

REVIEW

Perivascular fat, AMP-activated protein kinase and vascular diseases

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Perivascular adipose tissue (PVAT) is an active endocrine and paracrine organ that modulates vascular function, with implications for the pathophysiology of cardiovascular disease (CVD). Adipocytes and stromal cells contained within PVAT produce mediators (adipokines, cytokines, reactive oxygen species and gaseous compounds) with a range of paracrine effects modulating vascular smooth muscle cell contraction, proliferation and migration. However, the modulatory effect of PVAT on the vascular system in diseases, such as obesity, hypertension and atherosclerosis, remains poorly characterized. AMP-activated protein kinase (AMPK) regulates adipocyte metabolism, adipose biology and vascular function, and hence may be a potential therapeutic target for metabolic disorders such as type 2 diabetes mellitus (T2DM) and the vascular complications associated with obesity and T2DM. The role of AMPK in PVAT or the actions of PVAT have yet to be established, however. Activation of AMPK by pharmacological agents, such as metformin and thiazolidinediones, may modulate the activity of PVAT surrounding blood vessels and thereby contribute to their beneficial effect in cardiometabolic diseases. This review will provide a current perspective on how PVAT may influence vascular function via AMPK. We will also attempt to demonstrate how modulating AMPK activity using pharmacological agents could be exploited therapeutically to treat cardiometabolic diseases.

Abbreviations

ACC, acetyl-CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide 1- β -D-ribose nucleoside; AMPK, AMP-activated PK; BAT, brown adipose tissue; CAD, coronary artery disease; CTRP12, C1q/TNF-related protein-12; CVD, cardiovascular disease; DIO, diet-induced obesity; ER, endoplasmic reticulum; GLUT4, glucose transporter type 4; HDL, high-density lipoprotein; HFD, high-fat diet; HGF, hepatocyte growth factor; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LKB1, liver kinase B1; MPO, myeloperoxidase; mTOR, mammalian target of rapamycin; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; PVAT, perivascular adipose tissue; PVRFs, PVAT-derived relaxant factors; SMCs, smooth muscle cells; SOD, superoxide dismutase; T2DM, type 2 diabetes mellitus; TZDs, thiazolidinediones; WAT, white adipose tissue

Introduction

Cardiovascular disease (CVD) remains the most common cause of death worldwide. The inexorable rise in diabetes and obesity will continue to shorten many lives and be a huge burden on healthcare budgets throughout the world (Trujillo and Scherer, 2006). Evidence from several studies, including the Framingham Heart Study, has demonstrated that obesity

is closely associated with increased vulnerability to insulin resistance, type 2 diabetes mellitus (T2DM), hypertension, coronary artery disease (CAD), myocardial infarction (MI) and sudden death, congestive heart failure and stroke (Henry *et al.*, 2002; Galassi *et al.*, 2006). However, the pathophysiological mechanisms underlying the relationship between obesity and CVD remain poorly understood. Dysfunctional perivascular adipose tissue (PVAT) is a good candidate as a

link between obesity and cardiovascular risk due to the direct influence PVAT can exert on blood vessel health. AMP-activated protein kinase (AMPK) regulates adipocyte metabolism, adipose biology and vascular function, such that AMPK activation is an attractive therapeutic target for metabolic disorders such as T2DM and the vascular complications associated with obesity and T2DM. Recent studies have identified AMPK as a potential regulator of PVAT and also a target of PVAT action in the blood vessel.

Adipose tissue

Structurally, all adipose tissue consists of mature adipocytes containing lipid droplets, T lymphocytes, macrophages, collagen fibres, fibroblasts, preadipocytes, blood vessels and nerves. In general, adipose tissues have been classified into white adipose tissue (WAT) and brown adipose tissue (BAT) according to the size of lipid droplets and the number of mitochondria within the adipocytes. Adipocytes in WAT are small with single, large lipid droplets and variable numbers of mitochondria. Fat cells in BAT are larger in size with multiple small lipid droplets and larger numbers of mitochondria (Cinti, 2005; Nedergaard *et al.*, 2007). WAT is widely distributed in subcutaneous areas and around visceral organs, whereas BAT, which was originally thought to be present mainly in the intrascapular region in rodents and human infants, has recently been discovered in supraclavicular, suprarenal, cervical and periaortic areas (Cinti, 2005). BAT is classically linked to thermogenesis and is associated with metabolically active adipocytes that contain uncoupling protein-1 within the mitochondria (Cannon and Nedergaard, 2004; Frontini *et al.*, 2007). Whereas WAT can be considered to be a lipid storage depot, BAT is more vascularized and metabolically active, with greater noradrenergic innervation (Cannon and Nedergaard, 2004; Cinti, 2011).

Perivascular adipose tissue

PVAT is the adipose tissue surrounding the vasculature, which manifests a varied histological appearance according to the type of vessel it is surrounds. PVAT has been reported to be composed of both BAT and WAT, with different ratios depending on the location (Cinti, 2011). For instance, in Sv129 mice, PVAT around coronary arteries and thoracic aorta is composed mainly of BAT, PVAT in iliofemoral blood vessels has an equal amount of both types, whereas PVAT present around the abdominal aorta and mesenteric arteries consists mainly of WAT (Frontini and Cinti, 2010). In a similar manner, PVAT in Sprague-Dawley rats surrounding the thoracic aorta is mainly BAT, whereas PVAT in abdominal aorta and mesenteric vessels is predominantly WAT (Wang *et al.*, 2009a). Findings from animal studies are consistent with data from human PVAT studies, in which PVAT surrounding the aorta and its main branches including subclavian, carotid, intercostals and renal arteries has been reported to contain BAT (Frontini and Cinti, 2010). However, data from coronary arteries have been conflicting, with PVAT dissected from the origin of human right coronary arteries expressing a high

level of BAT-related genes, whereas others have reported gene expression around human coronary arteries to be related to WAT (Sacks *et al.*, 2009). Such a disparity may be due to the method of harvesting the adipose tissue or to inter-individual variations in fat tissue distribution (Miao and Li, 2012). Interestingly, a very recent study has identified a strong correlation between presence of brown adipocytes and atherosclerotic plaque in 271 samples of human coronary artery (Salisbury *et al.*, 2012). The authors speculate that as brown adipocytes are involved in neovascularization, they may have an important role to play in vascular remodelling in diseases such as atherosclerosis. However, another study, which investigated epicardial adipose tissue, found a positive correlation between the markers of BAT and circulating high-density lipoprotein (HDL) and a negative correlation with circulating triglycerides (Chechi *et al.*, 2012). The data of Chechi *et al.* suggest that depots of BAT around the heart may have a beneficial effect on the atherogenic profile of circulating lipids. Thus, further investigation is required to determine the precise role of BAT on atherosclerotic disease.

PVAT: function and autocrine/paracrine activity

In addition to the release of free fatty acids (FFAs)/non-esterified fatty acids by lipolysis, adipose tissue secretes bioactive proteins that are collectively termed adipokines and have endocrine, autocrine and paracrine actions. PVAT-derived adipokines participate in the regulation of vascular function under normal physiological conditions. Despite the obvious paracrine effect of PVAT on vascular tissues, the extent of their endocrine effect remains elusive and they appear to have both beneficial and detrimental effects on vascular function and pathophysiology (see Mattu and Randeve, 2013).

More than 50 adipokines have since been identified and shown to have a wide spectrum of haemodynamic, metabolic and immunological effects (Trayhurn, 2005; Wood and Trayhurn, 2006). Adipokines can be classified according to their effect on cytokine levels as either pro-inflammatory, such as leptin, or anti-inflammatory, such as adiponectin and adrenomedullin. As emerging vascular modulators, adipokines including adiponectin, omentin, nesfatin, vaspin and chemerin have been proposed to play a role in the regulation of cardiovascular function (Table 1). The classification of some adipokines, such as resistin, can be somewhat blurred since it can be expressed by other cell types such as macrophages and participate in inflammation throughout the body (Jamaluddin *et al.*, 2012). Others such as visfatin, originally considered as adipocytokines, may actually be produced in greater quantities by the stromal vascular tissue within PVAT (Stastny *et al.*, 2012). Nevertheless, studies seem to indicate that generation of visfatin is elevated in obesity and metabolic syndrome and so it is likely to be an important pro-inflammatory mediator in cardiometabolic disease (Figure 2). In addition to release of adipokines, adipocytes in PVAT also produce classical chemokines (or cytokines) including IL-6, IL-8, CCL2 (MCP-1) and plasminogen-activator inhibitor-1 (Thalmann and Meier, 2007; Rajsheker *et al.*,

Table 1

PVAT-derived biologically active molecules with influence on vascular function and related cardiometabolic disorders

Adipokines/ Cytokines	Physiological effect	Effect on vasculature	Associated disease	PVAT dysfunction
Leptin	Pro-atherogenic Pro-inflammatory	Direct vasodilator (Nakagawa <i>et al.</i> , 2002; Sahin and Bariskaner, 2007) ↑ VSMC proliferation/migration (Oda <i>et al.</i> , 2001; Huang <i>et al.</i> , 2010) ↓ VSMC proliferation (Bohlen <i>et al.</i> , 2007) ↑ Vascular permeability (Cao <i>et al.</i> , 2001) ↑ TNF- α , IL-6, IL-12, ROS (Fitzgibbons <i>et al.</i> , 2011; Rittig <i>et al.</i> , 2012) Indirect vasoconstrictor (Fruhbeck, 1999)	Obesity Hypertension Atherosclerosis Insulin resistance	↑ Production in obesity (Marchesi <i>et al.</i> , 2009; Ketonen <i>et al.</i> , 2010) ↓ Production in hypertension (Galvez <i>et al.</i> , 2006) Effect on VSMC contraction is lost in hypertension (Rodriguez <i>et al.</i> , 2007) ↑ Production in atherosclerosis (Henrichot <i>et al.</i> , 2005; Eiras <i>et al.</i> , 2008)
Adiponectin	Anti-atherogenic Anti-inflammatory	Direct vasodilator (Fésüs <i>et al.</i> , 2007; Zhu <i>et al.</i> , 2008) ↓ VSMC proliferation/migration (Wang <i>et al.</i> , 2005; Lamers <i>et al.</i> , 2011) ↓ IFN- γ , IL-6, NF- κ B, TNF- α phagocytosis, endothelial adhesion molecules (Ouchi <i>et al.</i> , 1999; Engeli <i>et al.</i> , 2003) ↑ IL-10, IL-1RA (Wolf <i>et al.</i> , 2004)	Obesity Hypertension Atherosclerosis Insulin resistance T2DM	↓ Production in obesity (Marchesi <i>et al.</i> , 2009; Ketonen <i>et al.</i> , 2010) ↓ Production in diabetes (Meijer <i>et al.</i> , 2013) ↓ Atherosclerosis (Henrichot <i>et al.</i> , 2005; Eiras <i>et al.</i> , 2008)
Resistin	Pro-atherogenic	↑ VSMC proliferation/migration (Shyu <i>et al.</i> , 2011) ↑ Endothelial adhesion molecule ↑ TNF- α , IL-6, NF- κ B (Tilg and Moschen, 2006)	Atherosclerosis Insulin resistance T2DM	↑ Endothelial injury (Shyu <i>et al.</i> , 2011)
Visfatin	Pro-atherogenic Pro-inflammatory	↑ VSMC proliferation/migration (Wang <i>et al.</i> , 2009a) ↑ Endothelium-dependent vasodilatation (Yamawaki <i>et al.</i> , 2009) ↑ TNF- α , IL-6, IL-8 (Tilg and Moschen, 2006) ↓ Apoptosis (Tilg and Moschen, 2006)	Atherosclerosis Insulin resistance T2DM	↑ Production in atherosclerosis (Henrichot <i>et al.</i> , 2005; Eiras <i>et al.</i> , 2008)
Omentin	Anti-atherogenic Anti-inflammatory	↑ eNOS (Tan <i>et al.</i> , 2010) ↓ NF- κ B (Tan <i>et al.</i> , 2010)	Atherosclerosis Metabolic syndrome	↓ Production in atherosclerosis, obesity and metabolic syndrome (Tan <i>et al.</i> , 2010)
Chemerin	Anti-atherogenic Anti-inflammatory	↓ TNF- α -induced VCAM-1 expression and ↓ Monocyte adhesion (Yamawaki <i>et al.</i> , 2012a)	Atherosclerosis	↑ Production in atherosclerosis (Yamawaki <i>et al.</i> , 2012a)
Nefastin	Contractile	Impair NO donor and SNP induced smooth muscle relaxation (Yamawaki <i>et al.</i> , 2012a)	Hypertension Obesity	↑ Levels in hypertension and obesity (Ramanjaneya <i>et al.</i> , 2010; Yamawaki <i>et al.</i> , 2012b)
Vaspin	Anti-inflammatory Anti-atherogenic	↓ SMC migration (Phalitakul <i>et al.</i> , 2012)	Atherosclerosis T2DM Obesity	↑ Levels in atherosclerosis, obesity and diabetes mellitus (Youn <i>et al.</i> , 2008; Phalitakul <i>et al.</i> , 2012)
Apelin	Anti-contractile Angiogenic	↑ Glucose utilization in skeletal muscle (Dray <i>et al.</i> , 2008) Antagonize the effect of Ang II (Chun <i>et al.</i> , 2008) ↑ NO production (Japp <i>et al.</i> , 2008)	Obesity Insulin resistance	↑ Levels in obesity and insulin resistance and heart failure (Chun <i>et al.</i> , 2008; Dray <i>et al.</i> , 2008)
Interleukins (IL-1, IL-6, IL-8), CCL2		↑ Endothelial proliferation (Henrichot <i>et al.</i> , 2005)	Atherosclerosis	↑ Production in atherosclerosis (Henrichot <i>et al.</i> , 2005; Eiras <i>et al.</i> , 2008)
HGF		↑ Endothelial proliferation (Rittig <i>et al.</i> , 2012) ↑ Cytokines release from SMCs (Rittig <i>et al.</i> , 2012)	Obesity	↑ Production in obesity (Bell <i>et al.</i> , 2006; Rittig <i>et al.</i> , 2012)
TNF- α	Pro-inflammatory	Vasodilator (Brian and Faraci, 1998) ↑ ROS ↑ Endothelial dysfunction (Zhang <i>et al.</i> , 2009a)	Obesity Hypertension Atherosclerosis T2DM	↑ Production in obesity, hypertension, atherosclerosis and T2DM (Zhang <i>et al.</i> , 2009a)

HGF, hepatic growth factor; NF- κ B, nuclear factor kappa-light-chain enhancer of activated beta cells; PVAT, perivascular adipose tissue; ROS, reactive oxygen species; T2DM, type 2 diabetes mellitus; VSMCs, vascular smooth muscle cells.

2010). Furthermore, inflammatory cells such as macrophages and T lymphocytes, fibroblasts and capillary endothelial cells, which are normally found in PVAT or attracted in response to inflammatory chemokines released by adipocytes, have also been demonstrated to contribute to the secretory profile of adipose tissue (see Szasz and Webb, 2012).

In addition to classical adipokines, PVAT can also produce angiotensin peptides, which, along with angiotensinogen, angiotensin-converting enzymes and receptors, are part of the renin-angiotensin-aldosterone system (Lu *et al.*, 2010). PVAT also releases reactive oxygen species (ROS), including superoxide (Gao *et al.*, 2006), H₂O₂ (Gao *et al.*, 2007) and gaseous molecules such as H₂S (Schleifenbaum *et al.*, 2010). In denuded vessels, generation of H₂O₂ induces relaxation via phosphorylation of smooth muscle contractile proteins (Gao *et al.*, 2007), whereas an enzyme present in PVAT, cystathionine γ -lyase, can generate H₂S. The H₂S induces relaxation by opening voltage-gated potassium channels in the vascular smooth muscle cells (SMCs) to cause hyperpolarization (Schleifenbaum *et al.*, 2010). In contrast, superoxide and angiotensin can potentiate vasoconstriction to electrical field stimulation *in vitro* (Gao *et al.*, 2006; Lu *et al.*, 2010). Another adipokine, apelin, has been found to induce NO-dependent vasorelaxation of peripheral and splanchnic human arteries both *in vitro* and *in vivo* (Salcedo *et al.*, 2007a; Japp *et al.*, 2008). As PVAT can have a dual effect on vessel tone, work has focused on how the function of PVAT changes in disease states as this is a key to understanding the role of PVAT in disease progression. Table 1 summarizes the major adipokines that are proposed to influence cardiovascular function.

The recent interest in adipose tissue as an active endocrine and paracrine organ has resulted in a number of studies examining the properties of different fat depots in the body. Production of adipokines and cytokines from the PVAT sur-

rounding small arteries, coronary arteries, aorta and systemic vessels can modulate inflammation, contractile activity of medial smooth muscle and endothelial function, all of which are likely to be important in diseases including atherosclerosis and hypertension (reviewed in Szasz *et al.*, 2013) (Table 1). The vasa vasorum present in the adventitia of the blood vessel promotes neovascularization following vascular damage and inflammation, facilitating transport of vasoactive factors released by PVAT to the inner layers of the vessel wall (Gossl *et al.*, 2009). A close structural communication between the PVAT and inner vasculature has been demonstrated in studies showing luminal termination of vasa vasorum in the inner vasculature in vessels such as the saphenous vein (Dashwood *et al.*, 2007).

Furthermore, the cellular composition of PVAT may vary according to age, nutritional status and environmental conditions. For instance, periaortic PVAT has been found to expand and contain more inflammatory cells in response to obesity (Skilton *et al.*, 2009). PVAT adipocyte size also shows variation and heterogeneity in CVD. For example, the size of pericardial adipocytes near the proximal part of the right coronary artery in coronary heart disease (CAD) patients is increased in contrast with non-CAD patients (Silaghi *et al.*, 2007). This implies that changes occur in PVAT in disease states, although the effect this has on the endocrine or paracrine functions of PVAT is still unclear.

PVAT in vascular diseases

Obesity accompanied by increased PVAT mass around blood vessels is a leading cause of vascular disease, leading to the hypothesis that dysfunctional PVAT in obese individuals may be an important inducer of vascular dysfunction (Figure 1). *In*

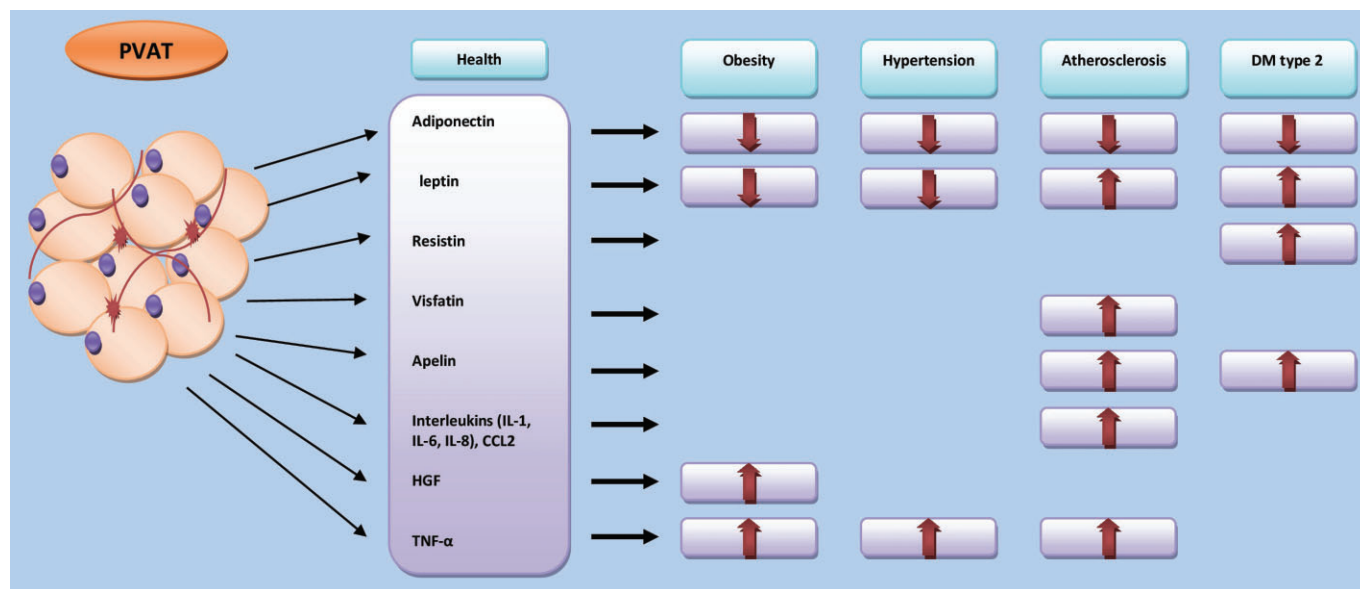


Figure 1

Changes in the PVAT production of adipokines and cytokines in cardiometabolic disease. The figure illustrates the alteration in the secretory profile of PVAT in obesity, hypertension and atherosclerosis. These diseases are associated with either reduction of adipokines release such as adiponectin or increased release of some other such as TNF- α and visfatin. DM, diabetes mellitus; HGF, hepatic growth factor; PVAT, perivascular adipose tissue.

vitro studies have shown that the vasoconstrictor effect of noradrenaline is attenuated in vessels with PVAT (Soltis and Cassis, 1991), whereas a study in lipotrophic mice, which are characterized by loss of adipose tissue (including PVAT), showed spontaneous vascular dysfunction leading to hypertension (Guzik *et al.*, 2007). However, it should be noted that lipotrophic mice exhibit a number of phenotypes, which could account for changes within the vasculature. A recent study using lipotrophic BAT-specific insulin receptor knockout mice found adipocytokine overexpression, insulin resistance and inflammatory marker expression in the endothelium and vascular dysfunction at 1 year (Gomez-Hernandez *et al.*, 2012). Treatment with an antibody to TNF- α reversed many of these effects, highlighting the importance of inflammatory adipocytokines in disease progression. Table 1 summarizes the major adipokines that are proposed to influence cardiovascular function and correlated disease.

PVAT in atherosclerosis

Atherosclerosis is characterized by a persistent inflammatory response that promotes development of atheromatous plaque in the tunica media and intima of the blood vessel wall (Ross, 1993). However, growing evidence suggests that the adventitia may also contribute to the development of atherosclerosis through accumulation of myofibroblasts, leading to constriction of the external elastic lamina and vascular remodelling (Scott *et al.*, 1996). Inhibition of myofibroblast proliferation by, for example, intravascular brachytherapy can induce marked vessel thinning (Wilcox *et al.*, 2001). Conversely, vascular proliferative disorders such as restenosis following balloon angioplasty are associated with inflammatory responses that extend into PVAT, suggesting an integral role of PVAT in remodelling and neointima formation (Okamoto *et al.*, 2001; Wilcox *et al.*, 2001). Wire or balloon injury to the lumen of mouse and rat vessels significantly increased the expression of pro-inflammatory adipocytokines and reduced adiponectin, an effect that was absent in TNF- α knockout (KO) mice and which could be replicated by TNF- α application to the perivascular area of the vessel (Takaoka *et al.*, 2010). Furthermore, the same group showed that TNF- α KO mice had reduced neointimal formation, reinforcing the potential importance of PVAT-derived inflammatory mediators in vascular remodelling. A very recently published study has indicated that the adipokine C1q/TNF-related protein-9 attenuates neointima formation in the wire-injured obese mouse femoral artery (Uemura *et al.*, 2013). The reduction in neointima was associated with reduced vascular SMC proliferation via a cAMP-PKA-dependent pathway. Conversely, neointimal area following femoral artery injury in both wild-type and leptin-deficit mice was enhanced by leptin and this was due to activation of the key regulator of protein and amino acid metabolism, mammalian target for rapamycin (mTOR) (Shan *et al.*, 2008).

In PVAT from organ donors without atherosclerosis, perivascular adipocytes are characterized by a reduced level of adipocytic differentiation as compared with adipocytes derived from subcutaneous and visceral (perirenal) adipose depots. Secretion of anti-inflammatory adiponectin is mark-

edly reduced, whereas that of pro-inflammatory cytokines IL-6, IL-8 and CCL2 is markedly increased in perivascular adipocytes. Likewise, PVAT harvested from murine aortic arch expresses lower levels of adipocyte-associated genes as compared with subcutaneous and visceral adipose tissues. Furthermore, 2 weeks of high-fat feeding caused further reductions in adipocyte-associated gene expression, while up-regulating pro-inflammatory gene expression, in PVATs. Changes in adipocyte differentiation and secretion were observed in the absence of macrophage recruitment to the PVAT, indicating that these properties are intrinsic to the adipocytes residing in this depot. Dysfunction of PVAT induced by fat feeding suggests that PVAT is capable of linking metabolic signals to inflammation in the blood vessel wall (Chatterjee *et al.*, 2009). Furthermore, the accumulation of macrophage and T lymphocytes at the interface of PVAT and the adventitia of human atherosclerotic aorta suggests pro-chemotactic activity of PVAT (Henrichot *et al.*, 2005). A recent study investigated the relationship between development of atherosclerosis and pro-inflammatory PVAT by transplantation of visceral fat to the mid-perivascular area of the carotid artery in apolipoprotein-E-deficient mice. Transplant of visceral WAT (inflammatory fat with a higher macrophage content) stimulated development of atherosclerotic lesions in carotid artery accompanied by an increased level of serum CCL2 and enhanced endothelial dysfunction. Such a detrimental effect was not seen with transplantation of subcutaneous fat and could be ameliorated by an antibody to P-selectin glycoprotein ligand (Ohman *et al.*, 2011). This important study demonstrates very elegantly that the pro-inflammatory and pro-atherogenic properties of adipose tissue in the hypercholesterolaemic state can have an adverse influence on vascular function and plaque formation.

PVAT may also contribute to atherosclerosis through release of angiogenic factors such as hepatocyte growth factor (HGF), acidic fibroblast growth factor, thrombospondin-1, serpin-E1, CCL2 and insulin-like growth factor-binding protein-3 (Rittig *et al.*, 2012). Indeed, as HGF is responsible for the stimulation of endothelial cell growth and cytokine secretion from SMCs, it is worth noting that PVAT mass, but not visceral or subcutaneous fat mass, is correlated with the level of HGF in patients with atherosclerosis, further reinforcing the link between increased PVAT, angiogenesis and remodelling in atherosclerosis (Rittig *et al.*, 2012).

Anti-atherosclerotic properties of PVAT

Atherosclerosis is well known to be associated with impaired energy metabolism and endothelial dysfunction in addition to inflammation (Hennig and Chow, 1988). BAT is involved in the regulation of energy expenditure in humans, and since some adipocytes within PVAT possess the morphological features of BAT, it might be anticipated that the fat surrounding the blood vessels can control the local energy metabolism of the vessel wall. Studies have shown that PVAT can generate heat, which helps in maintaining intravascular temperature and correlates with increased activity of metabolic enzymes in mice housed at 16°C (Chang *et al.*, 2012). In this study, development of atherosclerotic plaques in the mice was attenuated and serum triglyceride concentrations decreased

markedly. In mice lacking smooth muscle PPAR γ , an absence of PVAT was found, which caused endothelial dysfunction and temperature loss (Chang *et al.*, 2012). Epidemiological studies have reported that both extreme hot and cold environments increase the rate of death due to heart attack (Medina-Ramon and Schwartz, 2007); however, there are no clinical data correlating cold exposure with protection from the risk of atherosclerosis in humans. PVAT, by regulating temperature and metabolic activity in the vessel, may well have an important role in protection against adverse cardiovascular events. Indeed, many studies have noted an inverse correlation between adipose-derived adiponectin and risk of CAD. As adiponectin has anti-inflammatory and anti-atherogenic properties, it may have a predictive value for the genesis and development of acute coronary syndrome (Luo *et al.*, 2013). The role of PVAT-derived adiponectin compared with total serum adiponectin with respect to cardiovascular risk remains uncertain, however.

Effect of PVAT on vessel contractility and hypertension

Much evidence indicates that PVAT can exert anti-contractile effects in different vascular beds, an effect assumed to be due to release of PVAT-derived relaxant factors (PVRFs). Furthermore, the anti-contractile effect of PVAT is impaired in diseases such as hypertension (Lu *et al.*, 2011; Galvez-Prieto *et al.*, 2012) and metabolic syndrome (Payne *et al.*, 2010; Ma *et al.*, 2010b). However, to date, the identity of PVRF(s) has not been defined and the PVRFs may include adiponectin (Fésüs *et al.*, 2007), H₂S (Wojcicka *et al.*, 2011), NO (Gao *et al.*, 2007) and angiotensin (Ang) 1–7 (Lee *et al.*, 2009a). Recent studies have also demonstrated that palmitic acid methyl esters released by PVAT exert anti-contractile effects via opening of voltage-dependent K⁺ channels in SMCs (Lee *et al.*, 2011). Another study has indicated that prostacyclin secreted by PVAT may act as a PVRF and protect against endothelial dysfunction (Chang *et al.*, 2012). Although it is well established that prostacyclin is synthesized by endothelial cells of blood vessels, PVAT-derived prostacyclin may act as a secondary source of this vasodilator when the endothelium is dysfunctional in diseases such as atherosclerosis (Chang *et al.*, 2012). PVRFs may induce endothelium-dependent vasodilation by the release of NO followed by activation of smooth muscle K⁺ channels. A role for NO was supported by experiments where aortic rings devoid of PVAT were found to relax when transferred into an incubation solution containing PVAT in a manner sensitive to removal of the endothelium, NO synthase inhibition, high extracellular K⁺ or blockade of calcium-dependent K⁺ channels (Gao *et al.*, 2007). PVRF may also induce an anti-contractile effect by an endothelium-independent mechanism involving release of H₂O₂ and activation of GC activity. This was observed in aortic rings denuded of endothelium where the effect of PVAT on the contraction to phenylephrine was attenuated by catalase or GC inhibitors and abolished by superoxide dismutase (SOD) and iron (Gao *et al.*, 2007). In addition, Lu and colleagues found that SHR had enhanced contractile responses to phenylephrine when PVAT was present. Based on pharma-

cological data, the vessels from SHR were also less sensitive to the anti-contractile effects of Ang1-7 released from PVAT (Lu *et al.*, 2011). In lipotrophic mice, which also exhibit increases in BP, Takemori and co-workers also found enhanced contraction to phenylephrine and 5-HT and increased expression of angiotensin AT₁ receptors (receptor nomenclature follows Alexander *et al.*, 2013) in the aorta, all of which could contribute to the raised BP when PVAT is absent (Wartmann *et al.*, 1995).

PVAT may also exert contractile effects through release of diffusible factors. It has been reported that all components of the renin–angiotensin system, except renin, can be found in PVAT (Hermenegildo *et al.*, 2005). Moreover, it was reported that three isoforms of AT_{1a} receptors exist in PVAT with low expression of AT_{1b} receptors (Galvez-Prieto *et al.*, 2008). An early study by Soltis and Cassis (1991) also demonstrated a reduced sensitivity to noradrenaline in rat vessels containing PVAT but noted that electrical stimulation induced contraction only in vessels with PVAT. This response was blocked using the non-selective α -adrenoceptor antagonist phen-tolamine and attenuated by an Ang II receptor antagonist, indicating that PVAT may release angiotensin (Soltis and Cassis, 1991). Enalaprilat, an ACE inhibitor has also been reported to attenuate the response of rat mesenteric arteries to electrical stimulation. Interestingly, exogenous Ang II was found to elicit vascular contraction in blood vessels without PVAT, but not in arteries with intact PVAT, suggesting that Ang II is significantly involved in PVAT-induced contractile effects (Lu *et al.*, 2010). PVAT has also been reported to induce a contractile response to perivascular nerve stimulation via production of superoxide by NAD(P)H oxidase. This mechanism was proposed to involve activation of both tyrosine kinase and the ERK1/2 pathway (Gao *et al.*, 2006).

PVAT in obesity and diabetes

The metabolic balance in normal adipose tissue favours a non-inflammatory state. However, with the development of obesity, the metabolic balance changes and favours chronic low-grade inflammation. The latter has been proposed to be the link between obesity, insulin resistance and CVD (Yudkin *et al.*, 2005; Zhang and Zhang, 2009b). However, the cellular mechanisms leading to these changes are poorly understood. Obesity is defined as an increase in adipose tissue mass, and as this increases, there are also changes at morphological and functional levels in both BAT and WAT. Morphologically, there are changes in adipocyte type, fatty acid composition of lipid vesicles, cell size, cell number, inflammatory cell infiltration, extracellular matrix remodelling and structure. At a functional level, these changes result in an imbalance in adipokine production, oxidative stress, hypoxia and inflammation (Achike *et al.*, 2011).

Total PVAT mass is increased in obese humans, which is accompanied by a diminished anti-contractile effect and the appearance of signs of local inflammation and hypoxia. The latter effects are mimicked by IL-6 and TNF- α and alleviated upon application of SOD, catalase and TNF- α antagonists (Lee *et al.*, 2009b). Moreover, data from the Framingham Heart Study has demonstrated that periaortic fat mass is associated with diabetes, even if corrected for body mass index (Lehman

et al., 2010). Reports from animal models of obesity show an increased PVAT mass and adipocyte size (Ketonen *et al.*, 2010; Ma *et al.*, 2010b), coupled with a noticeable loss of PVAT-mediated anti-contractile activity. The loss of the anti-contractile effect was proposed to be due to impaired adipokine release, inflammation and oxidative stress. Secretion of leptin is also uniformly increased in diet-induced obesity (DIO) in animal models (Takemori *et al.*, 2007; Ketonen *et al.*, 2010). In coronary arteries from pigs with metabolic syndrome, release of leptin appears to induce endothelial dysfunction via a PKC α -dependent mechanism (Payne *et al.*, 2010). Obesity in mice, induced through either high-fat diet (HFD) or genetic modification, is also associated with reduced adiponectin, evidence of macrophage infiltration, ROS formation, decreased expression of SOD and signs of endothelial NOS (eNOS) uncoupling (Marchesi *et al.*, 2009).

ROS appear to play a significant role in PVAT-induced vascular dysfunction in obesity (Ketonen *et al.*, 2010). Attenuated endothelium-dependent relaxation in obese mice has been reported to be reversed by removal of PVAT or application of ROS scavengers and is associated with increased expression of leptin, CCL2 and NADPH oxidase in PVAT (Ketonen *et al.*, 2010). In another study, chronic (6 months) high-fat feeding of rats was associated with endothelial dysfunction and correlated with a decrease in AMPK, eNOS and up-regulation of mTOR, which was reversed by removal of PVAT (Ma *et al.*, 2010b).

Furthermore, an increased intake of fructose, as demonstrated in a study using the fructose-fed rat, induces multiple metabolic and endocrine alterations, which are concomitant with significant changes in PVAT fatty acyl composition and oxidative stress biomarkers, which are associated with changes in vascular reactivity. PVAT fatty acyl lipid composition showed significant changes in rats given a fructose-rich diet (FRD), mainly an increase in saturated fatty acids (FAs) and a decrease in monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), resulting in an increase of the saturated/MUFA and saturated/PUFA ratios. The PVAT redox state was also modified by the fructose overload, as shown by a reduced activity of antioxidant enzymes such as SOD and GSH-Px, which account for the excessive production of superoxide anion and organic peroxides, and a higher NO production, which may account for the activation of the inducible isoform of NO synthase in response to the augmented oxidative stress. It is likely that these findings account for the diminished anti-contractile effect of PVAT in the aortic vessels (Rebolledo *et al.*, 2010).

It has been proposed that dysfunction of PVAT is associated with a reduction in insulin-stimulated glucose transport by reducing muscle perfusion (Meijer *et al.*, 2011). Adiponectin released from PVAT has been reported to affect insulin sensitivity, inflammatory responses, appetite, atherosclerosis and haemostasis (see Tilg and Moschen, 2006). Adiponectin/ACRP30 deficiency and high TNF- α levels in adiponectin KO mice reduced muscle FATP-1 mRNA and IRS-1-mediated insulin signalling, resulting in severe diet-induced insulin resistance (Engeli *et al.*, 2003). PPAR γ agonists such as the thiazolidinediones (TZDs) may owe at least part of their beneficial effect on insulin sensitivity to increasing levels of adiponectin *in vivo* (Engeli *et al.*, 2003). Indeed, adiponectin-deficient mice have reduced responsiveness to PPAR γ

agonists, implying that adiponectin is an important mediator for improving insulin responsiveness (Nawrocki *et al.*, 2006). Furthermore, in obese animals, administration of adiponectin has been found to improve insulin sensitivity and to decrease hyperglycaemia and the level of fatty acids in the plasma (Berg *et al.*, 2002; Fantuzzi, 2005). As adipose tissue mass increases, the expression of adiponectin is reduced and release of pro-inflammatory cytokines such as TNF- α and IL-6 increases (see Tilg and Moschen, 2006). Conversely, there is a correlation between increases in plasma leptin and insulin resistance and cardiac dysfunction in a rat model of T2DM (Chen *et al.*, 2007). The effects of leptin may be indirectly mediated by the brain where it activates the sympathetic nervous system, thereby leading to elevation of BP via inducing vasoconstriction and Na⁺ retention, and also in the major metabolic organs where it induces insulin resistance (reviewed in Gu and Xu, 2013). Inflammatory cytokines such as TNF- α may be associated with obesity-related insulin resistance. Many studies have shown that *ob/ob* mice (leptin-deficient mice that have insulin resistance) also have a reduction of TNF- α or TNF receptors. In this study, the TNF- α -deficient obese mice had lower levels of circulating FFAs and were protected from the obesity-related reduction in the insulin receptor signalling in muscle and fat tissues. These findings demonstrated that TNF- α is an important mediator of insulin resistance in obesity through its effects on several important sites of insulin action (Fukada *et al.*, 2005).

Moreover, C1q/TNF-related protein-12 (CTRP12), a novel adipokine with anti-diabetic effects, has recently been shown to improve insulin sensitivity in the liver and adipose tissue in *ob/ob* and DIO mice. In cultured adipocytes, CTRP12 activated the PI3K-Akt signalling pathway to promote glucose uptake (Wei *et al.*, 2012). The study by Wei *et al.* provides further evidence linking adipose tissue to global glucose homeostasis and highlights the consequences of dysfunctional adipose tissue on insulin sensitivity and vascular function.

AMP-activated protein kinase (AMPK)

AMPK is a serine/threonine protein kinase that has been proposed to function as an intracellular energy sensor and is involved in the regulation of cellular and whole body metabolism (Hardie, 2011). AMPK is essential for the maintenance of cardiovascular health, has a regulatory role over vascular structure and function, and is activated by several pathological stimuli, including oxidative damage, ischaemia, hypoxia/anoxia and cardiac hypertrophy (Nagata and Hirata, 2010; Ewart and Kennedy, 2011). The mechanism of action of many drugs commonly used in cardiovascular and metabolic disease, such as statins, metformin and the TZDs, may also involve AMPK (see Ewart and Kennedy, 2011), although the precise upstream and downstream pathways remain poorly defined.

AMPK is a heterotrimeric protein complex comprising a catalytic α subunit and two regulatory β and γ subunits. Two or more isoforms of each subunit (α 1, α 2, β 1, β 2, γ 1, γ 2 and γ 3) encoded by different genes give rise to 12 possible heterotrimeric combinations, with splice variants further increasing diversity. The α subunit of AMPK consists of an N-terminal

catalytic kinase domain and a C-terminal regulatory domain. Within the activation loop of the kinase domain is a threonine residue (Thr172), whose phosphorylation by upstream kinases is required for activation (Hawley *et al.*, 1996). The C-terminal region of the α subunit comprises an auto-inhibitory domain (Crute *et al.*, 1998) and a region required for binding to the β and γ subunits. The β subunit comprises a C-terminal domain required for association with the α subunit (Wong and Lodish, 2006) and a central region for glycogen binding (Polekhina *et al.*, 2003). The γ subunit comprises of tandem repeat cystathionine β -synthase motifs, known as Bateman domains, which are responsible for AMP, ADP or ATP binding (Cheung *et al.*, 2000; Xiao *et al.*, 2011).

The catalytic and regulatory subunit isoforms of AMPK differ in their tissue distribution, subcellular location and regulation (Cheung *et al.*, 2000; Hardie, 2007). Thus, the tissue-specific subunit composition may be important to determine a specialized cellular and systemic response to different metabolic stimuli (Kahn *et al.*, 2005). In adipose tissue and endothelium, the most predominant catalytic subunit isoform is $\alpha 1$ (Daval *et al.*, 2005; Davis *et al.*, 2006), whereas the $\alpha 2$ catalytic subunit is the most commonly found in skeletal and cardiac muscle. Equal distribution of both $\alpha 1$ and $\alpha 2$ isoforms has been shown in rat liver (Woods *et al.*, 1996). Whereas the $\beta 1$ subunit is ubiquitously expressed, AMPK containing the $\beta 2$ subunit is abundant in skeletal muscle and heart. Notably, expression of the $\gamma 3$ subunit appears highly specific to glycolytic skeletal muscle, whereas $\gamma 1$ and $\gamma 2$ show broad tissue distribution (Cheung *et al.*, 2000).

Regulation of AMPK expression and activity

Under normal physiological conditions, ATP occupies some of the Bateman domains of the γ subunit and maintains AMPK in an inactive state (Adams *et al.*, 2004; Scott *et al.*, 2004). With depletion of ATP and elevation of AMP or ADP during periods of stress such as hypoxia or glucose shortage, ATP is displaced, leading to direct allosteric activation of the kinase (AMP). The degree of activation is determined by the subtypes of catalytic α and regulatory γ isoforms present (Cheung *et al.*, 2000), and activation also induces conformational change in the kinase domain that protects AMPK α from dephosphorylation of Thr¹⁷² (Riek *et al.*, 2008; Xiao *et al.*, 2011). Such protection from dephosphorylation has been proposed to be the major physiological mechanism for activation of AMPK (Sanders *et al.*, 2007). Two well-characterized upstream AMPK α -Thr¹⁷² kinases (AMPKK) have been identified to date. They are liver kinase B1 (LKB1) and Ca²⁺/calmodulin-dependent protein kinase kinase β . The combination of allosteric modulation and phosphorylation leads to a >1000-fold increase in kinase activity (Hawley *et al.*, 2005; Xie *et al.*, 2006). Another kinase, termed TGF- β -activated kinase 1 (TAK1), was recently identified as an upstream kinase for AMPK based on a genetic screen for mammalian kinases. TAK1 was shown to activate the Snf1 protein kinase *in vivo* and *in vitro* and co-expression of TAK1 and its binding partner TAB1 in HeLa cells stimulated

phosphorylation of AMPK-Thr¹⁷² (Momcilovic *et al.*, 2006). Mice carrying a cardiac-specific dominant negative mutation for TAK1 reported signs of the Wolff–Parkinson–White syndrome, which is similar to those associated with mutations in human AMPK $\gamma 2$. Moreover, TAK1-deficient MEFs had reduced AMPK activation by oligomycin, metformin and 5-aminoimidazole-4-carboxamide 1- β -D-ribo-nucleoside (AICAR), leading the authors to propose that TAK1 has a pivotal role in the regulation of LKB1/AMPK signalling axis, an essential pathway in the regulation of cell metabolism (Xie *et al.*, 2006).

Pharmacological activators of AMPK

In addition to physiological activation of AMPK by increases in the cellular AMP/ATP ratio (see Ewart and Kennedy, 2011), many pharmacological AMPK activators have been identified. Metformin is an oral biguanide that activates AMPK in intact cells and *in vivo* (Musi and Goodyear, 2002; Musi *et al.*, 2002). Activation of AMPK by metformin is dependent on its uptake by organic cation transporter 1 (Shu *et al.*, 2008), and recent studies utilizing AMPK γ mutants that are insensitive to AMP have shown that AMP increases are also essential for metformin-stimulated AMPK activity (Hawley *et al.*, 2010). Other studies have suggested that ROS and reactive nitrogen species are also involved in metformin activation of AMPK (Zou *et al.*, 2004; Fujita *et al.*, 2010). The main site of therapeutic action of metformin is the liver, where stimulation of AMPK was originally thought to underlie a metformin-induced suppression of glucose production and increased fatty acid oxidation in hepatocytes (Zhou *et al.*, 2001). However, more recent studies have demonstrated that AMPK activity is completely dispensable for the anti-gluconeogenic action (Foretz *et al.*, 2010), yet it remains possible that AMPK activation underlies other effects of metformin. In the vasculature, metformin-induced AMPK activation has been reported to up-regulate eNOS phosphorylation, increase NO bioavailability (Calvert *et al.*, 2008; Zhang *et al.*, 2011) and reduce SMC proliferation, migration and inflammatory responses (Kim and Choi, 2012; Vigetti *et al.*, 2011). In cardiac tissue, metformin-induced AMPK activity sustains energy balance, cardiomyocyte function and myocardial viability (Cha *et al.*, 2010; Fu *et al.*, 2011). The reported cardioprotective effect is principally achieved by the reduction of hypertrophic cell growth and endoplasmic reticulum (ER) stress (Dong *et al.*, 2010a).

TZDs are a class of anti-diabetic drugs, including rosiglitazone, troglitazone and pioglitazone, which have been used to counteract insulin resistance in patients with T2DM by increasing the sensitivity of peripheral tissues to insulin (Marx *et al.*, 2004). They act by binding to PPAR γ , which promotes the synthesis of glucose transporters and proteins regulating lipid metabolism, leading to storage of lipids in adipocytes rather than hepatocytes and muscle (Semple *et al.*, 2006; Burns and Vanden Heuvel, 2007). PPAR γ receptors are nuclear hormone receptors that are expressed widely not only in adipose tissue but also in skeletal muscle (Hevener *et al.*, 2003), liver (Gavrilova *et al.*, 2003) and macrophages (Hevener *et al.*, 2003). TZDs exert pleiotropic effects in a manner similar to statins, and some of these effects are

observed acutely and do not require gene transcription. The acute effects of TZDs have been proposed to be via activation of AMPK in adipose tissue, skeletal muscle and liver (LeBrasseur *et al.*, 2006). The mechanism of AMPK activation is reported to be due to an increase in intracellular AMP/ATP ratio (Fryer *et al.*, 2002a). Similar to metformin and phenformin, TZDs have been demonstrated to act as inhibitors of complex 1 of the respiratory chain (Salcedo *et al.*, 2007b); thus, an increase in the AMP/ATP ratio may be the mechanism for AMPK activation in response to these drugs. Notably, this effect was reported to be independent of PPAR γ (Guh *et al.*, 2010). TZDs can also activate AMPK by stimulating adiponectin release from adipose tissue (Yamauchi *et al.*, 2002) and this effect may be important in PVAT. Both rosiglitazone and pioglitazone have been reported to have beneficial anti-atherosclerotic and anti-inflammatory effects (Stocker *et al.*, 2007), as well as an additional beneficial influence on endothelium via AMPK-dependent and PPAR- γ -independent mechanisms (Polikandriotis *et al.*, 2005; Ceolotto *et al.*, 2007). However, it should be noted that TZD use is associated with the risk of fluid retention which may exacerbate heart failure (Hannan *et al.*, 2003). There is also a concern that rosiglitazone is associated with additional cardiovascular (MI and stroke) risk in patients with T2DM (Soares de Moura *et al.*, 2004).

Statins are HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitors used in the treatment of metabolic syndrome and hypercholesterolaemia. Besides their cholesterol-lowering effects, statins have also been reported to activate AMPK in human and bovine endothelial cells (Sun *et al.*, 2006). Sun *et al.* also reported that while atorvastatin and lovastatin rapidly stimulate AMPK and eNOS in mouse myocardium and endothelial cells, they do not alter the cellular AMP/ATP ratio, suggesting a different pathway of AMPK activation (Sun *et al.*, 2006; Goirand *et al.*, 2007). Two days of treatment with fluvastatin (at a dose of 10 mM) has also been reported to stimulate eNOS and AMPK in human iliac endothelial cells. This effect was blocked by an eNOS inhibitor, implying that AMPK up-regulation was dependent on NO synthesis (Xenos *et al.*, 2005). In another study conducted in rat aorta, simvastatin up-regulated both AMPK and the upstream kinase LKB1. They also reported that this activation of AMPK was dependent on PKC ζ -mediated phosphorylation of LKB1 (Choi *et al.*, 2008). However, another study using HeLa cells expressing mutant LKB1 reported that phosphorylation of Ser⁴³¹ on LKB1 was not required for AMPK up-regulation (Fogarty and Hardie, 2009). In addition to the beneficial effects of statins on endothelial function, which are likely due to eNOS up-regulation (Laufs *et al.*, 2000; 2002), statins exert anti-inflammatory (Cahoon and Crouch, 2007) and anti-atherogenic effects (Nissen *et al.*, 2005). These observations all indicate that AMPK activation might be a key to the pleiotropic effects of statins on cardiovascular protection.

6,7-Dihydro-4-hydroxy-3-(2'-hydroxy[1,1'-biphenyl]-4-yl)-6-oxo-thieno[2,3-*b*]pyridine-5-carbonitrile (A769662) is a member of the thienopyridone family that activates AMPK both allosterically and by inhibiting dephosphorylation of the kinase at Thr¹⁷² (Cool *et al.*, 2006). A769662 is dependent on the β -subunit carbohydrate-binding module and γ subunit (Guh *et al.*, 2010) of AMPK and, notably, exclusively activates trimers containing the β 1 subunit (Scott *et al.*, 2008). In *in*

vitro studies, A769662 has been shown to stimulate phosphorylation of acetyl-CoA carboxylase (ACC) independently of the upstream kinases LKB1 and CaMKK (Cool *et al.*, 2006; Goransson *et al.*, 2007). In cell-free assays, it has no direct effect on the ability of LKB1 or CaMKK to phosphorylate AMPK. The mechanism of AMPK activation by A769662 is thought to be distinct from that of AMP, as A769662 can still activate AMPK containing a mutation in the γ subunit, whereas AMP cannot. In addition, A769662 activation of AMPK was inhibited by a mutation in the β 1 AMPK subunit (Ser108 to Ala), which is an auto-phosphorylation site within the glycogen-binding domain; however, the same mutation only partially reduced AMPK activation by AMP (Cool *et al.*, 2006; Sanders *et al.*, 2007). A769662 stimulates AMPK directly in partially purified rat liver and suppresses fatty acid synthesis in primary rat hepatocytes. Short-term treatment of normal Sprague-Dawley rats with A769662 has been shown to reduce liver malonyl-CoA levels and the respiratory exchange ratio of CO₂ production to O₂ consumption, indicating an increased rate of whole-body fatty acid oxidation (Cool *et al.*, 2006). Treatment with compound A769662 reduced plasma glucose, weight gain, and both plasma and liver triacylglycerol (triglyceride) levels in *ob/ob* mice (Cool *et al.*, 2006).

Another widely used AMPK activator is AICAR, also known as Acadesine. AICAR is a pro-drug and analogue of adenosine that enters cells and stimulates AMPK, following its uptake and phosphorylation to its active nucleotide ZMP (5-aminoimidazole-4-carboxamide-1- β -D-furanosyl 5'-monophosphate), which mimics AMP (Merrill *et al.*, 1997; Kwon *et al.*, 1998). AICAR has been tested in human studies of ischaemic heart disease due its ability to block adenosine reuptake by cardiac cells, promoting stimulation of adenosine membrane receptors. In 1997, treatment with AICAR before and during surgery was shown to alleviate early cardiac death, MI and combined adverse cardiovascular consequences, although whether the effects were via AMPK was not investigated at that time (Mangano, 1997). AICAR has been demonstrated to reverse many aspects of the metabolic syndrome in animal models such as the *ob/ob* mouse, the *fa/fa* rat and high fat-fed rat (Buhl *et al.*, 2002; Iglesias *et al.*, 2002), and also in human subjects (Cuthbertson *et al.*, 2007). AICAR has also been reported to stimulate release of adiponectin and inhibit release of cytokines such as TNF- α and IL-6, which have been implicated in the development of obesity-induced insulin resistance (Kern *et al.*, 2001; Lihn *et al.*, 2004). AICAR is not suitable for clinical use because of its short half-life, requirement for i.v. infusion and variable effectiveness. It also causes bradycardia and can lead to hypoglycaemia when administered intravenously (Young *et al.*, 2005).

Downstream targets and role of AMPK

Once AMPK is activated, the enzyme phosphorylates several key proteins and mediates numerous downstream pathways that are involved in metabolic regulation. This includes decreasing gluconeogenesis, fatty acid and cholesterol synthesis, and increasing muscle glucose transport, fatty acid oxidation and mitochondrial biogenesis. The modulation of

these processes ultimately leads to the inhibition of energy-consuming anabolic pathways and the stimulation of ATP producing pathways, thus regulating cellular energy balance. In the vasculature, activation of endothelial AMPK has been shown to phosphorylate eNOS at Ser¹⁷⁷ and Ser⁶³³, stimulating NO release (Chen *et al.*, 2009; Mariman and Wang, 2010) and subsequent vasodilatation of both large conduit and resistance blood vessels (Wang *et al.*, 2009b; Bradley *et al.*, 2010). Purified AMPK has also been reported to phosphorylate eNOS at an inhibitory site (Thr⁴⁹⁵) *in vitro* (Hansen and Kristiansen, 2006). AMPK activation has been also found to exert anti-inflammatory effects in the endothelium by reducing TNF- α -stimulated monocyte adhesion to human aortic endothelial cells (Ewart *et al.*, 2008). AMPK activators, such as AICAR (Mariman and Wang, 2010), metformin (Xie *et al.*, 2008) and rosiglitazone (Boyle *et al.*, 2008), have been reported to directly activate AMPK and enhance NO production in human endothelial cells. In eNOS knockout mice, shear stress- and bradykinin-mediated activation of AMPK is attenuated (Zhang *et al.*, 2006; Wang *et al.*, 2009a) and pharmacological inhibition of NOS prevents activation of AMPK by shear stress, bradykinin and calcium ionophores (Zhang *et al.*, 2006). AMPK activation in vascular smooth muscle induces vasodilatation (Tirapelli *et al.*, 2006) and attenuates smooth muscle contraction (Yamawaki *et al.*, 2011). Metformin (O'Toole *et al.*, 2008) and adiponectin (Deng *et al.*, 2010) are other AMPK-activating agents that are reported to induce arterial vasorelaxation. Although this vasorelaxation *in vivo* may be at the level of the endothelium and is due to NO release, metformin was found to induce vasodilatation in blood vessels treated with an eNOS inhibitor. This suggests that AMPK in vascular smooth muscle can modulate vascular tone and this may be important where the endothelium is damaged or dysfunctional in terms of NO formation or release (Majithiya and Balaraman, 2006). At the level of the endothelium, AMPK activation with AICAR can also induce prostacyclin synthesis and subsequent vasodilatation (Chang *et al.*, 2010). In the same study, Chang and co-workers demonstrated that AICAR increases expression of the pro-inflammatory enzyme COX-2. The later was assumed to be a result of activation of p38 MAPK and TAK1 phosphorylation (Chang *et al.*, 2010).

In atherosclerosis, activation of AMPK by AICAR resulted in a reduction in ER stress and inhibition of macrophage proliferation, both induced by high levels of circulating oxidized low-density lipoprotein (LDL). This protects the endothelium from the pathological effect of modified LDL (Dong *et al.*, 2010b). AMPK has been associated with anti-inflammatory effects in neutrophils, which are the primary source of myeloperoxidase (MPO) and involved in the modification of LDL (Alba *et al.*, 2004). AMPK activation attenuated neutrophil activity and metformin also decreased MPO levels in lung tissue (Zhao *et al.*, 2008; Tsoyi *et al.*, 2011). In addition, AMPK activation has been shown to increase protein levels of the ATP-binding cassette transporters, ABCG1 and ABCA1, resulting in cholesterol efflux from macrophage-derived foam cells as well as reduced plaque formation in ApoE^{-/-} mice (Li *et al.*, 2010a; 2010b).

AMPK has also been implicated in restenosis. AMPK α 2 knockout mice had a dramatic increase in the formation of neointima after wire injury of the carotid artery compared

with wild-type controls (Song *et al.*, 2011). Local administration of the AMPK activator AICAR also reduced neointima formation 2 weeks after balloon injury in rat carotid arteries (Stone *et al.*, 2012). Furthermore, endothelium-selective expression of constitutively active AMPK was protective against vascular injury and promoted re-endothelialization in a murine diabetic model (Li *et al.*, 2012). Vascular smooth muscle proliferation is a critical event in the development and progression of vascular diseases, including atherosclerosis. It has been reported that AMPK suppresses vascular SMC proliferation associated with attenuated cell cycle regulation by p53 up-regulation (Dashwood *et al.*, 2007).

Acute administration of the AMPK activator AICAR reduces mean arterial pressure (MAP) in both rodents and humans (Foley *et al.*, 1989; Bosselaar *et al.*, 2011). SHR dosed with AICAR also showed an acute drop in MAP, which was not seen in the control group of Wistar-Kyoto rats, suggesting that AMPK could be a therapeutic target for novel antihypertensive agents (Ford *et al.*, 2012). Long-term administration of AICAR or resveratrol, which has been described as an activator of AMPK, also caused a reduction in MAP in obese Zucker rats (Buhl *et al.*, 2002; Rivera *et al.*, 2009). Furthermore, short-term calorie restriction in the SHRs resulted in increased activity of AMPK, which led to a reduction in MAP (Dolinsky *et al.*, 2010).

AMPK in obesity and inflammation

In many genetic models of rodent obesity, AMPK activity is down-regulated in peripheral tissues such as heart, skeletal muscle and liver (Barnes *et al.*, 2002; Liu *et al.*, 2006). However, it appears that AMPK activity is not altered in the hypothalamus (Fryer *et al.*, 2002a) or skeletal muscle (Fryer *et al.*, 2002a; 2002b) of animals rendered obese by an HFD. It has been claimed that AMPK protein expression and activity are unaltered in obese human muscle (Minokoshi *et al.*, 2002), although other studies have reported potential reductions (Zang *et al.*, 2004). In human type 2 diabetic skeletal muscle, AMPK expression and activity are also unchanged in patients with a similar body mass (Shaw *et al.*, 2005). The response of AMPK to allosteric activation by AMP (Minokoshi *et al.*, 2002) and LKB1 activity and expression (Chen *et al.*, 2005) is also unchanged in obesity and in obese type 2 diabetic skeletal muscle, suggesting that the muscle AMPK system is indeed intact at least in moderate obesity. Similarly, AICAR has been demonstrated to increase AMPK activity to a similar degree in obese skeletal muscle from rodents (Lin *et al.*, 2000; Pold *et al.*, 2005) and humans (Minokoshi *et al.*, 2002) in comparison with lean controls. Notably, activation of AMPK by AICAR increases glucose uptake and fatty acid oxidation in obese diabetic rodents (Barnes *et al.*, 2002) and humans (Minokoshi *et al.*, 2002).

The role of AMPK in adipose tissue remains relatively poorly defined, although, given the anti-inflammatory and metabolic role of AMPK in other tissues, it is clear that the importance of the influence of this kinase on fat should not be discounted. The remainder of this review will focus on the impact of AMPK action in adipose tissue and, in particular, PVAT.

Several physiological stimuli are reported to activate AMPK in adipose tissue, including starvation, exercise, circulating HDL and the adipocyte-derived cytokine adiponectin. The principal catalytic subunit of AMPK expressed in adipose tissue is $\alpha 1$ (Salt *et al.*, 2000; Daval *et al.*, 2005), although an important role for $\alpha 2$ cannot be excluded as down-regulation of this isoform can have marked effects on adipocyte function (Wang *et al.*, 2010). Whereas AMPK activation in skeletal muscle is proven to increase glucose transporter type 4 (GLUT-4) translocation and, therefore, both basal and insulin-stimulated glucose transport (Kramer *et al.*, 2006; Fazakerley *et al.*, 2010), AMPK activation in adipose tissue has yielded contrasting results. AICAR-stimulated AMPK activation has been reported to inhibit insulin-stimulated glucose uptake in adipocytes without affecting the early steps on the insulin signalling pathway (Salt *et al.*, 2000; Wu *et al.*, 2003) and is accompanied by a reduction in AS160 phosphorylation and reduced Rab-GAP activity (Gaidhu *et al.*, 2010). In contrast, adiponectin was reported to activate AMPK and increase both basal and insulin-stimulated glucose uptake (Wu *et al.*, 2003). Longer term AMPK activation (24–48 h) with metformin has been shown in 3T3-L1 adipocytes to increase basal glucose transport, thereby reducing the extent of stimulation in response to insulin (Boyle *et al.*, 2011), and similar results were found in adipocytes derived from human fat (Fischer *et al.*, 2010). In *ex vivo* experiments on human adipose tissue, GLUT-4 expression was found to be increased following prolonged incubation with AICAR (Lihn *et al.*, 2008); however, levels were unaffected in 3T3-L1 adipocytes following prolonged AICAR or metformin treatment (Boyle *et al.*, 2011). Biopsies from patients with type 2 diabetes treated for 10 weeks with metformin also showed no change in GLUT-4 expression levels (Boyle *et al.*, 2011).

Abnormal lipid profiles and lipotoxicity, as seen in obesity and T2DM, predispose individuals to CVD. The AMPK pathway is a major regulator of lipid metabolism and has been shown to up-regulate fatty acid oxidation (muscle, adipose, hypothalamus) and uptake, inhibit cholesterol biosynthesis (liver) and reduce lipogenesis (liver, hypothalamus) (reviewed in Viollet *et al.*, 2009). In adipocytes, AMPK phosphorylates and inhibits ACC and increases malonyl-CoA decarboxylase activity, resulting in lower malonyl-CoA levels and therefore promoting mitochondrial β -oxidation while simultaneously suppressing fatty acid synthesis (Viollet *et al.*, 2009). However, the role of AMPK in lipolysis has precipitated much discussion, as AMPK has been reported to up-regulate or inhibit lipolysis via phosphorylation of hormone-sensitive lipase. In isolated human adipocytes, TZDs and biguanides inhibited lipolysis in a manner thought to be through AMPK cascade activation. These effects were all found to be inhibited by the AMPK inhibitor compound C (Flechtner-Mors *et al.*, 1999). Experiments in rat adipocytes, however, have suggested that the impact of the biguanide metformin on lipolysis may, in fact, be due to PKA inhibition rather than AMPK activation (Zhang *et al.*, 2009c). AICAR (Gaidhu *et al.*, 2009) and A769662 (Hawley *et al.*, 2012)-induced AMPK activation has also been reported to inhibit adipocyte lipolysis, although this was only observed following short-term AICAR incubations (Gaidhu *et al.*, 2009). Overexpression of a constitutively active form of AMPK has been shown to inhibit β -adrenoceptor-induced lipolysis (Kwon *et al.*, 1998; Daval

et al., 2005). Notably, in AMPK $\alpha 1$ knockout mice, the size of adipocytes is smaller, and both basal and isoprenaline-induced lipolysis are higher than that of control adipocytes (Daval *et al.*, 2005). Furthermore, deletion of the AMPK $\alpha 2$ subunit is also accompanied by modifications in adipose tissue. AMPK $\alpha 2^{-/-}$ mice fed an HFD exhibited increased body weight and fat mass in relation to control mice fed similarly. The increase in adipose tissue mass was due to the enlargement of the adipocytes with increased lipid accumulation. This study showed that deletion of AMPK $\alpha 2$ subunit may be a factor contributing to the development of obesity (Villena *et al.*, 2004). Notably, it has been found that obesity increases PVAT mass and reduces its anti-contractile effect (Gao *et al.*, 2005). In contrast, exercise-induced AMPK activation has been shown to stimulate lipolysis via β -adrenoceptors (Koh *et al.*, 2007) and increased lipolysis was also observed after long-term stimulation with AICAR (Gaidhu *et al.*, 2009). Puzzlingly, additional recent evidence has shown that reducing AMPK activity does not appear to have any significant effect on lipolysis at all (Chakrabarti *et al.*, 2011), and it should also be noted that an increase in lipolysis itself may indirectly activate AMPK by increasing the AMP/ATP ratio (Gauthier *et al.*, 2008).

AMPK in inflammation

Several studies have revealed an irrefutable relationship between AMPK activity and inflammation in different tissues and cell types, including adipocytes, endothelial cells and macrophages (Galic *et al.*, 2011; Gauthier *et al.*, 2011). Activation of AMPK down-regulates pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6 in macrophages (Yang *et al.*, 2010; Galic *et al.*, 2011), and IL-6 and IL-8 in adipocytes (Lihn *et al.*, 2008). AMPK activation has also been demonstrated to up-regulate the expression of the anti-inflammatory cytokine IL-10 in macrophages (Sag *et al.*, 2008; Galic *et al.*, 2011). In cultured human SCAT, AICAR was reported to attenuate TNF- α and IL-6 secretion, while stimulating adipose tissue AMPK $\alpha 1$ activity and adiponectin gene expression (Lihn *et al.*, 2004). Berberine, an AMPK activator that inhibits pro-inflammatory signalling in isolated macrophages, has also been found to inhibit cytokine expression in adipose tissue of obese *db/db* mice (Jeong *et al.*, 2009). The activity of the NLRP3 inflammasome is markedly increased in myeloid cells from patients with type 2 diabetes, and it is reported that treatment with metformin decreased the maturation and secretion of IL-1 β and IL-18 in monocyte-derived macrophages. Metformin produces this effect through activation of AMPK; however, the mechanism remains elusive (Yang *et al.*, 2010; Lee *et al.*, 2013). Furthermore, it has been observed that IL-10 and TGF- β , two anti-inflammatory cytokines, induce a rapid phosphorylation and activation of AMPK, whereas a pro-inflammatory insult with LPS decreased the AMPK activity in both mouse and human macrophages (Sag *et al.*, 2008).

Nutrient stress induced by high-fat feeding has been found to suppress cardiac AMPK activity and induces inflammation, which is characterized by increased number of macrophages and up-regulation of IL-6 levels in the mouse heart. Furthermore, acute infusion of IL-6 profoundly lowered the

AMPK activity, decreased the glucose uptake by cardiac muscle and induced inflammation in the heart (Ko *et al.*, 2009). Ko *et al.* also demonstrated that diet-induced insulin resistance and inflammation were alleviated in IL-6-deficient mice (Ko *et al.*, 2009). However, IL-6 knockout in mice can be linked with the development of mature-onset obesity (Wallenius *et al.*, 2002) and diet-induced atherosclerosis (Madan *et al.*, 2008), an effect that can be attributed to the reduced AMPK activity in IL-6-deficient mice (Kelly *et al.*, 2004).

Several studies have reported that the activation of AMPK down-regulates the NF- κ B system, which is responsible for the development of low-grade chronic inflammation associated with obesity, T2DM and atherosclerosis (Rocha and Libby, 2009; Yang *et al.*, 2010). AMPK suppresses NF- κ B signalling indirectly via its downstream targets such as SIRT1 and PGC1 α , forkhead Box (FoxO) family and PPAR γ co-activator 1 α (PGC-1 α), which, in turn, activates the expression of inflammatory factors (Salminen *et al.*, 2011). Generally, stimulation of macrophages by pro-inflammatory stimuli decreases sirtuin levels, which is associated with increased activation of RelA/p65 subunit of NF- κ B and increased pro-inflammatory cytokine release (Yang *et al.*, 2007). Constitutively active AMPK α 1 has been found to mimic the effect of SIRT1 on de-acetylating NF- κ B, which consequently inhibits LPS- and FFA-induced TNF- α expression and restores the anti-inflammatory phenotype of macrophage (Yang *et al.*, 2010). Adenovirus-mediated overexpression of the PGC-1 α gene in human aortic SMCs and EC alleviates intracellular and mitochondrial ROS production, NF- κ B activity as well as CCL2 and VCAM-1 expression, supporting the possibility that signalling molecules stimulating PGC-1 α in the vasculature, such as AMPK, will exert beneficial effects on vascular inflammation and development of atherosclerosis (Kim *et al.*, 2007).

AMPK and autophagy

Autophagy is an essential mechanism not only for the recycling of amino acids and other macromolecules during periods of starvation but also for elimination of cellular components damaged by toxins, hypoxia or other noxious stimuli. Derangements in autophagy can lead to an exaggerated response to injury (Aki *et al.*, 2013). Macrophages in advanced atherosclerotic lesions are prone to apoptosis, which can contribute to plaque destabilization and cardiovascular events. Autophagy in macrophages appears to be protective against atherosclerosis development in two ways: by preventing apoptosis of the macrophage and by enhancing efferocytosis – clearance of apoptotic cells in the plaque (Liao *et al.*, 2012). The biochemical events involved in macrophage autophagy are still being actively investigated but, not surprisingly, energy sensing pathways such as AMPK and SIRT1 are involved. In human THP-1 cells, inhibition of SIRT1 with sirtinol was associated with up-regulation of pro-inflammatory genes, down-regulation of AMPK activity and impaired autophagy (Takeda-Watanabe *et al.*, 2012). AMPK also plays a pivotal role in hypoxia-induced autophagy in human cancer cell lines (Papandreou *et al.*, 2008) and human pulmonary artery SMCs exposed to hypoxia (Ibe *et al.*, 2013).

However, where the role of autophagy appears to be protective in macrophages, the situation is not so clear in vascular SMCs. In the study by Ibe *et al.*, AMPK isoforms had different effects on pulmonary artery SMCs, with AMPK α 1 inducing autophagy and promoting SMC survival. In terms of hypoxic pulmonary remodelling, this is an undesirable effect. Autophagy may also be responsible for removing the contractile proteins from vascular SMCs during their transition to a synthetic phenotype. In a recent study, spautin-1, an inhibitor of autophagy, prevented PDGF-induced disorganization of actin filaments, cell proliferation and migration, and production of extracellular matrix (Salabei *et al.*, 2013). Thus, in vascular lesions, autophagy in response to oxidative stress and growth factors may initiate deleterious changes in vascular function, which ultimately lead to adverse vascular remodelling. The role of AMPK in autophagy-induced phenotypic transformation of vascular SMCs has not been determined as yet.

AMPK and micro-RNAs

Micro-RNAs (miR) are small, non-coding RNAs 18–25 nucleotides in length that negatively regulate gene expression in a sequence-specific manner. Recent evidence has indicated that AMPK targets specific miRs and this may be of importance in the regulation of vascular homeostasis. The miR-144/451 cluster, for example, is down-regulated by stretch on the smooth muscle, which then allows phosphorylation of AMPK, leading to contractile differentiation (Turczynska *et al.*, 2013). At the level of the endothelial cell, shear stress was found to down-regulate ACE expression while activating AMPK α 2 had the same effect via phosphorylation of p53 and up-regulation of miR-143/145 (Kohlstedt *et al.*, 2013). Another miR that may impact on AMPK activity is miR-33. AMPK contains a predicted binding sequence for miR-33a/b and this miR appears to negatively regulate AMPK with the effect of increasing cellular cholesterol and triglyceride content (Fernandez-Hernando and Moore 2011). In terms of macrophage function, miR-223 appears to suppress the pro-inflammatory activation of macrophages in adipose tissue and thus may be a target to prevent adipose tissue inflammation and insulin resistance (Zhuang *et al.*, 2012). Adipocytes themselves can secrete miRs such as miR-130b and this correlates with the degree of obesity in a human population. Interestingly, the same study showed that inflammatory mediators such as TGF- β could stimulate miR release by adipocytes, suggesting that inflammation within the adipose tissue in obesity can generate miRs (Wang *et al.*, 2013). Taken together, these data indicate widespread generation of multiple miRs by cell types within the vasculature and surrounding adipose tissue, some of which may act through AMPK to influence vascular function. This may be mediated acutely through ACE, for example, or chronically via changes in vascular SMC differentiation or lipid content.

AMPK and PVAT

This review has shown that PVAT is implicated in vascular control (Figures 2 and 3) and function in health and disease

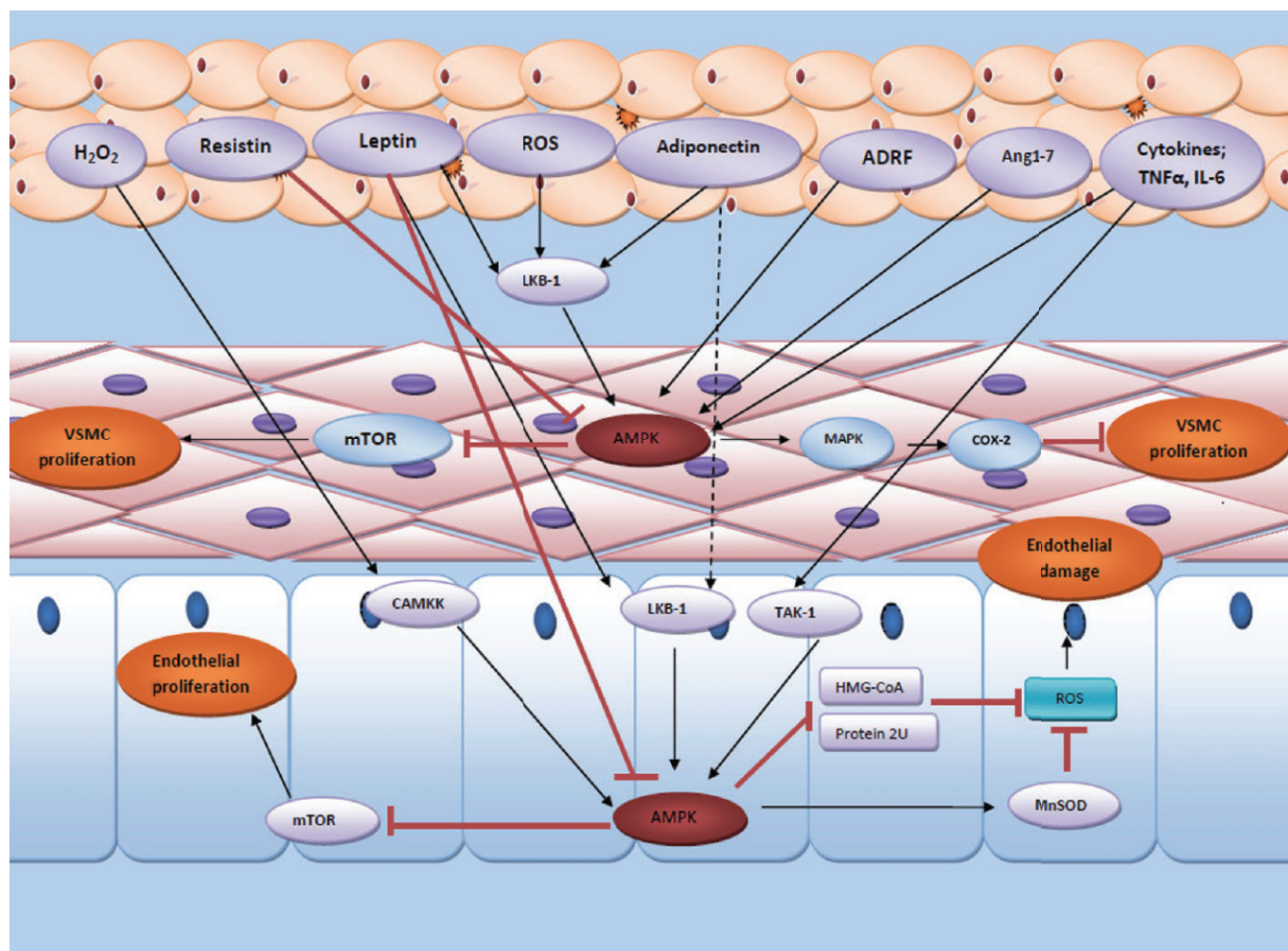


Figure 2

Schematic presentation of how PVAT modulates the function of AMPK in both endothelium and vascular smooth muscle and how these affect vascular function. PVAT is a highly active organ secreting various adipokines and cytokines implicating in the regulation of vascular contractility via modulation of AMPK activity. ADRF, adipose tissue-derived relaxation factor; Ang1-7, angiotensin 1–7; BK_{Ca}, calcium-dependent big potassium channel; CAMKK, calcium/calmodulin-dependent protein kinase kinase; eNOS, endothelial NOS; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LKB, liver kinase B; MAPK, mitogen-activated protein kinases; MnSOD, manganese superoxide dismutase; mTOR, mammalian target of rapamycin; PGI₂, prostacyclin; ROS, reactive oxygen species; TAK1, transforming growth factor β -activated kinase 1.

and, as discussed earlier, the metabolic kinase AMPK is also known to have vasculoprotective properties. As such, targeting AMPK may have therapeutic potential in treating some of the effects of obesity and insulin resistance on PVAT. Recent investigations have proposed that AMPK-stimulating adipokines, such as adiponectin, are the main mediators of the modulatory effect of PVAT on the vasculature. Adiponectin release is markedly reduced in obese PVAT and this leads to inhibited vasorelaxation. Experiments with an adiponectin receptor antagonist and the AMPK inhibitor compound C were shown to mimic the effect of obesity on vascular relaxation, and globular adiponectin also failed to increase vasorelaxation in AMPK2 α -deficient mice (Meijer *et al.*, 2013; Weston *et al.*, 2013).

It has been reported by Ma *et al.* (2010b) that PVAT can induce vascular dysfunction via dysregulation of the AMPK/mTOR pathway in diet-induced obese rats, with mesenteric

arterial rings incubated with periaortic fat from rats on an HFD demonstrating lower endothelium-dependent relaxation and down-regulation of AMPK and eNOS in the aorta with a concurrent up-regulation of mTOR. This effect was absent in periaortic fat from rats on a chow diet. Co-culture of vascular SMCs with periaortic adipocytes from rats on an HFD also reduced AMPK phosphorylation and increased mTOR phosphorylation. In obese PVAT, release of TNF- α and FFA are also increased (Ma *et al.*, 2010a), indicating further switching to a more pro-inflammatory vasoconstrictive phenotype. Adiponectin has been proposed to mediate crosstalk between PVAT and vascular endothelium and to contribute to the regulation of muscle perfusion (Bussey *et al.*, 2011). Adiponectin could also exert a pro-angiogenic effect in ischaemic tissue by acting directly on the vascular endothelium. It has been shown that AMPK signalling mediates adiponectin-induced angiogenic and anti-apoptotic cellular responses in

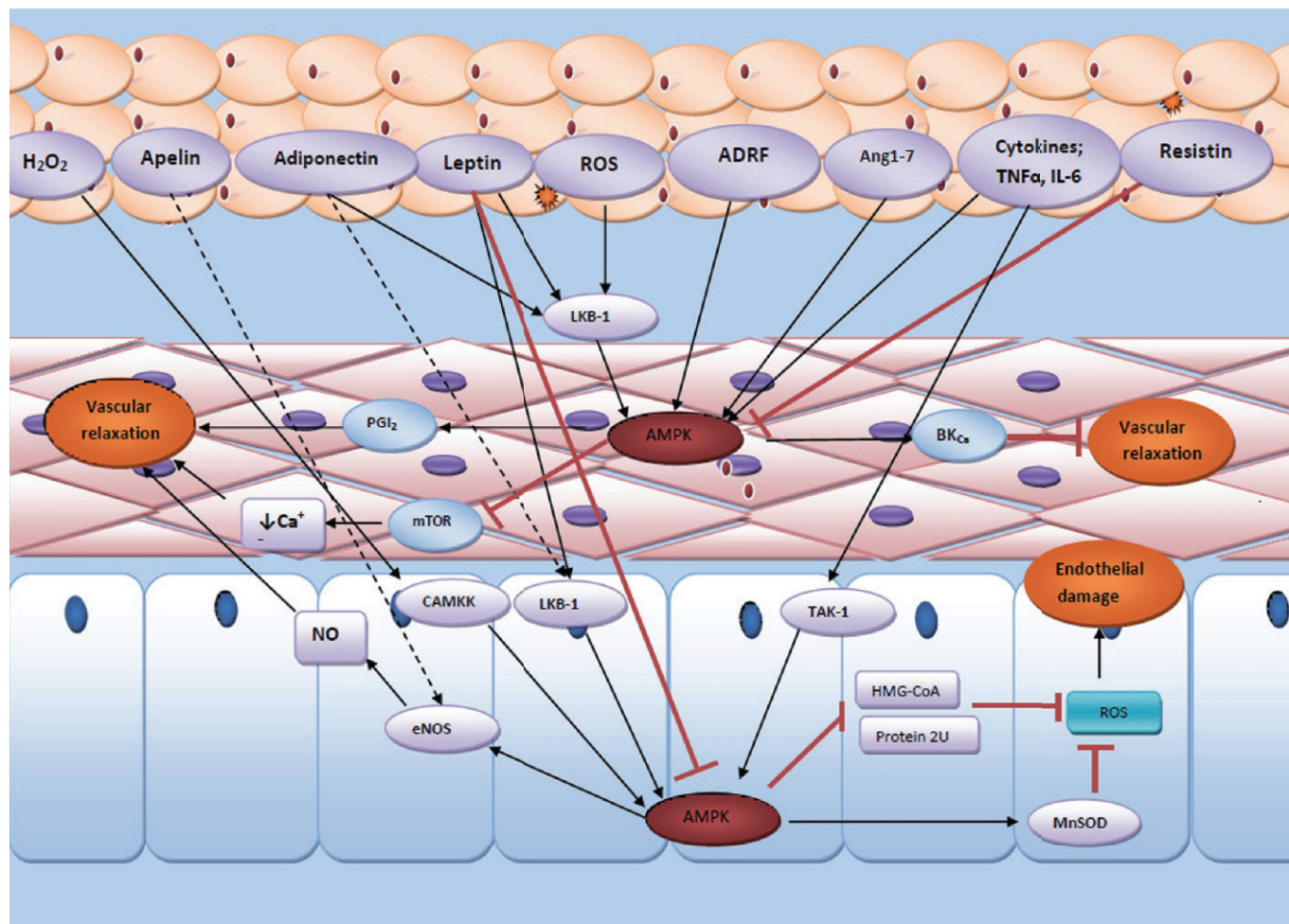


Figure 3

Schematic presentation of how PVAT modulates the function of AMPK in both endothelium and vascular smooth muscle and how these effects lead to vascular proliferation and atherosclerosis. PVAT is a highly active organ secreting various active molecules implicating in the regulation of vascular reactivity via modulation of AMPK function. ADRF, adipose tissue-derived relaxation factor; Ang1-7, angiotensin 1–7; CAMKK, calcium/calmodulin-dependent protein kinase kinase; eNOS, endothelial NOS; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; IP3R, inositol trisphosphate receptor; LKB, liver kinase B; MAPK, mitogen-activated protein kinase; MnSOD, manganese superoxide dismutase; mTOR, mammalian target of rapamycin; PGI₂, prostacyclin; ROS, reactive oxygen species; TAK1, transforming growth factor β-activated kinase 1.

endothelial cells (Kobayashi *et al.*, 2004; Ouchi *et al.*, 2004). However, secretion of adiponectin and other adipokines has been reported to change in association with obesity (Tilg and Moschen, 2006; Eringa *et al.*, 2007). Furthermore, activation of adipocyte β₃-adrenoceptors has been reported to stimulate release of a substance, which is assumed to be adiponectin and which indirectly opens myocyte BK_{Ca} channels (Weston *et al.*, 2013). This effect was reported to involve AMPK since it could be mimicked by the AMPK activator, A-769662, and blocked by the kinase inhibitor, dorsomorphin. This study has also demonstrated that glibenclamide and clotrimazole could block adipocyte-dependent myocyte hyperpolarization but not the response to a BK_{Ca} channel opener, NS1619, implying that AMPK may not activate BK_{Ca} directly (Weston *et al.*, 2013).

Cytokines generated from within the PVAT may also induce changes in vascular reactivity indirectly through up-regulation of iNOS. A recent *in vitro* study in 3T3 L1

adipocytes demonstrated that TNF-α treatment induced a 200-fold increase in iNOS gene expression (Digby *et al.*, 2010). AMPK activation within the adipose tissue may be beneficial in preventing the adverse effects of iNOS on vascular reactivity by inhibiting its up-regulation (Pilon *et al.*, 2004). Recent studies have supported this by demonstrating inhibition of iNOS expression in adipose tissue *in vivo* and in adipocytes and myocytes *in vitro* in response to AMPK activation by the plant polyphenol resveratrol (Centeno-Baez *et al.*, 2011). FFAs released by adipocytes may also be involved in changes in iNOS expression and vascular dysfunction. In human vascular SMCs, a combination of conditioned media from adipocytes and oleic acid induced not only iNOS up-regulation and NO formation but also a proliferative response (Lamers *et al.*, 2011). It has also been hypothesized that one of the beneficial effects of adiponectin is via activation of AMPK and suppression of iNOS expression in the vascular adventitia (Cai *et al.*, 2008). A follow-up study by the

same group examined the effects of adiponectin on adventitial fibroblast transition and migration. Their data showed that adiponectin induced AMPK phosphorylation and reduced the migration of fibroblasts, the expression of iNOS and the peroxynitrite marker nitrotyrosine in response to LPS treatment (Cai *et al.*, 2010). Taken together, these data indicate that PVAT cannot only modulate vascular reactivity via changes in iNOS and NO formation by adipocytes but also vascular structure through effects on VSMC and fibroblast proliferation and migration.

PVAT has also been reported to induce vasodilatation in muscle resistance arteries by increasing secretion of adiponectin and activation of AMPK α 2 in the blood vessels. Furthermore, this study reported that in obese *db/db* mice, PVAT mass increased dramatically in the muscle and was associated with loss of its ability to induce insulin-mediated vasodilatation. This was due to decreased release of adiponectin and disturbed insulin Akt activation. Notably, the effect of insulin-induced vascular reactivity can be restored by JNK (Meijer *et al.*, 2013). All of these studies highlight the importance of PVAT in modulating the activity of the underlying layers of blood vessels. Crucially, evidence is now accumulating that some of the beneficial effects of PVAT may become deranged in obesity and this can predispose to CVDs. AMPK present in the PVAT is likely to be an important intermediate in the actions of some PVAT-derived mediators. Targeting AMPK may have therapeutic potential in treating some of the effects of obesity and insulin resistance on PVAT.

Conclusions

In summary, given the influence PVAT has on the vasculature, there has been much interest in PVAT as a potential link between obesity and the development of CVD. Although the mechanisms explaining these results have not yet been elucidated, it is clear that they heavily and independently affect CVD. Although many drugs currently in use are known to activate AMPK, this is not always wholly responsible for their therapeutic action. As knowledge of the structure, function and distribution of the different subunit isoforms of AMPK increases, targeting drugs more specifically should be achievable. Salicylate and A769662 are specific to complexes containing the β 1 subunit, so developing pharmacological agents specific to other AMPK isoforms is likely to become a reality in the future.

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Conflict of interest

None of the authors have any conflict of interest to declare.

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