Perivascular adipose tissue as an endocrine organ: the role of statins

Fabio Sanna*, Marios Margaritis*, Charalambos Antoniades

Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of
Oxford United Kingdom

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*: equal author contribution

Corresponding author:

Fabio Sanna PhD

Division of Cardiovascular Medicine, Radcliffe Department of Medicine,

University of Oxford, West Wing L6 John Radcliffe Hospital, Headley Way

Oxford OX3 9DU, United Kingdom

e-mail: fabio.sanna@cardiov.ox.ac.uk

Abstract

Adipose tissue (AT), aside from being an energy storage site, functions as a source of cytokines, adipokines and other vasoactive molecules. Dysfunctional AT contributes to the development of cardiovascular disease by shifting to a pro-oxidant, pro-inflammatory phenotype. Perivascular AT (PVAT) is of particular importance to the development of vascular disease, due to its close proximity to the vascular wall. Molecules released from PVAT can exert both pro- and anti-contractile effects, the balance of which plays a role in controlling vascular tone. Recent evidence supports the existence of reciprocal, two-way interactions between PVAT and the vascular wall. Statins, with their pivotal role in cardiovascular disease prevention, have been shown to exert lipid-lowering independent, pleiotropic effects on the vascular wall, some of which may be mediated by modulatory effects on PVAT inflammation and secretome. These effects of statins provide a paradigm for the development of new therapeutic agents aimed at modulating PVAT function, as a novel treatment strategy against cardiovascular disease.

In the past, adipose tissue (AT) was regarded as a passive reservoir of energy storage. However, a vast amount of research in the last decades has established AT as a complex organ which exerts paracrine and endocrine effects via its complex secretome. Perivascular adipose tissue (PVAT) in particular is characterized by its unique role in modulation of vascular function. Due to its proximity with the vascular wall paracrine functions of PVAT could be of particular importance; however, differentiating paracrine from endocrine effects may be difficult. More interestingly, PVAT phenotype and its biological behaviour are affected by the presence of pathological conditions such as obesity, leading to changes in the nature of its secretome and alterations of the underlying vascular biology. Statins, widely used to prevent cardiovascular disease (CVD), have dual actions: they lower low-density lipoprotein (LDL) and cholesterol circulating levels and they also exert cholesterol-independent anti-inflammatory and antioxidant effects, called pleiotropic. In this review article we focus on PVAT as a target of statin therapy aimed at modulating the inflammatory state and function of the underlying vessel.

Traditional view of adipose tissue

One of the primary functions of AT is undoubtedly energy storage and control of the metabolic activities of the body. On the other hand, excessive accumulation of AT, defined as obesity, has been linked to metabolic disorders such as type 2 diabetes, which are causally related with CVD.

AT is composed of densely packed adipocytes, immune cells, such as macrophages and lymphocytes, as well as connective tissue mainly composed of fibroblasts. Depending on the nature of the adipocytes, AT can be divided into two categories, brown (BAT) and white AT (WAT). These two types of AT differ in their physiological features, cell type and body localization. WAT is widely distributed and is the most common type of AT, unlike BAT.

The two different types of AT exert opposite roles: WAT functions as an energy storage depot, while BAT expends energy in order to generate heat, in a process called non-shivering thermogenesis. BAT is characterized by higher expression level of uncoupling protein-1 (UCP-1) compared to WAT(1). UCP-1 uncouples the oxidative phosphorylation from ATP generation in the mitochondria, leading to energy dissipation through heat. From a histological point of view adipocytes in WAT are characterized by accumulation of a single big lipid droplet in the cytosol, whereas brown adipocytes contain multiple small lipid droplets (2). BAT is also characterized by abundant content of mitochondria and blood vessels. BAT was considered to be biologically active in neonates and young children generating heat to maintain normal body temperature (3). Imaging studies revealed the presence of UCP1-expressing adipocytes in adult humans (4). Activation of UCP1, followed heat production in BAT is triggered by sympathetic stimuli such as cold and exercise. In humans activation of UCP1 has been observed in the adipose tissue around neck, while in rodents this process can be also induced in subcutaneous adipose tissue (5, 6). Two recent clinical trials have demonstrated that WAT can be turned into BAT on cold acclimation, in a process defined a WAT browning (7-9). White adipose tissue browning is mainly driven by sympathetic stimulation. Other factors can also stimulate WAT browning including norepinephrine and thyroid hormones (10, 11). Interestingly, clinical studies as well as animal models have shown that hypothyroidism contributes to WAT browning, potentially as a compensatory mechanism of hypothermia resulting from reduced BAT activity(12, 13). Clinical studies proved that obesity is associated with a reduction in BAT activity (14, 15) and body mass index (BMI) also appears to negatively correlate with the amount of BAT, suggesting a possible role of BAT in obesity (8, 16). However, whether obesity is the cause of the effect of reduced BAT has not yet been clarified.

The traditional view of AT as an inert reservoir has been overturned in recent years. Obesity, characterized by WAT expansion and accumulation mainly in the visceral compartment, correlates with metabolic dysfunction and chronic low-grade inflammation, leading to type 2 diabetes, atherosclerosis and CVD. AT of obese patients is characterized by increased adipocytes size and production of several pro-inflammatory factors, including interleukin 6 (IL-6), TNF α and monocyte chemoattractant protein 1 (MCP-1) (17, 18).

Role of PVAT

In rodents, PVAT can be white, such as the PVAT surrounding the mesenteric artery, or it can resemble BAT, such as the depot surrounding the aorta (19, 20). In humans the morphological status of PVAT is not well defined, Brown *et al.* described PVAT differences between species, underlying that PVAT resembles both WAT and BAT(21). From a histological point of view PVAT is composed of adipocytes as well as stromal cells like fibroblasts and monocytes. Cellular composition of PVAT has been reported to be different depending on the anatomical region (22).

PVAT was originally thought to have the sole function of supporting the underlying vascular bed. However, it is now widely believed to be an active contributor in the regulation of vascular function. PVAT has important endocrine and paracrine roles which affect complex pathophysiological mechanisms such as vascular contractility, oxidative stress and inflammatory state. Several molecules released by PVAT (cytokines, adipokines, nitric oxide (NO) and hydrogen sulphide (H₂S)) can influence different functions on the underlying vasculature. Similarly, expression of pro-oxidant enzymes such as NADPH-oxidases (23), endothelial nitric oxide synthase (eNOS) (24, 25) as well as anti-oxidant enzymes like superoxide dismutase (SOD) has been reported in AT.

The ability of PVAT to affect vascular smooth muscle cell (VSMC) proliferation and migration has been extensively studied. Conditioned medium from differentiated 3T3-L1 adipocytes stimulates human VSMCs proliferation (26). In the same study, these effects were abolished by boiling or digesting the adipocyte-derived supernatant with proteinase K, suggesting a proteinous nature of the not yet identified adipocytes-derived growing factor(s) involved in VSMCs proliferation.

It has been shown that PVAT is able to inhibit vasoconstriction by releasing a not yet fully elucidated relaxing factor called adventitia-derived relaxing factor (ADRF). In rat aortas ADRF has been shown to reduce contraction via activation of K^+ channels in the underlying VSMCs (27). Adipokines, cytokines, H_2S and NO have been proposed as being ADRFs. It has been recently demonstrated that H_2S produced in rat's PVAT by the enzyme cystathionine γ -lyase (CSE) is responsible for the anticontractile effects of PVAT on its adjacent vessels(28). The mechanism through which H_2S improves vasodilation is not fully understood. However, there is evidence for involvement of K_{ATP} channels(29): H_2S induces rat aortic tissue relaxation via opening of K_{ATP} channels in VSMCs(30). Another important gaseous molecule produced by PVAT is NO, which can passively diffuse across membranes and increase endothelium-independent vasodilatory responses (31). Despite the fact that both H_2S and NO can be produced by PVAT and affect vascular function, the exact nature of ADRF has not yet been identified.

The AT secretome mainly consists of adipokines such as adiponectin, leptin, omentin, resistin and visfatin, which are produced predominantly by adipocytes. Moreover, cytokines like IL-6 and TNF α can be produced by immune cells residing in the AT in the vascular stromal fraction(32).

Influence of PVAT on cardiovascular disease

Several studies have recently reported the association of obesity with vascular inflammation. AT, in particular dysfunctional AT in obese subjects, is known to be related with CVD such atherosclerosis and type 2 diabetes. AT expansion and macrophage accumulation are characteristics of obesity, leading to activation of local and systemic inflammation. This, in turn, is associated with increased production of pro-inflammatory cytokines and adipokines. Adipocyte expansion also results in increased expression of chemotactic molecules such as MCP-1 which promotes monocyte migration into PVAT. Kanda *et al.* observed higher MCP-1 expression in adipocytes as well as increased plasma levels in transgenic obese *db/db* mice. Moreover, in the same study had shown a significant reduction of macrophage infiltration in the AT of a transgenic MCP-1 knock-out murine model (33). Macrophages in obese AT have also been demonstrated to display a pro-inflammatory phenotype characterized by activation of macrophages (34).

In obese patients with metabolic syndrome the anti-contractile effects of PVAT on small arteries have been shown to be completely abolished compared to normal subjects (35), while pro-inflammatory cytokines TNF α and IL-6 are mainly responsible for the attenuation of the anti-contractile effects of PVAT, which are fully restored by SOD and catalase (35). Therefore, PVAT is considered as a key modulator of vascular function and inflammation in the underlying vascular wall.

As mentioned previously, H₂S produced by PVAT exhibits vasodilatory properties. In both rodents and humans, obesity leads to significantly reduced production of H₂S (36, 37). In addition to H₂S, NO production in PVAT of obese mice is altered. Ning *et al.* demonstrated that in obese mice, NO produced by PVAT is reduced compared to lean mice. They also proved that NO reduction is due to eNOS uncoupling in PVAT, leading to reduced vasodilator response in mouse aorta(25). The adipokine adiponectin, encoded by *ADIPOQ* gene, is highly expressed in adipocytes and its plasma levels have been found to be lower in

obese subjects (38, 39) as well as in patients with type 2 diabetes (40). Several studies demonstrated that decreased adiponectin levels and increased oxidative stress in the vascular wall are linked. Our group has previously shown that adiponectin reduces NADPH-oxidases activation in human internal mammary arteries by preventing translocation of the small GTP-ase Rac1 from the cytoplasm to the plasma membrane via a PI3K/Akt dependent mechanism(40).

As discussed, PVAT can be considered as an endocrine and paracrine organ, able to affect vascular functions by producing vasoactive molecules. However, while it is now clear that PVAT affects vascular function through its complex secretory profile, a new concept of a mutual cross-talk between the underlying vessel and PVAT has been introduced. Our group has shown that adiponectin positively affects endothelial eNOS coupling by increasing vascular tetrahydrobiopterin (BH₄) in human arteries and veins (41). Moreover, we demonstrated that vascular superoxide (O₂⁻) is negatively correlated with serum adiponectin levels, but positively with *ADIPOQ* gene expression and release of adiponectin from PVAT (41). This seemingly contradictory finding can be explained by hypothesizing that PVAT acts as a sensor responding to changes coming from the vascular wall and adjusting its secretory profile to react to those changes. Our experiments showed that one of the possible mediators originating from the vascular wall in conditions of oxidative stress is 4-hydroxy-nonenal (4-HNE) and its protein adducts. 4-HNE has the ability to up-regulate adiponectin gene expression in PVAT, thus acting as a paracrine defence mechanism against increased oxidative stress originating from the underlying vasculature. This is an important and novel indication that the interactions between PVAT and vascular wall can be mutual and bi-directional (41).

Targeting PVAT: the role of statins

Statins are widely used to reduce cardiovascular risk. They exert their effects on the cardiovascular system through lipid lowering by inhibiting hydroxyl-methyl-glutaryl coenzyme A reductase enzyme (HMG-CoA reductase), which is a key enzyme in the biosynthesis of cholesterol. HMG-CoA reductase catalyses the conversion of HMG-CoA into mevalonic acid, which is the precursor of cholesterol. Inhibition of HMG-CoA-reductase leads to reduced biosynthesis of cholesterol.

Cholesterol lowering-independent effects have been reported to contribute to the beneficial effects of statins on the cardiovascular system. These effects have been named “pleiotropic”; this term refers to all anti-inflammatory(42, 43) and anti-oxidant (42, 44-46) properties attributed to the use of statins via mechanisms other than their cholesterol-lowering actions. Given the role of PVAT in regulating vascular homeostasis, it is rational to hypothesize that at least some of the beneficial effects of statins may be mediated by direct actions on PVAT.

Lipid soluble vs water-soluble statins: possible implications on AT biology

Whereas statins share a common mechanism of action, competitive inhibition of 3-HMG-CoA, they may be divided in two groups according to their water solubility. From a chemical point of view, lipophilicity of a drug can be expressed as log D (the logarithm of the ratio between the distribution of the chemical in octanol and distribution in water at pH 7.4). Atorvastatin, lovastatin and simvastatin belong to the lipophilic category, having log D respectively 1.53, 2.59 and 2.44, while pravastatin and rosuvastatin are hydrophilic, log D - 0.47 and -0.25 (47). Statins solubility affects parameters like pharmacokinetic and pharmacodynamic. Moreover, side effects as well as pleiotropic effects seem to be related to the chemical nature of the statins groups. Lipophilic statins can passively cross the plasma membrane, while hydrophilic statins need to be actively transported inside the cell by ion

channels (48). In this thus, hydrophilic statins are more hepatoselective and more unlikely provide side effects like central nervous system actions.

Distinct statins may also differently affect AT biology. Despite the fact that lipophilic statins can easily accumulate in the adipose tissue, several studies evidenced a more complex mechanism of action. In 3T3-L1 adipocytes, hydrophilic statin pravastatin increases the expression as well as secretion of adiponectin while the same effect has not been observed using the more lipophilic statin simvastatin. In the same paper the authors proved that prolonged pravastatin treatment increases plasma concentration of adiponectin in C57BL/6J mice (49). Nakata *et al.* studied the effect of different statins on adipocytes maturation and observed that 10ng/mL of atorvastatin inhibits 3T3-L1 cells differentiation into mature adipocytes, while simvastatin and pravastatin did not affect adipocytes maturation (50). The authors attributed pravastatin lack of effect to its hydrophilic properties. However, simvastatin which is lipophilic did not affect adipocytes maturation. Andreadou *et al.* demonstrated that short term administration of the lipophilic simvastatin in rabbits exerts different effects compared to hydrophilic pravastatin. Simvastatin reduced infarct size and activate eNOS and Akt only in normocholesterolaemic animals while pravastatin reduced infarct size both in normo- and in hyper-cholesterolaemic animals (51)

Whereas, lipophilic statins are expected to significantly diffuse into AT, further studies are still require to elucidate the mechanism through which lipophilic as well as hydrophilic statins have different implications on AT biology.

Anti-inflammatory effects of statins on PVAT

Several clinical trials have demonstrated that statins possess pleiotropic effects. One of the first pieces of evidence came from the PRINCE (pravastatin inflammation/CRP evaluation) trial (52). In this study treatment with pravastatin *per os* (40 mg/day) led to

significantly reduced serum levels of CRP in individuals with or without CVD, at 12 and 24 weeks of treatment. Importantly, this effect was independent of changes in LDL-C levels(52), in fact, the only independent predictors of CRP level change from baseline at 0 weeks were the baseline CRP levels themselves and treatment with the statin(52). Several years later, secondary analysis of the landmark JUPITER trial (Justification for the Use of Statin in Prevention: An Intervention Trial Evaluating Rosuvastatin) showed that higher CRP levels in apparently healthy, normo-lipidaemic individuals are associated with increased future vascular risk; treatment with rosuvastatin significantly reduced levels of CRP and cardiovascular risk, independently of LDL-C(53).

Statins exert their pleiotropic effects through mevalonate-mediated inhibition of isoprenoid formation, as stated previously. This leads to reduced activation of the pro-oxidant NADPH-oxidases, while also promoting endogenous antioxidant defense mechanisms. While this has been demonstrated in a large number of studies in vascular cells, cardiomyocytes and platelets, the existing literature focusing on PVAT is scarce. In an interesting animal study, the investigators used a rabbit model of high-fat diet-induced hyperlipidaemia (54). They demonstrated that high-fat diet leads to inflammatory changes in pericoronary adipose tissue, associated with increased inflammatory cell infiltration and pro-inflammatory gene expression pattern. Short-term treatment with high-dose atorvastatin led to a reversal of these effects, associated with improvement in the endothelial function of the underlying vessel. *In vitro* studies of adipocytes exposed to lipopolysaccharide showed that these effects of atorvastatin are mediated, at least in part, by inhibition of the 5-lipoxygenase pathway (54).

Stable atherosclerotic plaques are characterized by reduced NO bioavailability increased expression of H₂S generating enzymes. H₂S has been related to increased angiogenesis and this may result as a potential risk factor for plaque vulnerability (55). Simvastatin promotes atherosclerotic plaque stability by activating eNOS and restoring NO

balance. Simvastatin also reduces expression of the transcription factor HIF-1 α as well as reducing H₂S producing enzyme CSE, leading to lower levels of H₂S, preventing from its excessive accumulation, acting as a mitochondrial respiratory chain poison (56, 57).

Vasodilatory actions of statins through effects on PVAT

As described in detail previously, PVAT is a source of vasoactive molecules, some of which possess potent vasodilatory effects and play an important role in regulation of vascular tone. It has been hypothesized that some of the beneficial vasoprotective effects of statins may be mediated via actions on PVAT, by affecting its ability to produce vasodilatory mediators. Indeed, in a very interesting study, the researchers investigated the role of perivascular adipocyte-derived relaxation factor (PVRF) in a spontaneously hypertensive rat (SHR) model (58). In this model, thoracic aortic rings exhibit increased contractile responses to phenylephrine compared to controls. The investigators report that this pro-contractile effect is attenuated after co-incubation of SHR thoracic aortic rings PVAT obtained from normotensive control rats, but also from SHRs treated with atorvastatin (50 mg/Kg/day). This suggests that atorvastatin is able to restore the beneficial anti-contractile properties of PVAT, which are lost in the hypertensive model (58).

H₂S is increasingly being recognised as an important player in the regulation of vascular tone, as described previously in this article. Wójcicka *et al.* (59) investigated the effects of three-week atorvastatin and pravastatin treatment on H₂S release in male Wistar rats. They report that, even though both statins lead to elevated H₂S synthesis in liver, only atorvastatin treatment is linked to increased H₂S production in periaortic adipose tissue, with an associated attenuation of phenylephrine-induced vasoconstriction of endothelium-denuded aortic rings. These effects are linked to coenzyme-Q9 depletion, which is induced by the lipophilic atorvastatin in the PVAT studied, and reversed by addition of mevalonate, farnesylate and coenzyme-Q9. The researchers concluded that statins increase H₂S formation

not by increasing its synthesis, but by inhibiting its mitochondrial oxidation; this, in turn, enhances the protective anti-contractile properties of PVAT on the underlying vessel (60).

Statins and risk for Type2 diabetes: the role of AT

Despite the beneficial effects on the cardiovascular system, statin treatment has been linked to increased risk of type 2 diabetes. As mentioned previously, lipophilic statins can penetrate a large number of different cell types, in addition to hepatocytes. By inhibiting synthesis of isoprenoids and suppress ubiquinone (CoQ10) synthesis, they may reduce synthesis of ATP in β -cells leading of the pancreas, which could result in reduced insulin secretion (61). Indeed, simvastatin has been shown to inhibit glucose-induced insulin secretion from islets, whereas pravastatin did not demonstrate such effects(62). It has also been postulated that lipophilic statins can be up-taken by adipose tissue, where they may exert unwanted pleiotropic effects such as exacerbation of insulin resistance. Another mechanism by which statins may adverse influence glycaemic control is by reduction of isoprenoid formation which enhance glucose uptake via glucose transporter (GLUT) 4 in adipocytes (63). A large meta-analysis of 13 clinical trials did indeed reveal a slightly increased risk of development of diabetes; however, this risk was low in absolute value, especially when the reduction in coronary events was taken into account (64).

Conclusions

In this review we have discussed the physiological and pathophysiological roles of PVAT as an endocrine organ. We have reviewed recent literature which underlines the unique role of PVAT and its ability to affect vascular function and inflammation through its complex secretome. In pathological conditions like obesity, the PVAT secretome profile is altered leading to disease-states such as hypertension and atherosclerosis. It has recently

emerged that PVAT can act as sensor responding to the biochemical stimuli from the vascular wall by modifying its secretory profile. This mutual cross-talk between PVAT and vascular wall can provide a new potential target for cardiovascular risk prevention. Statins exert at least some of their pleiotropic effects via modulation of the PVAT secretome, by reducing oxidative stress and increasing net generation of vasodilatory H₂S (**Figure 1**). Given the mounting evidence in favour of the concept that statins affect cardiovascular risk prevention through more complex mechanisms than simple LDL and cholesterol lowering, research into how statins can affect PVAT secretome represents a step forward in the direction of targeting PVAT as a new pharmacological tool. The paradigm set by statins could form the basis for the development of novel therapeutic strategies against CVD.

Figure Legend

Figure 1. Pleiotropic effects of statins on adipose tissue

Statins inhibit the enzyme hydroxyl-methyl-glutaryl-Coenzyme A (HMG-CoA) reductase, responsible for catalysing the conversion of 3-hydroxyl-3-methylglutaryl-CoA in mevalonate, which is further converted through a multi-step process in cholesterol. Inhibition HMG-CoA leads to reduced formation of isoprenoids, subsequent reduced prenylation of the small GTP-ase Rac1 and reduced activation of NADPH-oxidases. Statins also increase bioavailability of the anti-contractile/anti-oxidant gasotransmitter H₂S, produced by the enzyme cystathionine γ -lyase in PVAT, by inhibiting H₂S mitochondria oxidation. At the same time, statins increase adiponectin expression in AT leading to reduced inflammation as well as reduced oxidative stress.

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