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Themed Section: Fat and Vascular Responsiveness

REVIEW

Perivascular adipose tissue from human systemic and coronary vessels: the emergence of a new pharmacotherapeutic target

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Fat cells or adipocytes are distributed ubiquitously throughout the body and are often regarded purely as energy stores. However, recently it has become clear that these adipocytes are engine rooms producing large numbers of metabolically active substances with both endocrine and paracrine actions. White adipocytes surround almost every blood vessel in the human body and are collectively termed perivascular adipose tissue (PVAT). It is now well recognized that PVAT not only provides mechanical support for any blood vessels it invests, but also secretes vasoactive and metabolically essential cytokines known as adipokines, which regulate vascular function. The emergence of obesity as a major challenge to our healthcare systems has contributed to the growing interest in adipocyte dysfunction with a view to discovering new pharmacotherapeutic agents to help rescue compromised PVAT function. Very few PVAT studies have been carried out on human tissue. This review will discuss these and the hypotheses generated from such research, as well as highlight the most significant and clinically relevant animal studies showing the most pharmacological promise.

LINKED ARTICLES
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Abbreviations
ADRF, adventitium-derived relaxing factor; BMI, body mass index; CAD, coronary artery disease; ICAM-1, intercellular adhesion molecule-1; L-NAME, NG-nitro-L-arginine methyl ester; MCP-1, monocyte chemotactic protein-1; MetS, metabolic syndrome; PVAT, perivascular adipose tissue; ROS, reactive oxygen species; VCAM-1, vascular cell adhesion molecule-1

Introduction: the clinical problem

Obesity, defined as a body mass index (BMI) of greater or equal to 30 $kg \cdot m^{-2}$ is a major problem in acculturated and developing societies. It often coexists with a number of other diseases including hypertension, dyslipidaemia and insulin resistance. Such a constellation has been labelled the metabolic syndrome (MetS). The international obesity taskforce (IOTF) estimates that approximately 1 billion adults are currently overweight (BMI 25–29.9 $kg \cdot m^{-2}$), and of these 475 million are obese ($\geq$30 $kg \cdot m^{-2}$) (IOTF, 2010).

The enormity of this epidemic highlights the need for novel approaches to obesity management and a furthering of our knowledge of the mechanisms responsible for the consequences of being overweight.

A number of reports has indicated that the distribution of fat around the body determines not only the obese phenotype but its consequences. For example, intra-abdominal and visceral fat depots have been linked with an increased cardiometabolic risk and the mortality associated with obesity (Fox *et al.*, 2007; Gesta *et al.*, 2007). The total amount of internal fat rises with increasing subcutaneous adipocity, but

even individuals classed as thin may have more visceral fat than some obese individuals. In addition, increased gluteofemoral fat mass has been negatively linked to levels of inflammatory cytokines and positively linked to raised concentrations of adipokines resulting in decreased metabolic and cardiovascular risk (Manolopoulos *et al.*, 2010).

Our current understanding of this problem focuses on our ancestors and the fact that fat was the energy store developed in times of plenty, which could then be burned during famine. Therefore genes which predisposed to obesity would confer survival benefits and such individuals would live long enough to reproduce. Several reviews have suggested that it is the breakdown of this system that is responsible for the contemporary problems associated with obesity, where susceptible individuals no longer have periods of famine and are instead constantly overeating readily available high energy foods (Neel, 1962; Diamond, 2003). Whilst in hibernating mammals, short-term obesity and insulin resistance have the beneficial effect of directing glucose to the brain, only man has developed chronic obesity with its associated cardiovascular morbidity and mortality (Scott and Grant, 2006).

Yudkin *et al.* (2005) originally postulated that perivascular adipose tissue (PVAT) might hold the key to linking obesity with the development of MetS and diabetes as a result of an adverse influence upon the vasculature (Yudkin *et al.*, 2005). In health, PVAT could produce adipokines that profoundly influence metabolism and the control of local vascular tone via vasocrine actions. It was suggested that the loss of such substances would result in a change in vascular function and development of insulin resistance. These authors suggested that the effect of circulating insulin on NO-mediated vasodilatation, which is of paramount importance in modulating the postprandial increase in nutritive flow, could be challenged by the paracrine action of adipokines released from local fat stores in obesity. They further highlighted the role inflammation may play and suggested elevated levels of TNF-α in obesity could disrupt the crosstalk between fat and blood vessels.

In this review we intend to focus on the vasoactive properties of PVAT as mediated by adipokines.

What are the principle adipokines released from adipocytes?

The recent interest in adipose tissue as a dynamic endocrine organ has resulted in a number of studies examining the shared, and in cases distinct, properties of different fat depots. PVAT surrounds subcutaneous small arteries, coronary vessels (peri-coronary and epicardial fat), aorta and systemic vessels and secretes a number of important adipokines (Figure 1).

Amongst the adipokines, leptin and adiponectin have been subject of recent reviews (Ren, 2004; Kadowaki and Yamauchi, 2005; Kadowaki *et al.*, 2006; Sweeney, 2010). Here, we review briefly the most significant findings on leptin, adiponectin and adrenomedullin. A more comprehensive list of adipokines and their roles with regards to the MetS has been published recently (Deng and Scherer, 2011).

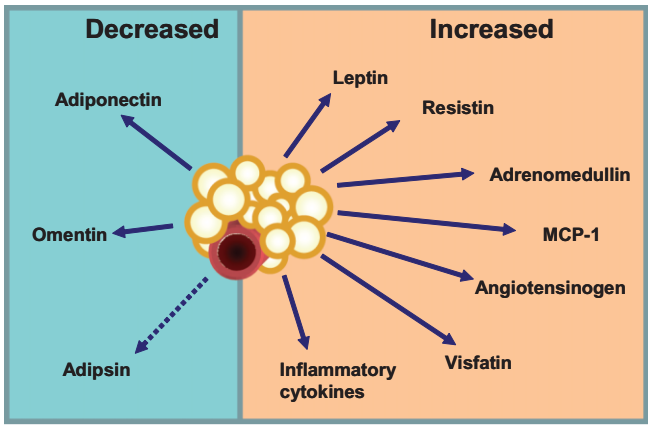

Figure 1

PVAT is the source of a number of vasoactive and metabolically significant adipokines.

Leptin

In rats leptin has a direct vasodilator effect acting via an endothelium- and NO-dependent mechanism (Lembo *et al.*, 2000). However, it has an endothelium-independent vasodilator effect on segments of human subclavian vein and internal mammary arteries harvested during surgery, thus highlighting the need for careful selection of vascular tissue in order to design clinically relevant studies (Momin *et al.*, 2006).

The role of leptin as an indirect vasoconstrictor has also been studied. Fruhbeck *et al.* have shown that leptin has a sympathoexcitatory effect leading to a rise in BP in Wistar rats (Fruhbeck, 1999). They demonstrated a significant rise in both systolic and diastolic BP when rats were given an intravenous bolus of the NO inhibitor NG-nitro-L-arginine methyl ester (L-NAME) followed by leptin, demonstrating the role NO plays in facilitating the vasorelaxant effects of leptin. In the same experiment they showed that leptin administration, following postganglionic blockade using chlorisondamine, resulted in a reduction in BP, which was abolished by NO inhibition.

Weight gain is often associated with increased insulin resistance and resultant hyperinsulinaemia. Interestingly, Vecchione *et al.* have demonstrated that insulin potentiates leptin-induced NO release and even at physiological levels, insulin enhances the vasodilator effects of leptin (Vecchione *et al.*, 2003).

That leptin, a pro-inflammatory adipokine, possesses vasodilator properties, and that obesity is associated with leptin resistance, implies its role in obesity is not understood fully. In MetS leptin levels are increased within the epicardial fat adjacent to the coronary vessels (Payne *et al.*, 2010). In dogs, hyperleptinaemia has been associated with endothelial dysfunction (Knudson *et al.*, 2005), and work on a swine model of MetS has shown that leptin exacerbates endothelial dysfunction via a protein kinase C-β dependent pathway (Payne *et al.*, 2009).

It is unclear whether elevated levels of leptin in epicardial fat are of benefit to the adjacent coronary vessels, or whether they may contribute to endothelial dysfunction in human arteries.

Adiponectin

Adiponectin is a 30 kDa protein made up of 244 amino acids. It is the most abundant adipokine (Dridi and Taouis, 2009; Liu *et al.*, 2010) and exists in two forms, full length, or a smaller globular fragment. (Kadowaki and Yamauchi, 2005; Kadowaki *et al.*, 2006; Dridi and Taouis, 2009). The full length form acts via the R2 receptor and the globular form via R1 (Yamauchi *et al.*, 2003a). In human adipocytes, the expression of Adipo R1 is ~15-fold higher than that of Adipo R2 (Rasmussen *et al.*, 2006). Circulating adiponectin levels are reduced in obesity (Sowers, 2008), diabetes (Kadowaki *et al.*, 2006), and there is a down-regulation of adiponectin receptors in the adipose tissue of obese individuals (Rasmussen *et al.*, 2006). Given its anti-diabetic (Berg *et al.*, 2001; Yamauchi *et al.*, 2001; 2003b; Maeda *et al.*, 2002; Pajvani and Scherer, 2003; Davis and Scherer, 2008), anti-atherosclerotic (Yamauchi *et al.*, 2003b; Han *et al.*, 2009) and vasodilator (Fesus *et al.*, 2007; Greenstein *et al.*, 2009) properties, adiponectin is believed to be the link between obesity and MetS. Its mechanism of action has yet to be determined fully.

In the liver, full-length adiponectin activates adenosine monophosphate-activated protein kinase (AMPK) and the trimeric form is known to activate AMPK in adipose tissue and muscle. Activation of AMPK leads to phosphorylation of Acetyl-Coenzyme-A Carboxylase (ACC), which results in fatty acid β-oxidation and inhibition of triacylglycerol and fatty acid synthesis (Kadowaki and Yamauchi, 2005; Liu *et al.*, 2010).

Deng *et al.* have demonstrated that adiponectin improves endothelial dysfunction by increasing NO production via phosphorylation of endothelial NO in the aorta of high-fat-fed obese Sprague-Dawley rats (Deng *et al.*, 2010). Moreover, in adiponectin receptor-knockout mice, there is a significant attenuation of endothelium-dependent vasodilatation in response to ACh (Ouchi *et al.*, 2003). Of relevance, adiponectin suppresses both basal and oxidized-LDL induced superoxide generation in bovine endothelial cells (Motoshima *et al.*, 2004), and also suppresses excess reactive oxygen species (ROS) production under high-glucose conditions via a cAMP/PKA-dependent pathway (Ouedraogo *et al.*, 2006). Given the elevated levels of oxidized-LDL (Holvoet *et al.*, 2008) and plasma glucose in patients with MetS, the concomitant reduction in adiponectin levels may explain partly the endothelial dysfunction observed by our group (Greenstein *et al.*, 2009) in the cohort of patients with MetS.

Incubation of healthy human vessels with a blocking peptide for the adiponectin R1 receptor almost abolished completely the anticontractile effect of PVAT in response to cumulative noradrenaline doses (Greenstein *et al.*, 2009). Our unpublished proteomic analysis of adipose tissue from obese patients shows a significant reduction in adiponectin levels as compared with lean individuals. Therefore, we suspect that the absence of the anticontractile function of PVAT in obesity is accounted for by a reduction in adiponectin levels in PVAT at least in part. It has been suggested that the high levels of adiponectin in some disease states may be a compensatory response to the development of 'adiponectin resistance' (i.e. a dysfunction in the adiponectin signalling pathway) (Sam and Walsh, 2010). Clearly, further research is required to describe the exact mechanisms of action of adiponectin in health and to explain how these pathways become affected in disease.

Adrenomedullin

Amongst the adipokines, adrenomedullin (AM) has received the least attention in recently published literature. It is a 52 amino acid peptide first isolated from a sample of human phaeochromocytoma, but later shown to be synthesized by adrenal, heart, and vascular endothelial and smooth muscle cells as well as white adipocytes of both rodents and humans (Fukai *et al.*, 2005; Silaghi *et al.*, 2007). Its dose-dependent vasodilator effect on the rat mesenteric vessels was first reported in 1993 (Nuki *et al.*, 1993). Its direct and potent vasodilatory action on blood vessels would suggest that it plays an important role in the control of vessel tone. Human studies have shown that intravenous infusion of the peptide leads to significant vasodilatation of pulmonary vessels providing a potential therapeutic strategy for pulmonary hypertension (Nagaya *et al.*, 2000; 2003). Also there is evidence of AM-induced human coronary (Terata *et al.*, 2000) and skeletal artery (Nakamura *et al.*, 1997) vasodilatation via an NO-dependent pathway.

The exact role of AM in obesity needs further evaluation, but we know that AM reduces levels of ROS in vascular smooth muscle cells (Yoshimoto *et al.*, 2005), and AM knockout mice express higher levels of ROS (Shimosawa *et al.*, 2003).

Catecholamine stimulation of β3 adrenoceptors on adipocytes results in lipolysis and release of stored adipokines (Robidoux *et al.*, 2006). AM inhibits lipolysis via an NO-dependent mechanism (Harmancey *et al.*, 2005) which in theory, would affect vessel tone indirectly by blocking the release of vasoactive adipokines such as adiponectin (Figure 2). Gettys *et al.* have reported that stimulation of the β3-adrenergic receptor on white rat adipocytes by the selective agonist CL316,243 leads to the inhibition of the release of leptin (Gettys *et al.*, 1996). The balance between the potential lipolysis-induced release of adiponectin and the inhibition of leptin release and their relevance to vascular tone needs further clarification.

Epicardial adipose tissue from patients with coronary artery disease (CAD) displays higher levels of AM than those without CAD. Given its vasorelaxant properties, one would assume this may serve as a protective mechanism for the diseased coronary vessels (Shibasaki *et al.*, 2010). Plasma AM concentrations are also elevated in disease states, which may provide further protection against oxidative stress and vasoconstrictors. Despite its antioxidant and vasorelaxant properties, it is possible that elevated AM levels in disease states may actually contribute to the vascular dysfunction.

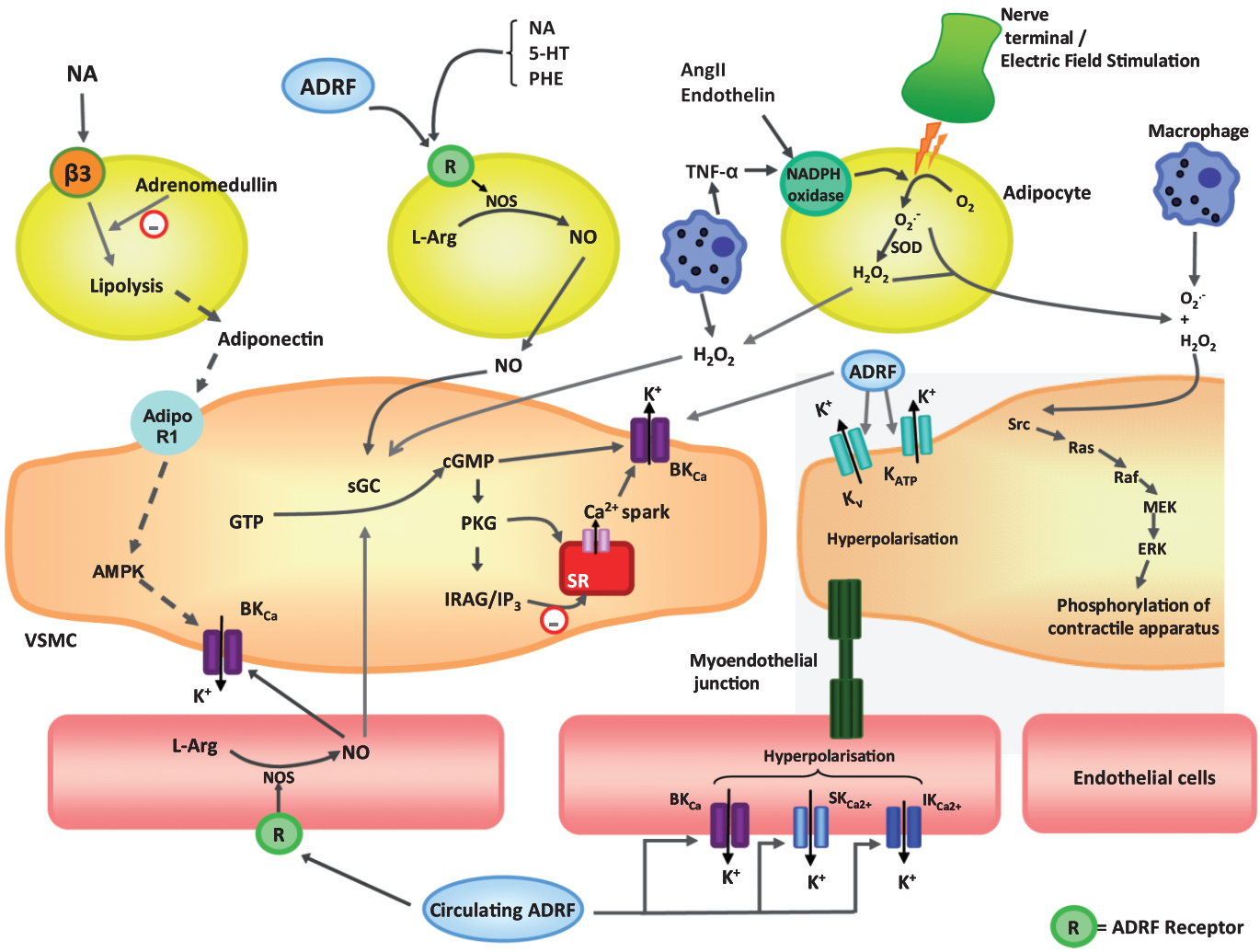

Figure 2

Potential mechanisms via which perivascular adipocytes, vascular smooth muscle cells and endothelial cells interact. Dotted lines represent unproven pathways. ADRF, adventitium-derived relaxing factor; AMPK, AMP-activated protein kinase; Ang II, angiotensin II; BK_{ca}, large conductance calcium-activated potassium channel; H_2O_2, hydrogen peroxide; ERK, extracellular signal-regulated kinases; 5-HT, 5-hydroxytryptamine (serotonin); GTP, guanosine triphosphate; cGMP, cyclic guanosine monophosphate; IP_3, inositol triphosphate; IRAG, IP_3 receptor-associated cGMP kinase substrate; IK_{ca}, intermediate conductance calcium-activated potassium channel; K_v, voltage-gated potassium channel; K_{ATP}, ATP-sensitive potassium channel; L-Arg, L-Argine; NA, noradrenaline; NO, nitric oxide; NOS, nitric oxide synthase; $O_2^{\cdot-}$, superoxide anion; ONOO, peroxynitrite; PCS, prostacyclin pathway; PHE, phenylephrine; PGH_2,prostaglandin H_2; PGI_2, prostaglandin I_2 (prostacyclin); PKG, protein kinase G; R, receptor; sGC, soluble guanylate cyclise; SK_{ca}, small conductance calcium-activated potassium channel; SOD, superoxide dismutase; SR, sarcoplasmic reticulum; TNF, tumour necrosis factor; VSMC, vascular smooth muscle cell.

PVAT and control of local vascular tone

PVAT function has been assessed in canine, swine and rodent models and demonstrated different functional and structural properties of PVAT, which vary both between species and anatomical site. Examples of structural differences include the fact that PVAT from rat aorta comprises smaller adipocytes compared with mesenteric vessels (Galvez-Prieto *et al.*, 2008). Whilst the murine thoracic aorta is surrounded by brown adipocytes, peri-abdominal fat is comprised of white adipocytes (Police *et al.*, 2009). From a functional perspective, coronary artery PVAT in healthy dogs attenuates acetylcholine-induced relaxation (marker of endothelial function) (Payne *et al.*, 2008; 2009), but does not affect bradykinin-mediated dilatation in healthy pig arteries (Payne *et al.*, 2010).

Three studies have shown that healthy human PVAT exerts an anticontractile effect on adjacent vessels. Rodent (both mouse and rat) mesenteric and aortic vascular beds have been the most frequently studied models of PVAT function. Data from these models have matched closely the data obtained from limited human studies.

In 1991, Soltis and Cassis were the first to report that vessels with intact PVAT were less responsive to noradrena-

line than naked vessels (Soltis and Cassis, 1991). Later studies used solution transfer protocols to demonstrate the existence of a transferrable adventitium-derived relaxing factor (ADRF) (Lohn *et al.*, 2002; Gao *et al.*, 2005b; Malinowski *et al.*, 2008; Greenstein *et al.*, 2009). The solution transfer experiments involved using a small volume of solution from a tissue bath with PVAT, adding it to a preconstricted vessel and measuring the vascular response. These suggest that the observed anticontractile property of PVAT is not merely a consequence of it acting as an obstacle to diffusion for vasoconstrictors, but as a dynamic tissue which secretes adipokines with anticontractile properties.

There are likely to be a number of substances which account for the anticontractile effects of PVAT. Indeed, both endothelium-dependent and endothelium-independent mechanisms have been demonstrated. Protocols using rat mesenteric vessels have shown that both PVAT and exogenous adiponectin exert anticontractile effects on preconstricted small arteries (Greenstein *et al.*, 2009); the effects of PVAT have been corroborated by experiments on rat aorta and its surrounding PVAT, which mostly consists of brown adipocytes (Lohn *et al.*, 2002; Gao *et al.*, 2007). Gao has shown that the endothelium-independent mechanism involves hydrogen peroxide (H_2O_2) and subsequent activation of soluble guanylyl cyclase. At low concentrations (10–100 μM) of H_2O_2, mesenteric vessels preconstricted with phenylephrine undergo further constriction, but higher concentrations of H_2O_2 (0.3–1 mM) result in a biphasic response, with an early constriction followed by relaxation. Hydrogen peroxide released from both adipocytes and macrophages can act via the raf/MEK/ERK pathway and influence the phosphorylation of contractile apparatus in vascular smooth myocytes (Figure 2).

Interestingly, exposure of rat mesenteric arteries to electric field stimulation (EFS) leads to a contractile response via stimulation of α_1-adrenoceptors by the perivascular sympathetic nerves. PVAT enhances this contractile response by stimulating superoxide generation and activation of tyrosine kinase and MAPK/ERK pathway (Gao *et al.*, 2006) (Figure 2). More recently it has been shown that Angiotensin II derived from adipocytes potentiates the contractile response to EFS (Lu *et al.*, 2010), thereby highlighting the role of the renin-angiotensin system (RAS) within PVAT with regards to local vascular tone modulation.

Physiological release and action of ADRF: possible role of the potassium channel

The exact mechanisms by which adipocytes exert their effects on adjacent arteries are not well understood. Figure 2 shows a number of the potential mechanisms involved. Downstream in the PVAT anticontractile pathway are vascular potassium (K) channels, which occupy a central position in the maintenance and regulation of vascular tone. Early experiments on potassium channels have identified roles for a number of K channels. In rat mesentery the anticontractile effect is attenuated by blockade of the delayed rectifier K channel (K_V) (Verlohren *et al.*, 2004). Studies of rat aorta suggest roles for adenosine triphosphate (ATP)-sensitive K channels (K_{ATP}) (Lohn *et al.*, 2002) as well as small and intermediate conductance calcium-sensitive potassium channels (SK_{Ca} and IK_{Ca}) (Gao *et al.*, 2007). In human internal mammary artery, the relaxing factor appears to work via large conductance calcium-sensitive K channels (BK_{Ca}) (Gao *et al.*, 2005b).

More recent experiments have highlighted the pivotal role of the BK_{Ca} channel in mediating the PVAT effect. Work from our group supports the role of the BK_{Ca} channel in the vasodilator function of PVAT. Pharmacological inhibition of the channel using paxilline or using mesenteric arteries from BK_{Ca} knockout mice ($Slo^{-/-}$, $BK\beta1^{-/-}$) results in a significant impairment of the response (Lynch *et al.*, 2010b). This highlights the role of BK_{Ca} channels in facilitating the ADRF effect, but further work is needed to clarify whether BK_{Ca} channels are also present on adipocytes as well as the vascular myocytes. Additionally, the removal of endothelium or inhibition of NOS can significantly attenuate the response in these mouse models (Lynch *et al.*, 2010a).

Elegant micro-electrode studies of de-endothelialized rat mesenteric arteries have shown that in non-constricted vessels the hyperpolarization to exogenous adiponectin is inhibited by selective blockade of BK_{Ca} (Egner *et al.*, 2010). This group has reported also that stimulation of β3 adrenoceptors releases a factor which indirectly activates myocyte BK_{Ca} channels. They have suggested that this factor is adiponectin working via myocyte adipoR1 receptors to activate AMPK. These protocols were performed in the absence of endothelium. However, there are BK_{Ca} channels present on the endothelium (Hughes *et al.*, 2010) and stimulation of these channels by circulating ADRF could result in hyperpolarization of endothelial cells and subsequent hyperpolarization of vascular myocytes via the myoendothelial junction, thus leading to their relaxation (Figure 2).

Obesity is a state of adrenergic overdrive with increased circulating noradrenaline levels (Prezio *et al.*, 1964) and an overactive sympathetic nervous system (Lambert *et al.*, 2010) releasing noradrenaline at nerve terminals which are present in PVAT. Noradrenaline can bind to β3 adrenoceptors, although with lower affinity than to β1 and β2 adrenoceptors. In obesity one would expect that overstimulation of the β3 adrenoceptor by noradrenaline would result in an increase in adiponectin release from adipocytes thus lowering vessel tone and enhancing metabolic homeostasis. However, in obesity, there are reduced blood and adipocyte adiponectin levels (Asayama *et al.*, 2003; Kern *et al.*, 2003) as well as down-regulation of adiponectin receptors (Kadowaki and Yamauchi, 2005). Further research is required to explain the reduced levels of adiponectin and its receptors. There may be an increase in breakdown of adiponectin secondary to oxidative stress and inflammation or a reduction in its production due to obesity-induced damage to the intracellular mechanisms involved in production of the protein. We shall discuss further evidence for inflammation-induced PVAT damage later in this review.

PVAT and human vessels

Harvesting human arteries for PVAT studies is fraught with difficulty, which may explain the limited number of pub-

lished studies. The most accessible human blood vessel is the internal thoracic artery, obtained during coronary artery bypass graft operations (Gao *et al.*, 2005b; Malinowski *et al.*, 2008) and small arteries from gluteal fat biopsies (Greenstein *et al.*, 2009). Both are clinically relevant vascular beds. However, the atraumatic harvest of saphenous vein grafts, via the 'no-touch' technique has been shown to result in vessels with intact PVAT and immunohistochemical evidence of the potent vasodilator NO (Dashwood *et al.*, 2007; 2009). Venous PVAT has been shown to exert an anticontractile effect on adjacent tissue by releasing Ang-(1–7) which activates Kv channels and relaxes vascular myocytes through eNOS release (Lu *et al.*, 2011b).

Gao *et al.* first studied the anticontractile properties of human PVAT using segments of human internal thoracic artery. It was shown that human PVAT exerted an anticontractile effect on its adjacent vessel upon exposure to contractile agents including U46619, which is a thromboxane A_2/prostaglandin H_2 receptor antagonist (Gao *et al.*, 2005b). This is a significant finding because thromboxane A_2 and its stable metabolite thromboxane B_2 (TxB_2) are known to be potent vasoconstrictors and the levels of TxB_2 are known to be increased during cardiopulmonary bypass (Davies *et al.*, 1980). Perioperative spasm of the internal thoracic artery is a commonly encountered issue (He *et al.*, 1994), therefore leaving the perivascular fat intact may help counteract the vasoconstricting effects of the increased plasma thromboxane levels. In this study, the anticontractile effect of PVAT was shown to be due to a transferrable relaxing factor (ADRF) that activated BK_{Ca} channels.

Malinowski *et al.* also used segments of human internal thoracic artery and investigated whether it is PVAT *per se*, or adipose tissue in general, that is capable of exerting an anticontractile effect (Malinowski *et al.*, 2008). Following a coronary artery bypass operation, the *in situ* graft (taken from the internal thoracic artery) heals in close proximity to pleural tissue. Dose-responses to serotonin were constructed for skeletonized vessel segments with and without incubation with pleural fat. The study showed that, despite the presence of white adipocytes in pleural fat, there was no anticontractile effect. The study also demonstrated clearly that adipokines released by PVAT are able to affect the blood vessel even if the fat tissue has been loosely added to the tissue bath and is not in direct contact with the artery.

In order to assess whether ADRF acts via the endothelium, Malinowski *et al.* incubated their vessels with inhibitors of NO synthase and cyclooxygenase (COX). It is well known that NO and prostacyclin produced in the endothelium have vasodilatory effects. This study showed that inhibition of NO and COX did not abolish the anticontractile effect of PVAT. This does not mean that PVAT function is endothelium-independent; rather that it can exert its effect independent of the NO and COX pathways.

The most recent study of human PVAT has been carried out on small arteries harvested from adipose tissue samples obtained by gluteal biopsies in patients with MetS. Figure 3A demonstrates the anticontractile function of PVAT in healthy individuals whilst Figure 3B shows that PVAT did not exhibit an anticontractile function in the group with MetS (Greenstein *et al.*, 2009). Incubating healthy PVAT intact human vessels with a fragment of the human type 1 adiponectin

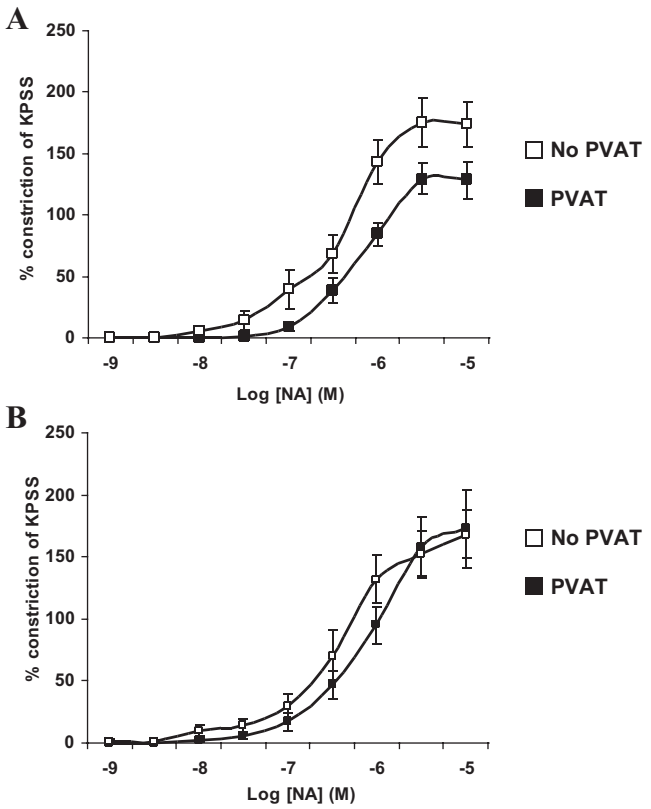

Figure 3

Effect of obesity and the metabolic syndrome on anticontractile capacity of PVAT on small arteries from subcutaneous gluteal fat, *$P < 0.01$ (Greenstein *et al.*, 2009). (A) In healthy control participants PVAT exerts a significant anticontractile effect ($P = 0.009$, multiple ANOVA) when compared with contractility of arteries without PVAT ($n = 10$). (B) In patients with obesity and metabolic syndrome, presence of PVAT has no effect on contractility ($n = 10$). KPSS, high potassium physiological saline solution; NA, noradrenaline.

receptor completely abolished PVAT anticontractile function, thus identifying adiponectin as an ADRF in human PVAT. Also presence of NO in human PVAT was demonstrated by incubating PVAT intact healthy vessels with L-NMMA which resulted in attenuation of the anticontractile property of PVAT. This study will be discussed further in the next section.

PVAT, obesity and inflammation

The effect of obesity on PVAT function was first reported in 2005. It was shown that, prenatal exposure of rats to nicotine caused postnatal obesity and an increased amount of PVAT. However, the rise in PVAT volume which was associated with weight gain resulted in a reduction in the anticontractile function of PVAT surrounding the aorta. It was thought that this may be due to a change in the nature of PVAT or a reduction in secretion of relaxation factor(s) from PVAT (Gao *et al.*, 2005a).

Often obesity is associated with hyperglycaemia and hyperinsulinaemia in the context of the MetS. The role of

circulating insulin on vascular tone has been well documented (Eringa *et al.*, 2004), but its effect on PVAT-mediated vascular responses has not been established. However, both acute and chronic hyperglycaemia enhance the PVAT anticontractile effect (Lee *et al.*, 2009b). This highlights the complex nature of the MetS and the difficulty of controlling for variables in *ex vivo* experiments.

Adipocytes undergo significant hypertrophy in obesity. The cross-sectional area of adipocytes in obese individuals with MetS is up to 1000 μm^2 larger than that of healthy individuals (Greenstein *et al.*, 2009). Given that the diffusion limit of oxygen is thought to be around 100 µm (Hosogai *et al.*, 2007), the hypertrophied adipocytes are likely to be subjected to a decreased oxygen tension. In obese individuals, there is no increase in blood supply to match the increase in adipocyte size, and the postprandial increase of blood flow that occurs in lean subjects is also absent in obesity (Bulow *et al.*, 1987; Coppack *et al.*, 1992). This implies that hypertrophied adipocytes exist in a state of relative hypoxia. This has been confirmed by pimonidazole staining (a 2-nitroimidazole which is activated at low oxygen concentrations) of adipocytes from obese mice (Hosogai *et al.*, 2007), as well as measurements of partial pressures of oxygen in abdominal subcutaneous adipose tissue of obese humans (Pasarica *et al.*, 2009). In the context of hypoxia, vasa vasorum in the adipose tissue surrounding vessels plays an important role in providing oxygen to the hypoxic PVAT. Recent work has shown that PVAT induces the formation of vasa vasorum (Manka *et al.*, 2010), which would be beneficial in obesity.

Hypoxia reduces PVAT anticontractile effects in rat mesenteric arteries (Greenstein *et al.*, 2009); however, in aorta the opposite occurs, namely an enhancement of PVAT function (Maenhaut *et al.*, 2010). Clearly, these regional differences merit further evaluation.

From a therapeutic perspective, experimental hypoxia-induced damage to PVAT function can be rescued using free radical scavengers (Greenstein *et al.*, 2009) and Eplerenone (Withers *et al.*, 2011). Future clinical trials can explore the possibility of using such drugs in the treatment of patients with obesity.

The chronic low-grade inflammatory state which develops as a consequence of hypoxia in obesity is marked by an increase in blood levels of ROS and pro-inflammatory cytokines such as CRP, IL-6 and TNF-α (Trayhurn *et al.*, 2008). Incubation with TNF reduces the anticontractile effect of PVAT in vessels harvested from healthy individuals, suggesting that the higher levels of the inflammatory cytokine in obesity may partly account for the reduced anticontractile function of PVAT. Similar results were shown in healthy rat tissue where incubation with TNF and IL-6 resulted in a reduction of the anticontractile function of PVAT (Greenstein *et al.*, 2009).

Further human studies have shown a link between high levels of IL-6 and increased risk of CAD (Pai *et al.*, 2004). Epicardial adipose tissue IL-6 mRNA levels have been shown to be higher in CAD than non-CAD patients, with higher levels correlating with greater degrees of angiographically defined vascular injury (Eiras *et al.*, 2008). The up-regulation of inflammatory cytokines in the adipose tissue suggest a role for oxidative stress as a mechanism for damage to PVAT function. Proteomic analysis of epicardial adipose tissue and subcutaneous adipose tissue obtained from CAD patients has demonstrated higher levels of ROS in epicardial adipose tissue, and mRNA analysis has revealed lower levels of the antioxidant enzyme catalase (Salgado-Somoza *et al.*, 2010).

The role of the macrophage

Increased macrophage numbers in adipose tissue of obese animals (Rausch *et al.*, 2008) and humans (Weisberg *et al.*, 2003) also support the hypoxia theory, as hypoxic cells secrete chemokines to attract macrophages (Pasarica *et al.*, 2009). Further data from our group suggest that when PVAT from mouse vessels is rendered hypoxic, the presence and activation of macrophages is the key modulator of increased vascular contractility. Moreover, following the conditional ablation of macrophages, hypoxic insult has no effect on contractility of the vessels (Figure 4), further implicating a role for macrophages in mediating the response to inflammatory stimuli (Withers *et al.*, 2011).

The levels of monocyte chemotactic protein-1 (MCP-1) are raised in plasma and adipose tissue of both genetically

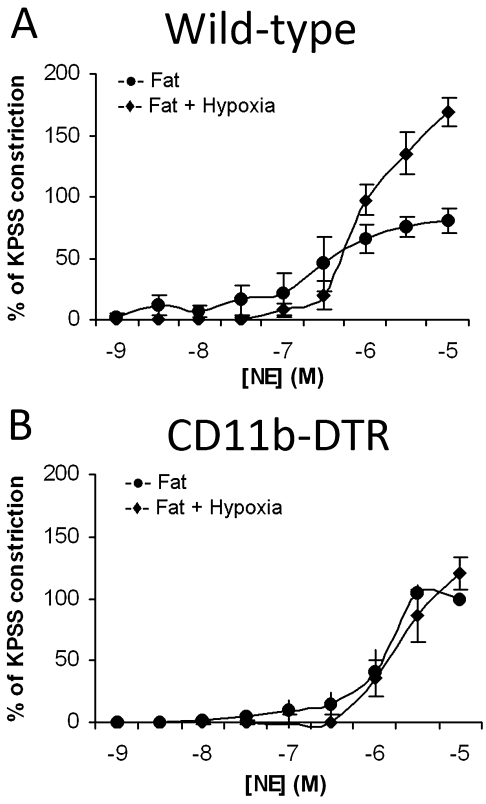

Figure 4

The presence of macrophages is the key modulator of increased vascular contractility in vessels with hypoxic PVAT (Withers *et al.*, 2011). (A) When PVAT from mouse vessels is rendered hypoxic, there is increased sensitivity of the vessel to cumulative doses of noradrenaline. (B) In CD11b–diphtheria toxin (DT) receptor (DTR) transgenic mice (DT administration selectively kills monocytes/macrophages), hypoxia has no effect on vascular contractility. KPSS, high potassium physiological saline solution; NE, norepinephrine.

obese and diet-induced obese mice. In MCP-1 knockout diet-induced obese mice, there is a significant reduction in numbers of macrophages in the adipose tissue as well as improved insulin sensitivity (Kanda *et al.*, 2006). Paradoxically, however, while it is likely that activated macrophages in obese patients contribute to PVAT damage, in health, perivascular macrophages enable structural remodelling of the small artery wall in response to flow. Bakker *et al.* have shown that in mouse mesenteric arteries, inactivation of macrophages by clodronate, reduces flow-induced remodelling (Bakker *et al.*, 2006) which is observed in arteries from diabetic animals (Belin de Chantemele *et al.*, 2009).

We propose that reduced levels of adiponectin in obesity enhances macrophage effects on PVAT function as adiponectin suppresses macrophage phagocytosis and TNF-α production (Yokota *et al.*, 2000). Moreover, it has been shown that hypoxia and ROS decrease adiponectin production from 3T3-L1 adipocytes (Chen *et al.*, 2006).

A major process in the development of atherosclerosis is the recruitment of leucocytes to the endothelium and their subsequent migration into the subendothelial space. Vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) are two of the adhesion molecules expressed on vascular endothelial cells and are responsible for facilitating the recruitment of the white blood cells. In patients with coronary heart disease and carotid artery atherosclerosis, there are increased levels of ICAM-1 (Hwang *et al.*, 1997). Resistin is produced by adipocytes and is also expressed in macrophages which are present in larger numbers in the PVAT of obese animals and humans. It has been shown that resistin induces the expression of ICAM-1 and VCAM-1 in human endothelial cells and that adiponectin can block this effect of resistin (Kawanami *et al.*, 2004). PPARγ agonists have been shown to reduce the expression of resistin mRNA in macrophages (Patel *et al.*, 2003) whilst increasing plasma levels of adiponectin (Long *et al.*, 2010). Ouchi *et al.* have also reported that adiponectin can dose-dependently inhibit the expression of VCAM-1 and ICAM-1 in human aortic endothelial cells (Ouchi *et al.*, 1999).

The RAS within PVAT

The RAS is an important regulator of blood pressure and vascular function. Circulating angiotensinogen is cleaved by renin to produce angiotensin 1 (Ang I). Angiotensin-converting enzyme (ACE) converts this to Ang II (Engeli *et al.*, 1999) which binds to two receptors, AT1 and AT2. Adipocytes secrete products of the RAS and the functional significance of this has been reviewed in depth (Gorzelniak *et al.*, 2002; Engeli *et al.*, 2003). RAS products such as ACE, angiotensinogen and AT1 and AT2 receptors have been identified on human and rodent adipocytes (Cassis *et al.*, 1988; Harp and DiGirolamo, 1995; Crandall *et al.*, 1999; Schling *et al.*, 1999; Cassis, 2000). However, it is becoming apparent that the adipocyte RAS system influences tissues beyond its local environment and that it plays a role in the development of vascular diseases such as obesity and hypertension. Much is known about adipose RAS *per se*; however, this section will focus on the RAS component of PVAT.

There appears to be differential distribution of components of the RAS system expressed in both white and brown PVAT (Cassis *et al.*, 1988; Campbell *et al.*, 1993; Campbell *et al.*, 1995; Engeli *et al.*, 1999; Galvez *et al.*, 2006). Angiotensinogen and Ang I expression is similar between white and brown tissue (Galvez *et al.*, 2006); however, Ang II expression appears to vary between brown and white perivascular adipocytes (Jonsson *et al.*, 1994; Engeli *et al.*, 1999; Schling *et al.*, 1999; Giacchetti *et al.*, 2002). There is conflicting evidence of renin expression in perivascular brown or white adipose tissue with some groups reporting no detectable levels of the protein and others reporting its presence (Engeli *et al.*, 1999; Giacchetti *et al.*, 2002; Galvez-Prieto *et al.*, 2008).

There is a growing body of evidence to suggest that elements of adipocyte-derived RAS play vital roles in the normal and pathogenic responses of blood vessels. Ang 1–7 has recently been identified as an important PVAT diffusible RAS product (Lee *et al.*, 2009a). It is released by PVAT and acts on the endothelium to stimulate the release of NO, which in turn hyperpolarizes smooth muscle cells through K_{Ca} channels. Ang 1–7 is thought to counterbalance the contractile influence of Ang II (Yagil and Yagil, 2003; Ferrario *et al.*, 2005; Reudelhuber, 2006). Increased expression of Ang II and decreased expression of Ang 1–7 has been reported in hypertensive models. Indeed treatment of hypertensive models with ACE inhibitors lowers Ang II and increases Ang 1–7 (Yagil and Yagil, 2003). Ang I antagonism using Losartan restored PVAT-induced anticontractility in fructose fed rats (Huang *et al.*, 2010). Ang II type 1 receptor antagonism using olmesartan can reduce blood pressure and increase adiponectin levels in a rat model of MetS (Mizukawa *et al.*, 2009). Lu *et al.* have recently shown that PVAT-induced anticontractility is impaired in spontaneously hypertensive rats and that the anticontractile response could be inhibited with an Ang 1–7 antagonist in normotensive rats (Lu *et al.*, 2011a). Interestingly Ang 1–7 is also thought to be vital for the PVAT-induced anticontractile response observed in venous rings (Lu *et al.*, 2011b).

Epicardial adipose tissue and its clinical correlates

Given its proximity to the coronary vessels, epicardial and pericoronary fat present an attractive therapeutic target for CAD and the treatment of its consequent symptoms. Epicardial adipose tissue thickness correlates well with waist circumference, visceral adipose tissue mass, fasting insulin and diastolic blood pressure (Iacobellis *et al.*, 2003a,b) and has been shown to be significantly greater in patients with MetS than those without (Iacobellis *et al.*, 2008).

In man, Chatterjee *et al.* have shown that PVAT surrounding coronary vessels is made up of smaller, more irregularly shaped adipocytes which exhibit a reduced differentiation state as compared with visceral and subcutaneous fat depots. They also report that PVAT from the coronary vessels secretes lower levels of the anti-inflammatory cytokine adiponectin and higher levels of pro-inflammatory cytokines IL-8 and IL-6 as compared with subcutaneous and visceral adipocytes (Chatterjee *et al.*, 2009). Moreover, exposure to IL-6 has been linked with a reduction in adiponectin production by human adipocytes (Simons *et al.*, 2007). There is a high level of macrophage

infiltration in the epicardial fat tissue of CAD patients (Baker *et al.*, 2006); indeed there is a greater pro-inflammatory profile of the epicardial adipose tissue as compared with the subcutaneous adipose tissue of individuals with CAD (Mazurek *et al.*, 2003). Also it has been shown that peri-coronary adipose tissue contains higher levels of MCP-1 as compared with visceral and subcutaneous tissue (Chatterjee *et al.*, 2009).

There are lower adiponectin mRNA levels in the epicardial adipose tissue of patients with CAD as compared with samples from those undergoing thoracic operations for non-CAD-related indications (Iacobellis *et al.*, 2005; 2009; Eiras *et al.*, 2008). Lower levels of adiponectin in epicardial tissue have also been associated with hypertension (Ohashi *et al.*, 2006; Teijeira-Fernandez *et al.*, 2008) and increased risk of myocardial infarction (Pischon *et al.*, 2004).

Greif *et al*. (Greif *et al.*, 2009) have studied the relationship between epicardial adipose tissue and coronary atherosclerosis in 286 consecutive patients with an intermediate pretest likelihood for CAD using dual-source multi-slice CT coronary angiography. Interestingly, they found no correlation between BMI and coronary atherosclerosis, but those with atherosclerotic lesions were found to have higher volumes of pericardial adipose tissue (226 $\pm$ 92 cm^3) than those without lesions (134 $\pm$ 56 cm^3; $P < 0.001$). Those with larger volumes of pericardial adipose tissue had lower levels of plasma adiponectin and HDL, but higher levels of the pro-inflammatory cytokine TNF and highly sensitive CRP.

A recent comprehensive post-mortem study on 41 human cadavers carried out by Spiroglou *et al*. has shown that adiponectin is present in both peri-aortic and peri-coronary human fat depots and its levels inversely correlate with the degree of atherosclerosis (Spiroglou *et al.*, 2010). In animal models of vascular injury, adiponectin knockout mice exhibit more severe neointimal thickening and increased proliferation of vascular smooth muscle cells than wild-type mice (Matsuda *et al.*, 2002).

Conclusions and perspectives

In this review we have discussed clinically significant PVAT studies and highlighted a number of potential mechanisms via which PVAT may exert its anticontractile effect in health and how these can become disordered in obesity (Figure 2).

Our current understanding of the intricate nature of interactions between adipocytes, vascular myocytes and endothelial cells is not sufficient to explain precisely the role adipokines play and the receptors and signalling pathways involved in facilitating their actions. We can conclude that in response to vasoconstrictor challenges, adipocytes release adipokines with anticontractile effects on the smooth myocytes of adjacent vessels. The mechanism of action of the ADRF(s) may involve direct action on potassium channels of vascular myocytes. Circulating ADRF(s) can also work via potassium channels on the endothelium and there is also the possibility that the potassium channels present on white adipocytes may be involved in facilitating the ADRF action. ADRF action on its receptors can also lead to NO release from adipocytes and endothelial cells. We have also discussed the contribution of macrophages and ROS in compromising the anticontractile effect of PVAT in hypoxia.

It is clear that animal studies have contributed significantly to our current understanding of the mechanism of action of PVAT. However, the studies have been conducted using different vascular beds in different animal models using different pharmacological approaches. Also it has become apparent that PVAT property varies amongst species and vascular beds, and not all animal studies are clinically relevant thus highlighting the paramount importance of research using human tissue.

Obesity and its associated co-morbidities pose a huge threat to public health and to our increasingly fragile economies. Clearly, prevention of obesity by effective educational programmes needs to be coupled with treatments for those currently suffering from the consequences of the disorder. Currently, the most radical and arguably the only effective intervention in treatment of obesity is bariatric surgery which is available only to a limited number of patients worldwide. The recent appreciation of the contribution of PVAT to metabolic homeostasis and control of local vessel tone, as well as its involvement in disease states such as obesity, hypertension and atherosclerosis has generated great interest in the possibility of reversing the PVAT damage and rescuing its pre-morbid properties. This field of research will no doubt continue to provide new challenges and opportunities in the years to come.

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Conflict of interest

The authors declare no conflict of interest.

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