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REVIEW

Cross-talk between adipose tissue and vasculature: role of adiponectin

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Abstract

Adipose tissue is a highly dynamic endocrine organ, secreting a number of bioactive substances (adipokines) regulating insulin sensitivity, energy metabolism and vascular homeostasis. Dysfunctional adipose tissue is a key mediator that links obesity with insulin resistance, hypertension and cardiovascular disease. Obese adipose tissue is characterized by adipocyte hypertrophy and infiltration of inflammatory macrophages and lymphocytes, leading to the augmented production of pro-inflammatory adipokines and vasoconstrictors that induce endothelial dysfunction and vascular inflammation through their paracrine and endocrine actions. By contrast, the secretion of adiponectin, an adipokine with insulin sensitizing and anti-inflammatory activities, is decreased in obesity and its related pathologies. Emerging evidence suggests that adiponectin is protective against vascular dysfunction induced by obesity and diabetes, through its multiple favourable effects on glucose and lipid metabolism as well as on vascular function. Adiponectin improves insulin sensitivity and metabolic profiles, thus reducing the classical risk factors for cardiovascular disease. Furthermore, adiponectin protects the vasculature through its pleiotropic actions on endothelial cells, endothelial progenitor cells, smooth muscle cells and macrophages. Data from both animal and human investigations demonstrate that adiponectin is an important component of the adipo-vascular axis that mediates the cross-talk between adipose tissue and vasculature. This review highlights recent work on the vascular protective activities of adiponectin and discusses the molecular pathways underlying the vascular actions of this adipokine.

Keywords Adipokine, endothelial function, inflammation, obesity, vascular homeostasis.

Obesity is closely associated with an increased risk for a cluster of metabolic and cardiovascular diseases, including insulin resistance, type 2 diabetes, hypertension, coronary heart disease and stroke (Alberti *et al.* 2009). A recent report by the Prospective Studies Collaboration (PSC), with data obtained in four continents from almost 900 000 participants during 57 prospective studies, shows that the median survival rate is reduced by 8–10 years for morbidly obese subjects with body weight index (BMI) at 40–45 kg m^{-2} compared to those with normal BMI, primarily because of the increased death from cardiovascular disease (Whitlock *et al.* 2009).

The hallmark of obesity is excess accumulation of adipose tissue, which is intimately associated with the structural and physiological functions of the

vasculature. In addition to its role as a depot for storage of excess energy, adipose tissue is a highly dynamic endocrine organ and an important metabolic sensor, participating in the regulation of insulin sensitivity, glucose and lipid metabolism and vascular homeostasis. Since the discovery of leptin in 1994, several dozens of adipokines have been identified and characterized. These adipokines are the key components of the 'adipo-vascular axis' that mediate the dialogue between adipose tissue and the vasculature. The majority of adipokines, such as tumour necrosis factor α (TNFα), monocyte chemoattractant protein (MCP)-1, resistin, serum amyloid A3, interleukin (IL)-6 and lipocalin-2, are of pro-inflammatory nature and contribute to the pathogenesis of obesity-related insulin resistance and vascular dysfunction (Hoo *et al.* 2008). By contrast, adiponectin is one of few adipokines with favourable effects on insulin sensitivity and cardiovascular function, and has been proposed as a promising therapeutic target for combating obesity-associated vascular disease (Berg & Scherer 2005). Furthermore, adipose tissue can also modulate vascular tone by secreting vasoactive substances, such as angiotensin, endothelin-1, adrenomedullin and nitric oxide (NO) (Linscheid *et al.* 2005, Eringa *et al.* 2007).

This review will discuss the regulation of vascular function by adipose tissue through the adipose-vascular axis, and its relevance to obesity-related insulin resistance and vascular disease, with a particular focus on perivascular adipose tissue (PVAT). It will also summarize the role of adiponectin in mediating the cross-talk between adipose tissue and the vasculature, and highlight recent work on the receptor involved and the post-receptor signalling pathways underlying the vascular protective effects of adiponectin.

Modulation of vascular functions by adipose tissue

A growing body of evidence suggests the existence of a reciprocal interplay between adipose tissue and vasculature. Both white adipose and brown adipose tissue are highly vascularized organs, and this vascularization plays a crucial role in determining adipocyte differentiation and growth, as well as the physiological function of adipose tissue, by supplying nutrients, growth factors and circulating stem cells (Cao 2010). Conversely, adipose tissue, especially PVAT, exerts profound effects on vascular tone in particular endothelium-dependent vasodilatation, inflammation and remodelling by secreting a large number of bioactive substances, including adipocyte-derived relaxing factor (ADRF), vasoconstrictors and various cytokines and chemokines (Rajsheker *et al.* 2010) (Fig. 1). The aberrant production of these factors is an important contributor to obesity-associated endothelial dysfunction and vascular inflammation.

Vasodilator and vasoconstrictor factors released by PVAT

Perivascular adipose tissue is present around almost all blood vessels, particularly conduit arteries (Thalmann & Meier 2007). A unique feature of PVAT is that

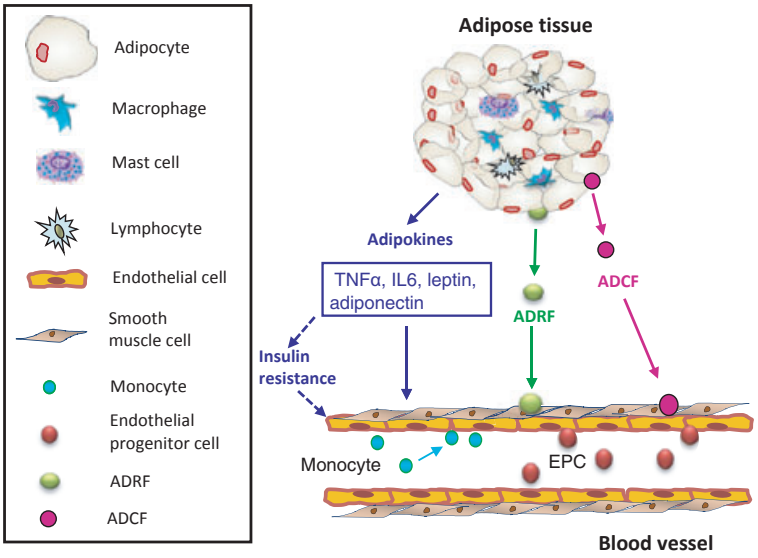

Figure 1 Adipose tissue modulates vascular functions by releasing a broad range of bioactive factors. In obesity, adipose tissue is infiltrated with several types of inflammatory cells, including macrophages, lymphocytes and mast cells, all of which contribute to the secretion of pro-inflammatory factors. Adipose tissue released factors act either in an autocrine/paracrine manner to modulate vascular tone and inflammation, or in an endocrine manner to regulate insulin sensitivity and metabolism, thereby influencing vascular function indirectly.

adipocytes are not separated from the blood vessel wall by a fascial layer, but encroach into the adventitial region, thus allowing easy access for factors secreted from PVAT into blood vessels (Chatterjee *et al.* 2009).

Regulation of vascular tone by PVAT was first demonstrated by Soltis & Cassis (1991), and was subsequently confirmed by several independent studies in different species including rats and humans (Lohn *et al.* 2002, Dubrovska *et al.* 2004, Gao *et al.* 2007). These studies demonstrated that pre-incubation with culture medium of PVAT has a relaxing effect on arteries without PVAT, suggesting the existence of a transferrable ADRF released from this adipose tissue. The release of ADRF depends on extracellular calcium and its effect is mediated by intracellular tyrosine kinases and voltage-dependent K^+ channels in the vascular smooth muscle, while NO and prostaglandins are not involved (Dubrovska *et al.* 2004). The anti-contractile activity of mesenteric adipose tissue is diminished in spontaneously hypertensive rats, suggesting that impaired functions of PVAT might contribute to hypertension in this model (Galvez *et al.* 2006).

In addition to its direct effects on vascular smooth muscle cells (VSMC), there is emerging evidence demonstrating that ADRF elicits endothelium-dependent vasodilatation by promoting the production of endothelium-derived NO (Gao *et al.* 2007). Thus, the relaxation of aortic rings induced by incubation with culture medium of PVAT derived from Wistar rats is abrogated upon removal of the endothelium or pharmacological inhibition of endothelial NO synthase (eNOS) (Gao *et al.* 2007). Likewise, a recent study in humans demonstrates that PVAT from healthy individuals exerts anti-contractile activity on small arteries by increasing NO bioavailability (Greenstein *et al.* 2009). However, this anti-contractile activity of PVAT is lost in obese subjects with metabolic syndrome, primarily because of increased inflammation and oxidative stress in this tissue.

A number of adipose tissue-released factors with anti-contractile activity, including leptin (Lembo *et al.* 2000), adiponectin (see further discussion below) and angiotensin (Ang) 1–7 (Lee *et al.* 2009), have been proposed as potential candidates for ADRF. Leptin induces relaxation of the aorta and mesenteric arteries by inducing eNOS activity and NO release, and also by enhancing the production of endothelium-derived hyperpolarizing factor (Lembo *et al.* 2000). However, the anti-contractile property of leptin seems to be different from that of ADRF, which involves K^+ channel-dependent vasodilatation. In addition, the lack of functional leptin receptors in the Zucker fa/fa rats does not alter the anti-contractile effect of PVAT (Dubrovska *et al.* 2004), implying that leptin may not be an ADRF. Adiponectin inhibits serotonin-induced vasoconstriction, and this effect is abolished by the inhibition of K_v channels (Fesus *et al.* 2007). Nevertheless, adiponectin is not ADRF, since the anti-contractile effects of PVAT are not different in aortic rings and mesenteric arteries isolated from adiponectin knockout and wild-type mice (Fesus *et al.* 2007). Obviously, the exact nature of ADRF remains to be determined.

Besides ADRF, PVAT also releases a number of vasoconstrictors (termed adipose tissue-derived constricting factor, ADCF) (Gao 2007). Upon stimulation by Ang II, periaortic PVAT secretes endothelin (ET)-1 (An *et al.* 2006). In addition, PVAT of conduit arteries synthesizes angiotensinogen (Rogerson *et al.* 1992). Ang II enhances the generation of reactive oxygen species (ROS), which in turn impairs endothelium-dependent vasodilatation (Rey *et al.* 2002). The ADCF-dependent vasoconstriction is mediated by ROS and is blocked by inhibitors of NADPH oxidase (Rey *et al.* 2002). Furthermore, periaortic PVAT expresses angiotensin-converting enzyme, suggesting the existence of the functional renin–angiotensin system in this tissue (Zhuo *et al.* 1997).

In conclusion, PVAT plays a dual role in modulating vascular tone by releasing a number of vasoactive substances that act on endothelial and VSMC. In obesity, dysfunction of adipose tissue and local inflammation result in impaired production of ADRF and elevated release of ADCF, thereby leading to endothelial dysfunction which may contribute to hypertension (Gao 2007).

On the other side of the adipo-vascular axis, adipose tissue itself is also a target of vasoactive factors released by the endothelium. For example, it has been shown that adipocytes express endothelin-1 receptor subtype A (ETA), which mediates the inhibitory action of endothelin-1 on TNFα-induced inducible nitric oxide synthase (iNOS) expression (Merial-Kieny *et al.* 2003). Endothelin-1 has been reported to stimulate IL-6 secretion in 3T3-L1 adipocytes (Chai *et al.* 2009). A recent study suggests that NO released from the endothelium may exert its actions on adipocytes to modulate adipokine production (Koh *et al.* 2010). Adiponectin biosynthesis in adipose tissue of eNOS knockout mice is much lower than in wild-type littermates.

Regulation of vascular inflammation by adipose tissue

Chronic inflammation plays a central role in the pathogenesis of vascular disease associated with obesity and diabetes (Berg & Scherer 2005). Transplantation of visceral adipose tissue into apolipoprotein (apo) $E^{-/-}$ mice results in a marked acceleration of the formation of atherosclerotic lesions by inducing the production of pro-inflammatory factors, suggesting that adipose tissue is a major contributor to vascular inflammation (Ohman *et al.* 2008). In obesity, adipose tissue serves as a harbour for a broad range of inflammatory cells,

including activated macrophages, T cells and B cells, which act in a synergistic manner to produce a number of pro-inflammatory cytokines (including TNFα, IL6 and IL8), chemokines [including MCP-1 and Regulated upon Activation, Normal T Cell Expressed and Secreted (RANTES)] and adipokines [including resistin, adipocyte fatty acid-binding protein (A-FABP) and lipocalin-2] (Libby *et al.* 2010). Among different types of adipose tissues, PVAT is the primary contributor to vascular inflammation due to both its proximity to the blood vessel wall and its pro-inflammatory properties (Chatterjee *et al.* 2009). PVAT appears to secrete inflammatory mediators more actively compared to other adipose tissue depots in humans and in diet-induced obese mice (Henrichot *et al.* 2005, Chatterjee *et al.* 2009). Inflammatory cell infiltration is markedly increased in PVAT surrounding atherosclerotic compared to healthy aortae (Chatterjee *et al.* 2009), and this change is companied by a markedly increased expression of inflammatory genes in PVAT, but not in subcutaneous and omental fat. Moreover, secretion of MCP-1, a key pathological cytokine in vascular inflammation and atherosclerosis, is increased 40-fold in perivascular compared to subcutaneous adipocytes (Braunersreuther *et al.* 2007).

The pro-inflammatory factors released from adipose tissue exert adverse effects on the vasculature *via* both direct and indirect mechanisms. First, a number of pro-inflammatory adipokines and cytokines act in an endocrine manner to induce insulin resistance and metabolic derangement, which are classical risk factors for vascular disease (Hotamisligil & Erbay 2008). Second, several chemokines and adipokines, such as MCP-1 and IL8, induce the recruitment and infiltration of monocytes, lymphocytes and neutrophils into the blood vessel wall to instigate the local inflammation (Libby *et al.* 2010). Third, many adipokines and cytokines can act on endothelial cells to impair NO production and endothelium-dependent vasodilatation.

Tumour necrosis factor α is one of the best characterized adipokines/cytokines that is causally involved in obesity-related vascular dysfunction. Adipose tissue-derived TNFα is an important contributor to systemic insulin resistance by impeding insulin's actions in major metabolic targets (liver and skeletal muscle) (Hotamisligil & Erbay 2008). In addition, TNFα impairs insulin-induced vasodilatation through activation of c-JUN NH2-terminal kinase (JNK) and inhibition of insulin signalling in microvascular endothelium (Eringa *et al.* 2006). TNFα also inhibits eNOS, therefore causing a reduced NO bioavailability (Scherrer & Sartori 2000). The impaired vasodilatation by TNFα leads to reduced blood flow and capillary recruitment in skeletal muscle (de Jongh *et al.* 2006), thereby ultimately contributing to insulin resistance by limiting the substrate supply.

Another emerging adipokine that plays a key role in vascular inflammation is A-FABP, which is abundantly expressed in both adipocytes and macrophages (Hoo *et al.* 2008). A-FABP potentiates toxic lipids-induced inflammation in macrophages by inducing endoplasmic reticulum stress, thereby leading to the activation of JNK and nuclear factor-kappa B (NF-κB) signalling pathways (Erbay *et al.* 2009). Both genetic and pharmacological inhibition of A-FABP prevents diet-induced atherosclerosis in apoE$^{-/-}$ mice by blocking the inflammatory responses in the vessel wall (Furuhashi *et al.* 2007). In addition, the pharmacological inhibition of A-FABP alleviates endothelial dysfunction in diet-induced obese mice (M. Lee, A. Xu and P. Vanhoutte, unpublished observation). Taken in conjunction, these findings support the central role of adipose tissue in instigating obesity-associated vascular inflammation, by releasing pro-inflammatory factors.

Adiponectin: a key component of the 'adipo-vascular axis'

Adiponectin is one of the most abundant adipokines secreted from adipocytes, accounting for approx. 0.01% of the total protein content of human plasma (Arita *et al.* 1999). This adipokine is composed of a NH_2-terminal hyper-variable region, followed by a collagenous domain consisting of 22 Gly-X-Y repeats and a COOH-terminal C1q-like globular domain (Scherer *et al.* 1995). Circulating adiponectin exists predominantly as three distinct oligomeric complexes (Xu *et al.* 2005, Wang *et al.* 2008). The basic building block of oligomeric adiponectin is a tightly associated homotrimer, which is formed *via* hydrophobic interactions within its globular domains. Two trimers self-assemble into a disulfide-linked hexamer, which further associates into a bouquet-like high molecular weight (HMW) multimeric complex that consists of 12–18 protomers (Tsao *et al.* 2003). The post-translational modifications, especially hydroxylation and subsequent glycosylation of several highly conserved lysine residues within its collagenous domain, is crucial for the formation of HMW oligomeric adiponectin, the major bioactive isoform responsible for its insulin-sensitizing activity. Adiponectin is also modified by sialic acids through O-linked glycosylation situated on threonine residues within the hypervariable region (Richards *et al.* 2010), which determines the circulating half-life of the adipokine by modulating its clearance from the bloodstream.

The adiponectin receptors and their post-receptor signalling pathways

Two subtypes of adiponectin receptors (adipoR1 and adipoR2) have been identified by Yamauchi *et al.*

(2003). Both receptors contain seven transmembrane domains, but they are structurally and functionally distinct from classical G-protein coupled receptors (Kadowaki & Yamauchi 2005). Both adipoR1 and adipoR2 have an inverted membrane topology with a cytoplasmic NH_2 terminus and a short extracellular COOH terminus of approx. 25 amino acids. Several groups have recently investigated the phenotypic changes in adipoR1 and adipoR2 knockout mice. Yamauchi *et al.* (2007) reported that the targeted disruption of adipoR1 results in the abrogation of adiponectin-induced activation of AMPK, whereas ablation of adipoR2 diminished adiponectin-stimulated peroxisome proliferator-activated receptor (PPARα) signalling. Simultaneous disruption of both adipoR1 and R2 abolished the binding and actions of adiponectin, leading to insulin resistance and marked glucose intolerance (Yamauchi *et al.* 2007). AdipoR1-null mice generated by Bjursell *et al.* (2007) showed increased adiposity with increased glucose intolerance, while adipoR2-null mice were lean and resistant to diet-induced glucose intolerance, indicating that adipoR1 and adipoR2 may have opposing effects. On the other hand, Liu *et al.* (2007) reported that ablation of adipoR2 reduced diet-induced insulin resistance, but promoted type 2 diabetes. Although both adipoR1 and adipoR2 are expressed in several types of vascular cells, including endothelial cells (Cheng *et al.* 2007), VSMC (Wang *et al.* 2005) and macrophages, the physiological functions of these two receptors in modulating vascular tone remain unclear.

The binding of adiponectin to adipoR1 and adipoR2 leads to the activation of AMPK and PPAR-α respectively (Kadowaki & Yamauchi 2005). APPL1, an adaptor protein containing a pleckstrin homology domain, a phosphotyrosine binding domain and a leucine zipper motif, is a direct interacting partner of adipoR1 and adipoR2 (Mao *et al.* 2006, Cheng *et al.* 2007). APPL1 appears to play a key role in coupling the adiponectin receptors to their downstream signalling cascades, although the detailed molecular events involved remain to be elucidated.

Adiponectin induces calcium (Ca^{2+}) influx into myotubes by binding to adipoR1, leading to the activation of Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ), which in turn activate AMPK by its phosphorylation (Iwabu *et al.* 2010). Activated AMPK enhances the activity of NAD+-dependent type III deacetylase sirtuin 1 (SIRT1), leading to increased mitochondrial oxidative capacity following deacetylation and activation of PPAR γ coactivator-1α (PGC-1α), a master regulator of mitochondrial biogenesis (Canto & Auwerx 2009). However, whether or not these adiponectin-mediated signalling cascades also operate in the vasculature and mediate the vascular actions of adiponectin awaits further demonstration.

Cardiometabolic protection by adiponectin

Unlike most adipokines with pro-inflammatory activities, adiponectin possesses anti-inflammatory and insulin-sensitizing properties. Adiponectin protects against almost all the major obesity-related pathologies, including insulin resistance (Berg *et al.* 2001), hypertension (Ohashi *et al.* 2006), atherosclerosis (Okamoto *et al.* 2002), non-alcoholic fatty liver disease and steatohepatitis (Xu *et al.* 2003), heart failure (Shibata *et al.* 2005), airway inflammation (Shore *et al.* 2006) and several types of obesity-related cancers (Wang *et al.* 2006, 2007). Among these favourable effects, the vascular protective activities of adiponectin have been documented repeatedly in a large number of animal and human studies. Epidemiological studies on different ethnic groups have identified low level of circulating adiponectin, especially its HMW oligomeric complex, as an independent risk for type 2 diabetes, hypertension, atherosclerosis and myocardial infarction (Zhu *et al.* 2008). Likewise, adiponectin knockout mice are more susceptible to diet-induced insulin resistance (Berg *et al.* 2001, Yamauchi *et al.* 2002), endothelial dysfunction (Ouchi *et al.* 2003), hypertension (Ohashi *et al.* 2006), atherosclerosis (Kubota *et al.* 2002) and heart failure (Shibata *et al.* 2005). On the other hand, elevating serum adiponectin concentrations by either genetic or pharmacological intervention alleviates these disorders (Berg *et al.* 2001, Yamauchi *et al.* 2003, Shibata *et al.* 2005, Ohashi *et al.* 2006).

Overall, the vascular protective effects of adiponectin are attributed to its pleiotropic actions on multiple targets (Fig. 2). First, it enhances insulin sensitivity and improves the metabolic profile, primarily through its actions on liver and skeletal muscle. Second, as a key component of the 'adipo-vascular axis', adiponectin acts on both adipose tissue and vasculature to exert its anti-inflammatory and anti-oxidant activities, thus preventing endothelial injury and dysfunction. Additionally, an indirect effect of adiponectin on sympathetic nervous system has been proposed, in which adiponectin negatively regulates blood pressure via suppressing sympathetic nerve activity (Tanida *et al.* 2007, Wang & Scherer 2008). It was recently demonstrated that adiponectin receptors are present in the nucleus of the solitary tract in medulla and injection of adiponectin to this area reduced blood pressure (Hoyda *et al.* 2009). However, it has been reported that adiponectin does not pass through the blood–brain barrier (Spranger *et al.* 2006). Therefore, further studies are needed to clarify whether or not the sympathetic nerve system is the direct target of adiponectin.

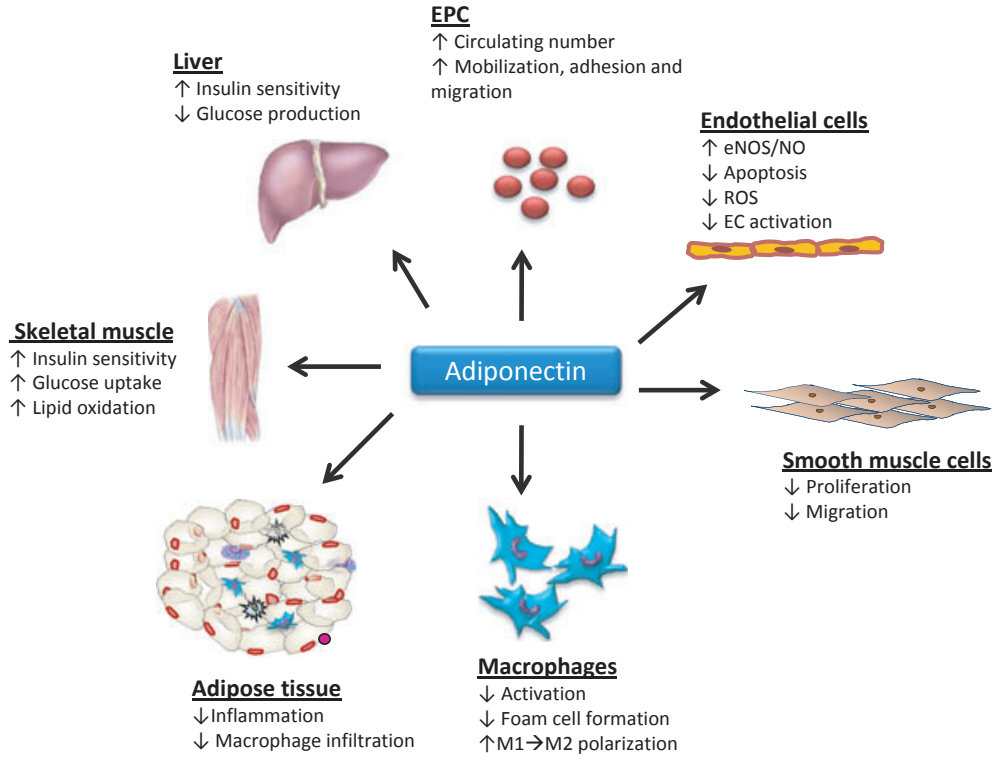

Figure 2 Adiponectin exerts its vascular protective effects through its pleiotropic actions on multiple targets. EC, endothelial cells; EPC, endothelial progenitor cells; NO, nitric oxide; ROS, reactive oxygen species.

Suppression of adipose tissue inflammation by adiponectin

Several animal studies demonstrate that adiponectin acts in an autocrine/paracrine manner to inhibit obesity-induced macrophage infiltration and production of pro-inflammatory cytokines in adipose tissue (Kim *et al.* 2007, Ohashi *et al.* 2010), thereby interfering with the transmission of the 'inflammatory signal' from adipose tissue to the vasculature. The transgenic over-expression of adiponectin in *ob/ob* obese mice results in a significantly decreased number of infiltrated macrophages in adipose tissue as well as reduced systemic inflammation compared with wild-type obese mice, despite a marked expansion of adipose tissue in the transgenic mice (Kim *et al.* 2007).

Altered macrophage polarization plays a key role in instigating obesity-induced inflammation in adipose tissue (Mantovani *et al.* 2009). Macrophages in lean adipose tissue behave as 'alternatively activated' macrophages with an anti-inflammatory phenotype that expresses M2-markers and is associated with wound repair and angiogenesis (Lumeng *et al.* 2007). By contrast, obesity leads to a reduction of these M2 markers with an increased expression of genes associated with M1 markers leading to a phenotype of 'classically activated' macrophages with pro-inflammatory properties. Adiponectin promotes macrophage polarization towards an anti-inflammatory phenotype in adipose tissue (Ohashi *et al.* 2010). Peritoneal macrophages and the stromal vascular fractions (SVF) cells of adipose tissue isolated from adiponectin-knockout mice exhibit elevated M1-markers including TNFα, IL6 and MCP-1 and decreased M2 markers such as arginase-1 and the anti-inflammatory cytokine IL10. These changes are reversed by adenovirus-mediated systemic administration of adiponectin. Furthermore, both full-length and globular recombinant adiponectin stimulate the expression of M2- but inhibit that of M1-markers in monocyte-derived macrophages and SVF cells derived from human adipose tissue (Ohashi *et al.* 2010). Thus, adiponectin counteracts obesity-induced inflammation in adipose tissue by decreasing macrophage infiltration as well as modulating macrophage polarization.

Direct effects of adiponectin on the vasculature

Adiponectin inhibits almost every pathological event involved in vascular disease, ranging from endothelial injury and dysfunction to atherosclerotic lesion formation (Zhu *et al.* 2008). These favourable effects of adiponectin are mediated through its pleiotropic actions on several types of cells in the vasculature, including

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mature endothelial cells, endothelial progenitor cells (EPC), monocytes and smooth muscle cells.

Promoting endothelium-dependent vasodilatation. Both *in vitro* and *in vivo* animal studies as well as clinical data consistently support the role of adiponectin as a vasodilator that induces eNOS activation and endothelial NO production (Hattori *et al.* 2003, Ouchi *et al.* 2004, Tan *et al.* 2004, Xi *et al.* 2005). In humans, circulating levels of adiponectin are associated positively with endothelium-dependent vasodilatation of the brachial artery, measured by high-resolution ultrasound (Tan *et al.* 2004). Aortic rings isolated from adiponectin-knockout mice display a reduced eNOS phosphorylation and NO production and impaired relaxation compared to those from wild-type controls, and these changes are reversed by supplementation with recombinant adiponectin (Cao *et al.* 2009). Likewise, systemic administration of recombinant adiponectin in Sprague-Dawley rats with high fat diet-induced obesity increases eNOS activity, NO production and relaxation of aortic rings *ex vivo* (Deng *et al.* 2010). Furthermore, direct addition of recombinant adiponectin into the organ chambers elicits endothelium-dependent relaxation of both aortic rings and small mesenteric arteries isolated from C57 mice (Cheng *et al.* 2007).

In endothelial cells, adiponectin enhances eNOS activity and NO production *via* AMP-activated protein kinase (AMPK)-mediated phosphorylation of eNOS at Ser^{1177} (Chen *et al.* 2003) and Ser^{633} (Chen *et al.* 2009). Both types of adiponectin receptors (adipoR1 and adipoR2) are expressed in endothelial cells and mediate adiponectin-induced phosphorylation of AMPK and eNOS in a complementary manner (Cheng *et al.* 2007). Adiponectin also promotes the complex formation between heat shock protein (HSP) 90 and eNOS, which is required for the maximal activation of the enzyme (Cheng *et al.* 2007). However, the precise signalling mechanism whereby adiponectin induces eNOS activation following binding to its receptors needs further definition.

Protecting the endothelium from oxidative stress. Production of ROS reduces the bioavailability of NO as a result of the uncoupling of eNOS. In addition, the increased level of superoxide anions leads to the resultant formation of peroxynitrite, which further aggravates the impairment of eNOS activity and reduces NO production (Grattagliano *et al.* 2008, Hajer *et al.* 2008). Adiponectin inhibits both the basal and oxidized low density lipoprotein (LDL)-induced ROS generation possibly through the inhibition of NADPH oxidase in bovine endothelial cells (Motoshima *et al.* 2004), and in human umbilical vein endothelial cells (HUVECs) by increasing glutathione levels (Plant *et al.* 2008). Furthermore, adiponectin counteracts high glucose-induced ROS production in HUVECs through a mechanism dependent on the cAMP/PKA pathway (Ouedraogo *et al.* 2006).

Consistent with the above *in vitro* observations, aortic segments isolated from adiponectin knockout mice exhibit an increase in both superoxide anion and peroxynitrite levels, and these changes are reversed by treatment with recombinant adiponectin (Cao *et al.* 2009). In aortic rings isolated from rats fed with a high-fat diet for 14 weeks, recombinant adiponectin markedly suppresses the obesity-induced elevation in superoxide anion and peroxynitrite production (Li *et al.* 2007b), resulting in an increase in eNOS activity and a reduction in iNOS activity in the hyperlipidaemic blood vessels (Li *et al.* 2007b). Likewise, both the cardio-protective and hepato-protective effects of adiponectin have been attributed to its anti-oxidant actions (Tao *et al.* 2007, Zhou *et al.* 2008), possibly through the induction of mitochondrial uncoupling protein-2 (UCP-2). These findings in animals are supported by clinical data showing a negative association between the plasma level of adiponectin and markers of oxidative stress such as the urinary secretion of 8-epi-prostaglandin-F2α (Zhu *et al.* 2008). However, little is known about the molecular pathways underlying the anti-oxidant actions of adiponectin at this stage.

Preventing endothelial cell activation and monocyte adhesion. Endothelial cell activation, characterized by the elevated expression of adhesion molecules [such intercellular adhesion molecule-1 (ICAM-1), cell adhesion molecule-1 (VCAM-1) and E-selectin] and the production of pro-inflammatory cytokines (TNFα, IL1 and IL8), is a key pathological event leading to vascular inflammation and atherosclerosis. Adiponectin suppresses the TNFα and resistin-induced expression of adhesion molecules as well as the production of IL8, resulting in the attenuation of monocyte attachment to the endothelial cells (Kobashi *et al.* 2005). This anti-inflammatory effect of adiponectin in endothelial cells appears to be mediated by PKA-dependent suppression of NF-κB activation (Ouchi *et al.* 2000). In human aortic endothelial cells, adiponectin suppresses high glucose (15 mM)-induced IkappaB (IκB) phosphorylation and NF-κB binding activity, leading to a reduction in mRNA and protein expression of the pro-inflammatory C-reactive protein (Devaraj *et al.* 2008). In animals, the adenovirus-mediated expression of adiponectin decreases the expression of adhesion molecules in the aortic tissue of apoE-deficient mice (Okamoto *et al.* 2002) and in a rabbit model of atherosclerosis (Li *et al.* 2007a). In addition, the over-expression of adiponectin receptors (adipoR1 and adipoR2) in rat carotid arteries significantly reduces

the expression of adhesion molecules such as ICAM-1 (Zhang *et al.* 2009). In the microcirculation of adiponectin-deficient mice, an increase in leukocyte rolling and adhesion has also been observed as a result of the reduced NO production and the augmented expression of E-selectin and VCAM-1 in the endothelium. System administration of recombinant adiponectin to adiponectin-deficient mice abolishes the leukocyte-endothelial cells interactions by reducing the expression of adhesion molecules in an eNOS-dependent manner (Ouedraogo *et al.* 2007).

Improving EPC functions and vascular repair. Endothelial progenitor cells are active players in endothelial repair after vascular injury. A reduced number of circulating EPC is an independent risk factor for cardiovascular disease, supporting the important roles of those cells in maintaining cardiovascular health (Hill *et al.* 2003, Tousoulis *et al.* 2008, Sibal *et al.* 2009). Adiponectin has been implicated in modulating EPC function, as evidenced by the increase in EPC number and differentiation upon adenovirus-mediated delivery of adiponectin in adiponectin-knockout mice (Shibata *et al.* 2008). In diabetic rats, the reduction in circulating EPCs and endothelial repair are associated with a decreased serum level of adiponectin (Sambuceti *et al.* 2009). Treatment of diabetic mice with cobalt protoporphyrin (CoPP), an inducer of the anti-oxidant heme oxygenase-1 (HO-1), results in up-regulation of adiponectin expression, leading to the restoration of vascular repair by improving both the number and the function of EPC. The favourable effects of adiponectin on EPC function appear to be mediated by eNOS activation (Wegiel *et al.* 2010).

The above experimental observations are also supported by clinical data showing that hypoadiponectinaemia is correlated with defective EPC function and low EPC numbers in diabetic patients (Makino *et al.* 2008). In addition, the circulating number of EPC is up-regulated by treatment with the PPAR-γ agonist pioglitazone in patients with coronary heart disease (Werner *et al.* 2007) and type 2 diabetes (Makino *et al.* 2008). Nevertheless, whether or not the beneficial effects of the PPARγ agonists on EPC number and function are mediated by the induction of adiponectin is currently unclear.

Inhibiting macrophage activation and foam cell formation. Endothelial dysfunction progresses into the development of atherosclerotic plaques when monocytes infiltrate into the subendothelial space of the arterial wall and differentiate into macrophages (Libby 2002). Activated macrophages express scavenger receptors that bind to internalized lipoprotein particles modified by oxidation or glycation, transforming themselves into arterial foam cells. The pro-inflammatory cytokines that mediate the local inflammatory response in the lesion are released by the foam cells, further amplifying the atherosclerotic progression (Libby *et al.* 2010).

Besides modulating macrophage polarization, adiponectin dampens macrophage inflammation and foam cell formation (Zhu *et al.* 2008). The inhibitory effects of adiponectin on macrophage-to-foam cell transformation may be mediated by its ability to suppress class A scavenger receptor expression, resulting in reduced uptake of acetylated LDL particles (Ouchi *et al.* 2001). In addition, adiponectin decreases the activity of acyl-coenzyme A: cholesterol acyltransferase, the enzyme that catalyses cholesteryl ester formation and enhances macrophage-to-foam cell transformation (Furukawa *et al.* 2004).

Both globular and full-length forms of adiponectin suppress leptin- and/or lipopolysaccharide (LPS)-induced macrophage secretion of pro-inflammatory cytokines, such as TNFα, IL1, IL6 and IL8 (Saijo *et al.* 2005, Tsatsanis *et al.* 2005, Zhang *et al.* 2005, Thakur *et al.* 2006, Park *et al.* 2007). Prolonged treatment of macrophages with adiponectin desensitizes LPS-induced activation of NF-κB and extracellular-signal-regulated kinase (ERK) 1/2 (Wulster-Radcliffe *et al.* 2004, Yamaguchi *et al.* 2005). By contrast, acute treatment with adiponectin triggers the release of TNFα and IL6 (Tsatsanis *et al.* 2005, Park *et al.* 2007) *via* NF-κB and ERK1/2 activation, which subsequently causes an induction of IL10, an anti-inflammatory cytokine that renders macrophages tolerant to further stimulation by LPS or other pro-inflammatory cytokines (Park *et al.* 2007). Globular adiponectin induces IL10 production by stimulating the phosphorylation of cAMP-response element binding protein, consequently leading to the transactivation of the IL10 promoter in macrophages (Park *et al.* 2008). Both adipoR1 and adipoR2 are expressed in monocytes and macrophages. However, there is currently no evidence suggesting that these two receptors mediate the actions of adiponectin in macrophages.

Reducing the proliferation and migration of vascular smooth muscle cells. Adiponectin exerts an inhibitory effect on the proliferation and migration of VSMC (Arita *et al.* 2002, Okamoto *et al.* 2002, Wang *et al.* 2005), through two distinct mechanisms. First, adiponectin interacts in an oligomerization-dependent manner with different atherogenic growth factors, including heparin-binding epidermal growth factor-like growth factor, platelet-derived growth factor and basic fibroblast growth factor (Arita *et al.* 2002, Wang *et al.* 2005), subsequently blocking the binding of these growth factors to their respective cell membrane

receptors (Wang *et al.* 2005). Second, adiponectin inhibits insulin growth factor-1-induced ERK1/2 activation and proliferation of VSMC derived from aortic rings through AMPK phosphorylation (Motobayashi *et al.* 2009). Adiponectin-deficient mice display enhanced proliferation of VSMC and increased neointimal thickening after mechanical injury compared with wild-type controls (Kubota *et al.* 2002), and these changes are reversed by adenovirus-mediated expression of adiponectin (Okamoto *et al.* 2002).

Conclusions and future perspectives

Both the animal studies and clinical investigations at hand demonstrate that the cross-talk between adipose tissue and the vasculature appears to play an important role in maintaining vascular homeostasis. Adipose tissue, particularly PVAT, modulates vascular reactivity, inflammation and remodelling through the release of a broad range of bioactive substances, including ADRF, ADCF, adipokines and chemokines.

Unlike most adipose-released adipokines with detrimental effects on the vasculature, adiponectin exerts multiple favourable effects through its pleiotropic actions on the adipo-vascular axis, as it targets adipocytes, macrophages, mature endothelial cells, EPCs and VSMCs. Therefore, adiponectin is a key component of the 'adipo-vascular axis' that mediates the cross-talk between adipose tissue and the blood vessel wall, and a reduced production and/or impaired action of adiponectin represents a key mechanism that links obesity and cardiovascular disease.

In light of the aetiological roles of adiponectin deficiency (hypoadiponectinaemia) in the pathogenesis of obesity-related vascular disease, pharmacological interventions to increase adiponectin production represent an attractive strategy for the treatment of these diseases. Indeed, many anti-diabetic and cardiovascular drugs, including PPARγ agonists (thialozidinediones) and the PPARα agonists (fenofibrates), renin–angiotensin system blocking drugs (angiotensin-converting-enzyme inhibitors and angiotensin-II receptor blockers), glucose-lowering agents (glimepiride), third-generation β-adrenergic blockers (nebivolol) and cannabinoid CB1 receptor antagonists (rimonabant) increase circulating levels of adiponectin in patients with diabetes and cardiovascular disease (Zhu *et al.* 2008). However, whether or not the cardiovascular benefits of these drugs are mediated in part by the up-regulation of adiponectin remain unclear and warrants further investigation.

Although the vascular functions of adiponectin have been extensively investigated, most of the available studies are based on the use of genetically modified mice. However, the vascular structure and physiology in mice is quite different from that in humans. Therefore, further studies on large animal models, such as rabbits and pigs, are needed to confirm the vascular functions of adiponectin. In addition, it is also of paramount importance to determine the physiological role of the two adiponectin receptors (adipoR1 and adipoR2) and their downstream signalling in the vascular system in both small and large animals. Further studies in this exciting field will provide important information to develop new therapeutic approaches for treating and/or preventing obesity-related diabetic and vascular disease by targeting the 'adipo-vascular axis'.

Conflict of interest

The authors declare no conflict of interest.

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