of monocytes into the subendothelial space of arteries where they differentiate into macrophages [77]. Activated macrophages express scavenger receptors and internalize modified lipoproteins, thereby transforming themselves into foam cells. The pro-inflammatory factors produced from activated macrophages are the major contributors to endothelial cell activation and atherosclerotic lesion formation. Recent studies have demonstrated that adiponectin suppresses both macrophage activation and foam cell formation.

Both AdipoR1 and AdipoR2 are expressed in monocytes and macrophages [78]. Adiponectin has been shown to dampen the early phases of macrophage inflammatory responses [79], acting to inhibit the growth of myelomonocytic progenitor cells and decrease the ability of mature macrophages to respond to various activations [79,80]. Both full-length and globular forms of adiponectin inhibited leptin- and LPS (lipopolysaccharide)-induced macrophage production of pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6 and IL-8 [81–85]. In addition, adiponectin binds to LPS and other chemokines,
such as MIP-1α (macrophage-inflammatory protein-1α) and MCP-1, in vitro, which may lead to the decreased bioavailability of these pro-inflammatory factors [86,87].

Prolonged treatment of macrophages with adiponectin (6–24 h) caused desensitization of LPS-stimulated NF-κB and ERK1/2 (extracellular-signal-regulated kinase 1/2) activation [79,80,88]. On the other hand, short-term treatment with adiponectin (<30 min) activated the NF-κB and ERK1/2 pathways, and induced the production of TNF-α and IL-6 in various types of macrophages [81,85]. These apparently paradoxical observations can be explained by the ability of adiponectin to induce ‘macrophage tolerance’ (Figure 3). This hypothesis was initially proposed by Tsatsanis and co-workers [85], who demonstrated that pre-exposure of macrophages with 10 µg/ml adiponectin rendered the cells tolerant to further adiponectin exposure or to other pro-inflammatory stimuli, such as the TLR3 (Toll-like receptor 3) ligand polyI:C (polynosinic:polycytidylic acid) and the TLR4 ligand LPS. A more recent report by Park et al. [81] provided further a detailed molecular basis to support this hypothesis. This study showed that transient activation of NF-κB and ERK1/2 by adiponectin increased the expression of Erg-1 (early growth response protein-1), which consequently transactivated TNF-α gene transcription and protein production. Transient elevation of TNF-α by adiponectin was obligatory for the subsequent induction of IL-10, an anti-inflammatory cytokine which renders macrophages tolerant to LPS and other pro-inflammatory stimuli [81]. Indeed, adiponectin has been shown to induce IL-10 production in both macrophages and human leucocytes [89,90]. Co-culture of macrophages with a neutralizing antibody against IL-10 during 18 h of exposure to adiponectin prevented the adiponectin-mediated desensitization of LPS-stimulated TNF-α mRNA accumulation [81]. These results collectively suggest that induction of IL-10 is a key step in establishing adiponectin-induced macrophage tolerance to activation by various pro-inflammatory stimuli. Furthermore, induction of IL-10 by adiponectin
also resulted in the augmented expression of TIMP-1 (tissue inhibitor of metalloproteinases-1), a physiological inhibitor of MMPs (matrix metalloproteinases) that is involved in the rupture of atherosclerotic plaques [91]. Therefore adiponectin-mediated induction of TIMP-1 expression through IL-10 might contribute to its vasculoprotective activity by the stabilization of atherosclerotic lesions. Consistent with these in vitro findings, a positive correlation between plasma adiponectin and IL-10 expression was observed in a clinical study [93].

In addition to macrophages, it is interesting to note that the rapid activation of NF-κB by adiponectin has recently been reported in human endothelial cells [94]. Although this study did not investigate the long-term effects of adiponectin, previous findings published from other groups have demonstrated that chronic treatment with this adipokine inhibited TNF-α or high-glucose-mediated NF-κB activation in endothelial cells [69,95]. Therefore further studies are warranted to investigate whether or not the rapid and transient activation of NF-κB is also an obligatory step in adiponectin-mediated long-term tolerance of endothelial cells to the activation induced by pro-inflammatory factors. Although the hypothesis that the short-term activation of NF-κB by adiponectin renders the long-term tolerance to inflammation is attractive, the pathophysiological relevance of these in vitro observations remains unclear at this stage. Plasma adiponectin concentrations in both humans and rodents are relatively stable and do not vary substantially in response to acute stimuli such as transient nutritional changes. Therefore adiponectin-induced short-term and rapid activation of NF-κB observed in these in vitro studies might not occur under physiological conditions.

The inhibitory effects of adiponectin on macrophage-to-foam cell transformation might be mediated by its ability to suppress class A scavenger receptor expression, resulting in reduced uptake of acetylated LDL particles [96]. In addition, adiponectin decreased ACTA (acyl-CoA:cholesterol acyltransferase) activity, the enzyme that catalyses cholesteryl ester formation and enhances macrophage-to-foam cell transformation [66].

**Regulation of phagocytosis by adiponectin**

Mounting evidence suggests that defects in the phagocytic function of macrophages contribute to progression of atherosclerosis [97,98]. Impaired clearance of early apoptotic cells by macrophages has been observed in atherosclerotic lesions of humans and rabbits [99]. Although an earlier report demonstrated an inhibitory effect of adiponectin on phagocytosis in response to stimulation with LPS in vitro [79], more recent studies have provided both in vitro and in vivo evidence showing that adiponectin enhances phagocytic activities of macrophages [84,100]. In comparison with wild-type mice, adiponectin-deficient mice had a markedly impaired ability to clear apoptotic thymocytes in response to dexamethasone treatment and to remove early apoptotic cells that were injected into their intraperitoneal cavities [100]. In addition, adiponectin deficiency in lpr mice led to a further reduction in apoptotic cell clearance, which was accompanied by exacerbated systemic inflammation. Conversely, replenishment with recombinant adiponectin promoted the clearance of apoptotic cells by macrophages in both adiponectin-deficient and wild-type mice, and also reduced features of autoimmunity in lpr mice [100]. The stimulatory effects of adiponectin on phagocytosis are attributed to the ability of this adipokine to opsonize apoptotic debris and to facilitate its binding to the macrophage cell surface, through interaction with calreticulin and CD91 (Figure 3). Taken together, these results suggest that adiponectin protects the organism from systemic inflammation by promoting the clearance of early apoptotic cells by macrophages through a mechanism involving calreticulin. Notably, none of the putative adiponectin receptors (AdipoR1, AdipoR2 and T-cadherin) are required for the phagocytotic activities of adiponectin [100].

**Antithrombotic activities of adiponectin**

Platelet activation plays a crucial role in the progression of atherosclerosis and plaque rupture. Inhibition of platelet aggregation has been suggested to prevent arterial thrombosis [101]. A clinical study has demonstrated that the plasma adiponectin level was negatively associated with platelet activation independently of other risk factors [102]. Adiponectin-deficient mice had accelerated thrombus formation following laser-induced carotid arterial injury, whereas adenovirus-mediated expression of adiponectin reversed these changes [103]. In vitro, adiponectin inhibited collagen-induced platelet aggregation in platelet cells harvested from human subjects and adiponectin-deficient mice [103]. It is well-established that endothelial-derived NO inhibits platelet activation and, hence, suppresses platelet adhesion and aggregation [104]. The inhibitory effects of adiponectin on thrombosis may be attributed, at least in part, to its ability to stimulate endothelial NO production.

**Inhibition of smooth muscle proliferation**

VSMC (vascular SMC) proliferation and migration toward the intima contribute to intimal thickening of the arteries during the development and progression of vascular lesions. Adiponectin inhibits both proliferation and migration of human aortic SMCs induced by several atherogenic growth factors, including HB-EGF [heparin-binding EGF (epidermal growth factor)-like growth factor], PDGF-BB (platelet-derived growth factor-BB) and bFGF (basic fibroblast growth factor) [105,106]. A more recent study has also demonstrated the suppressive effects of adiponectin on PDGF-BB-induced proliferation of primary pulmonary arterial SMCs harvested from apoE-deficient mice [107]. The antiproliferative effect of
Vascular effects of adiponectin

Adiponectin is attributed primarily to its oligomerization-dependent interaction with these growth factors, leading subsequently to the blockade of their binding to the respective cell-membrane receptors [106]. Consistent with these in vitro findings, adiponectin-deficient mice, as compared with wild-type controls, had enhanced proliferation of VSMCs and increased neointimal thickening after mechanical injury [108]. Adenovirus-mediated expression of adiponectin in these mice attenuated the extent of neointimal proliferation [54].

ADIPONECTIN AND ITS PATHWAYS AS A THERAPEUTIC TARGET FOR VASCULAR DISEASES

According to the clinical and experimental evidence discussed above, therapeutic interventions that can enhance the actions of adiponectin, such as increasing plasma adiponectin concentrations or up-regulating/activating adiponectin receptors, could represent attractive strategies to combat obesity-related diabetes and vascular diseases.

Elevation of plasma adiponectin concentrations

Many currently available therapies for cardiovascular diseases, such as lifestyle modifications, calorie restriction, and pharmacological and dietary interventions, have been shown to increase plasma levels of adiponectin in rodents and/or humans (Figure 4). There is also growing interest in the pharmaceutical industry to search for natural or synthetic compounds that can increase adiponectin production [109].

Prolonged weight loss through either gastric banding surgery or calorie restriction increased circulating levels of adiponectin in obese subjects [110]. A combination of a Mediterranean-type diet with moderate physical activity also induced a significant increase in adiponectin in pre-menopausal obese women [111]. In addition, weight loss led to changes in the oligomeric distribution of adiponectin [75]. HMW adiponectin was significantly increased, whereas the hexameric and trimeric forms were decreased. Dietary fish oils and polyunsaturated fatty acids increased adipose tissue mRNA expression and plasma levels of adiponectin in several animal models of obesity [112–114]. Furthermore, Oolong tea [115], green tea extracts [116] and (+)–catechin (a type of green tea polyphenol) [117] have been shown to elevate plasma adiponectin in humans and rodent models.

The PPARγ agonists TZDs (thiazolidinediones) are widely used antidiabetic drugs that also possess vasculoprotective and anti-inflammatory properties. TZDs, such as rosiglitazone and pioglitazone, increase adiponectin production in humans and rodents in vivo, and in adipocytes in vitro [118–120]. In patients with diabetes, TZD-mediated increases in adiponectin, especially its HMW oligomeric forms, correlated well with improvements in insulin sensitivity [121]. The TZD-induced increase in circulating adiponectin is probably mediated by their ability to transactivate adiponectin gene expression [119] and selectively enhance the secretion of the HMW oligomeric form of this adipokine from adipocytes [122]. Two recent independent studies on adiponectin-deficient mice have demonstrated that the insulin-sensitizing effects of TZDs were mediated, at least in part, by induction of adiponectin production [50,123].
PPARα agonists have also been shown to increase adiponectin production. Fenofibrate therapy significantly increased plasma adiponectin levels and insulin sensitivity in patients with primary hypertriglyceridaemia [121]. Significant correlations between the degree of changes in serum adiponectin concentrations and insulin levels, CRP levels and insulin sensitivity were observed after fenofibrate therapy [124]. Notably, fenofibrate therapy for 2 months elevated serum adiponectin levels without a change in body weight [125]. This raises the possibility that the therapeutic effect of this drug may be, in part, mediated through the induction of adiponectin.

Drugs blocking the RAS (renin–angiotensin system) blocking, including ACE (angiotensin-converting enzyme) inhibitors and ARBs (AngII-receptor blockers), have repeatedly been shown to elevate plasma adiponectin levels without affecting adiposity [126–129]. Losartan alone or a combined therapy with simvastatin and losartan in patients with hypercholesterolaemia and hypertension significantly increased plasma adiponectin levels and insulin sensitivity relative to baseline measurements [125]. In addition, several other agents with either antidiabetic and/or vasculoprotective activities, including glimepiride (a glucose-lowering drug) [130], nebivolol (a new β-adrenergic blocker) [131] and rimonabant (a cannabinoid CB1 receptor antagonist) [132], have also been shown to increase plasma adiponectin concentrations in humans. However, it is currently unclear whether or not the beneficial effects of these drugs on cardiovascular disease are mediated by adiponectin.

**Up-regulation or activation of adiponectin receptors**

In addition to hypoadiponectinaemia, decreased expression of the two adiponectin receptors (AdipoR1/AdipoR2) have also been reported in obese animals with insulin resistance and endothelial dysfunction [133], and in patients with T2DM [134]. Therefore another logical approach to combat obesity-related metabolic and vascular diseases is to induce AdipoR1/AdipoR2 expression or to develop receptor agonists that can mimic adiponectin actions. A recent study [135] has demonstrated that exercise combined with a hypocaloric diet increased both AdipoR1 and AdipoR2 expression in skeletal muscle in older obese adults (>60 years of age). In addition, intensive physical training has been shown to elevate AdipoR2 expression in adipose tissue [136]. In human macrophages, both PPARα and PPARγ agonists increased AdipoR2 expression, whereas a synthetic LXR (liver X receptor) agonist induced the expression of both AdipoR1 and AdipoR2 [78]. In KKAγ obese mice, a PPARα agonist reversed the decreases in AdipoR1 and AdipoR2 expression in adipose tissue [137]. These findings raise the possibility that up-regulation of adiponectin and its receptors might represent one of the mechanisms accounting for the vasculoprotective activities of PPARα and PPARγ agonists.

There are no currently available therapeutic strategies that have been shown to mimic the actions of adiponectin in activating its receptors. A recent in vitro study has identified osmotin, a member of the PR-5 (pathogenesis-related-5) family of plant defence proteins, as a potential adiponectin receptor agonist [138]. The three-dimensional structure of osmotin is similar to globular adiponectin, both of which consist of antiparallel β-strands arranged in the shape of a β-barrel. Interestingly, osmotin activates AMPK via adiponectin receptors in mammalian C2C12 myocytes.

**CONCLUDING REMARKS**

Recent research provides compelling evidence supporting the role of adiponectin as a physiological regulator of vascular homeostasis in both animal models and humans. Major progress has been made in unveiling the molecular mechanisms that underlie the multiple vasculoprotective actions of this adipokine. However, it should be noted that many mechanisms proposed are based on in vitro studies and, thus, the physiological relevance of these findings remains to be confirmed. Our understanding of the adiponectin receptors, especially with respect to structure-function relationships and their pathophysiological role in vascular dysfunction, is still at a very early stage. Further investigations in this exciting field will facilitate the development of selective adiponectin agonists that can be potentially used for the therapeutic intervention of diabetes and vascular disease.

**ACKNOWLEDGMENTS**

This work was supported by NSFC (Natural Science Foundation of China)/RGC (Research Grants Council) Joint Research Scheme (N_HKU 727/05), Matching Funding for National ‘973’ Basic Research Project, and an Outstanding Young Research Award from University of Hong Kong (to A.X).

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Received 2 October 2007/22 October 2007; accepted 24 October 2007
Published on the Internet 1 February 2008, doi:10.1042/CS20070347