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Review

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*Review*

# The Role of Perivascular Adipose Tissue in the Pathogenesis of Endothelial Dysfunction in Cardiovascular Diseases and Type 2 Diabetes Mellitus

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**Abstract:** Cardiovascular diseases (CVDs) and type 2 diabetes mellitus (T2DM) are two of the four major chronic non-communicable diseases (NCDs), representing the leading cause of death worldwide. Several studies demonstrate that endothelial dysfunction (ED) plays a central role in the pathogenesis of these chronic diseases. Although it is well known that systemic chronic inflammation and oxidative stress are primarily involved in the development of ED, recent studies have shown that perivascular adipose tissue (PVAT) is implicated in its pathogenesis, also contributing to the progression of atherosclerosis and to insulin resistance (IR). In this review we describe the relationship between PVAT and ED, and we also analyse the role of PVAT in the pathogenesis of CVDs and T2DM, further assessing its potential therapeutic target with the aim of restoring normal ED and reducing global cardiovascular risk.

**Keywords:** non-communicable diseases (NCDs); perivascular adipose tissue (PVAT); endothelial dysfunction (ED); cardiovascular diseases (CVDs); insulin resistance (IR); type 2 diabetes mellitus (T2DM)

## 1. Introduction

Non-communicable diseases (NCDs) are chronic medical conditions characterized by long duration and slow progression [1–3], resulting from a combination of genetic, physiological, environmental and behavioural factors [3]. They represent the leading cause of death worldwide [4], being responsible of 90% of deaths and 85% of disability in European countries [5].

The four major NCDs are cardiovascular diseases (CVDs), cancer, chronic respiratory diseases and diabetes [4,5]. According to data reported by the World Health Organization (WHO) in 2016, CVDs account for about 44% of deaths related to NCDs [4], representing the leading cause of morbidity and mortality among these chronic diseases [4,6,7], whereas diabetes accounts for about 4% of all NCDs deaths [4].

Several risk factors contribute to the pathogenesis of NCDs, the most common of which is obesity, which is itself particularly linked to the increased risk of CVDs and type 2 diabetes mellitus (T2DM) [5].

As it is well known, obesity is *per se* associated with an altered endothelium-dependent vascular reactivity, even in metabolically healthy obesity, but the presence of metabolic abnormalities contributes to enhance worsening of the endothelial dysfunction (ED) [8,9], which represents the common feature of CVDs and T2DM [10]. ED is defined as the lack of the vasoprotective homeostatic function of the endothelium [10–12], and is characterized by vasoconstriction and by pro-thrombotic and pro-inflammatory state [12,13].

Although the first description of ED has been made in the mid-1980s [10,13] and subsequent researches proved that systemic oxidative stress is the major mechanism involved in its pathogenesis [13–15], recent studies have shown that perivascular adipose tissue (PVAT) also contributes to the development of ED [16,17] by producing a variety of bioactive molecules, like adipokines, involved in the regulation of vascular tone and local inflammation [16,18], thus promoting the development and progression of atherosclerosis and CVDs [19,20]. Moreover, some of these adipokines affect insulin sensitivity, contributing to the pathogenesis of T2DM and also to the worsening of ED in this chronic disease [18,19,21].

In this review, we describe the relationship between PVAT and ED, and we analyse the role of PVAT in the pathogenesis of CVDs and T2DM, further evaluating PVAT as a potential therapeutic target with the aim of restoring normal endothelial function and reducing global cardiovascular risk.

## 2. Endothelial dysfunction (ED)

The endothelium, a continuous inner monolayer of blood vessels, was initially described as a selectively permeable membrane, separating the vascular and the interstitial compartments and regulating the transport of fluids and macromolecules between these two different sections [14,22–24], but it is currently known that it plays a central role in the modulation of vascular homeostasis [25,26], by producing and releasing a wide range of vasoactive substances, implicated in both vasodilation and vasoconstriction [24–26]. Moreover, endothelial cells secrete a variety of molecules involved in the regulation of blood coagulation, platelet function, inflammation and smooth muscle cells proliferation [24,26].

The key mediator in the maintenance of vascular homeostasis by modulating vascular tone and inhibiting inflammation, thrombosis and cellular proliferation is nitric oxide (NO) [26,27], a soluble gas synthetized from L-arginina by the endothelial nitric oxide synthase (eNOS) enzyme, constitutively expressed in the endothelial cells [27].

The reduced bioavailability of NO, observed in the presence of risk factors such as smoking, aging, hypertension, hyperglycaemia, hypercholesterolemia and obesity [12,23,28], induces the development of ED [23,29], in whose pathogenesis oxidative stress, which is characterized by a higher production and decreased degradation of reactive oxygen species (ROS), plays a critical role [13,30] by mediating the endothelial production of cytokines, which in turn contribute to the development and progression of atherosclerotic lesions secondary to the endothelial activation [15,26,31].

Although endothelium has been described as the main regulator of vascular homeostasis, recent studies showed that also the perivascular adipose tissue (PVAT) is an active component of the vasculature [32,33], being involved in the maintenance of vascular function and contributing to the prevention of vascular inflammation and atherosclerosis in healthy condition [34,35].

## 3. Perivascular adipose tissue (PVAT)

Perivascular adipose tissue (PVAT) refers to adipose tissue surrounding blood vessels, with the exception of capillaries and pulmonary and cerebral vasculature, and it is composed of adipocytes, which are predominant, nerves and stromal cells [36].

Although until recently it was seen as a simply connective tissue providing mechanical support to adjacent structures [36,37], it is now considered a metabolically active tissue, with specific properties and secretory patterns depending on its localization, involved in the modulation of vascular homeostasis and endothelial function and in the development of insulin resistance (IR) [35,36,38].

### 3.1. PVAT modulation of endothelial dysfunction

PVAT is tightly associated with the blood vessel wall [39,40], directly in contact with the adventitia [38,41], and this close interaction promotes the crosstalk between PVAT and all the cellular components of the vascular wall [40], including endothelial cells, on which PVAT exerts its effects in endocrine, autocrine and paracrine manner by secreting a wide range of bioactive molecules like

vasoregulators, including PVAT-derived relaxing factors (PVRFs) and PVAT-derived contracting factors (PVCFs), adipokines, including adiponectin and leptin, chemokines and cytokines, ROS and angiotensin peptides [32,37–39].

The role of PVAT in the modulation of vascular homeostasis was first described by Soltis and Cassis after observing a significant decrease to norepinephrine response of the intact aortic ring in vitro, and suggesting that PVAT contributes to the removal of catecholamines [42]. However, subsequent studies showed that the vasodilatory effect of PVAT was independent of adrenergic neuronal reuptake of catecholamines and was mediated by a transferable substance, called adipocyte-derived relaxing factor (ADRF), that is released in a $Ca^+$-dependent manner and acts by activating ATP-dependent $K^+$ channels of the vascular smooth muscle cells (VSMCs) [43–45]. As the chemical nature of ADRF is still unknown and there might be more than one single molecule responsible for this effect [44,46], the term PVAT-derived relaxing factors (PVRFs) was coined to identify several bioactive molecules involved in the PVAT-induced vasodilation [32,46].

In addition to ADRF, other possible PVRFs are NO, hydrogen sulphide ($H_2S$), hydrogen peroxide ($H_2O_2$), angiotensin 1-7 (Ang1-7) and palmitic acid methyl ester (PAME) [41,46,47].

Once reached the endothelial layer of blood vessels, these molecules modulate vascular tone through an endothelium-dependent mechanism, mediated by the release of endothelial NO which in turn induces the activation of $K^+$ channels leading to vasodilation, or through an endothelium-independent mechanism [48–51].

PVAT-induced vasodilation can also be directly mediated by PVAT-derived NO [50]. Indeed, similarly to the endothelial cells, also adipocytes express eNOS enzyme which induce the production of vasoactive NO and consequently the modulation of vasodilation, by acting on VSMCs, and the endothelial protection, by inhibiting platelet aggregation and leukocyte adhesion [48]. However, in addition to PVRFs, PVAT also produces PVCFs, including superoxide anion, catecholamines, prostaglandins, angiotensin II and resistin, which contribute to counteract the effects of PVRFs primarily reducing NO bioavailability and thus inducing vasoconstriction and ED [41,52].

As previously described, besides PVRFs and PVCFs, PVAT secretes a wide range of adipocytokines, including adipokines and cytokines, involved in the regulation of vascular tone and in the maintenance of the metabolic homeostasis [52]. Among the adipokines, adiponectin is the most abundant molecule produced and released by PVAT [50], and plays a critical role in the modulation of vascular tone through the activation of its receptor, particularly of the adiponectin receptor 1 (Adpo1), widely expressed on endothelial cells and VSMCs [53]. Upon adiponectin binding to the Adipo1 receptor of the endothelial cells, the adaptor protein APPL1 is recruited to the receptor site, leading to the phosphorylation of AMP-activated protein kinase (AMPK) and then to the activation of the eNOS, promoting NO production [53,54]. As well as adiponectin, leptin contributes to vascular homeostasis through the enhanced NO production by eNOS activation in endothelial cells [55,56].

As mentioned above, PVAT also secretes cytokines, such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and interleukin-6 (IL-6), which reduce NO bioavailability contributing to ED [52]. Indeed, PVAT-derived TNF-$\alpha$ primarily interferes with endothelial function through enhancing ROS production, via activation of nicotinamide dinucleotide phosphate oxidase (NADPH oxidase), leading to NO breakdown and to reduced NO production as the result of eNOS uncoupling [57–59]. Moreover, PVAT-derived TNF-$\alpha$ directly activates the c-Jun N-terminal kinase pathway contributing to the increased production of the endothelin-1 (ET-1), which in turn promotes ROS production and consequently ED [57]. Similarly to TNF-$\alpha$, also IL-6 promotes ED by inhibiting eNOS expression and activity [60,61], leading to a decreased NO bioavailability [62].

Thus, under physiological condition, PVAT exerts a protective effect on endothelial function contributing to maintain the balance between PVAT-derived anti-contractile/anti-inflammatory and pro-contractile/pro-inflammatory bioactive molecules and ensuring a greater production of adiponectin and NO than TNF-$\alpha$ and IL-6, finally preventing the development of CVDs [63].

*3.2. PVAT regulation of insulin sensitivity*

Insulin is an endocrine peptide hormone involved in glucose homeostasis by binding its specific receptors expressed on target tissues such as skeletal muscle [64], which is responsible for most of the insulin-mediated glucose uptake in the post prandial state [65], and which also promotes glycogen synthesis [64].

Apart from its role in inducing myocellular signalling cascade leading to the translocation of glucose transporter 4 (GLUT4) to the plasma membrane, resulting in glucose uptake [64], insulin enhances glucose disposal in the skeletal muscle also by binding its receptors expressed on the endothelial cells of the skeletal muscle microvasculature thus inducing vasodilation and consequently an increased total blood flow to the muscle [66]. Insulin mediated vasodilation on the skeletal muscle microvasculature depends on the activation of phosphatidyl inositol 3-kinase (PI3-K) pathway which induces eNOS, increasing NO production [67] and consequently promoting muscle microvascular dilation, and thus improving muscle glucose uptake [66,67].

As for other vessels, also skeletal microvasculature is surrounded by PVAT [38], involved in the modulation of muscle insulin-sensitivity by releasing adipocytokines, which play a critical role in the regulation of insulin-mediated vasodilation [21,68,69].

Among these adipocytokines, adiponectin is widely known to be an insulin sensitizer [70], acting on the skeletal muscle cells by binding Adipo1 receptor and thus activating an intracellular signalling cascade, leading to GLUT4 membrane translocation and glucose uptake [70,71]. Furthermore, adiponectin modulates muscle microvascular recruitment and insulin delivery to skeletal muscle [71] through the direct activation of AMPK-eNOS pathway and the indirect phosphorylation of the protein kinase B (PKB or Akt), which is involved in most of the metabolic actions of insulin [67,72], leading to microvasculature NO-mediated vasodilation and to an increased muscle glucose disposal [70,73,74].

Taking into account that PVAT secretes a copious amount of adiponectin and that adiponectin modulates skeletal muscle microvascular perfusion and glucose uptake, it has been demonstrated that adiponectin derived from PVAT surrounding muscle microvasculature regulates insulin-induced vasodilation through the activation of AMPK pathway in the microvessels wall [75,76], emphasizing the close interaction between PVAT and muscle microvasculature, and then the pivotal role of PVAT in ensuring glucose homeostasis and insulin sensitivity.

Furthermore, PVAT is able to release Ang1-7, which acts not only as an endothelium-dependent relaxing factor, as previously described, but also as an insulin sensitizer, by binding and activating the G-protein-coupled receptor Mas, thus inducing the phosphorylation of the insulin receptor substrate-1 (IRS-1) and consequently the activation of Akt pathway, which mediates insulin metabolic action [77]. Importantly, by activating Mas in the skeletal muscle microvasculature, Ang1-7 also increases the endothelial surface area available for glucose exchange leading to an increased muscle glucose disposal and enhanced metabolic action of insulin [78,79]. Thus, similarly to adiponectin, PVAT-derived Ang1-7 could be involved in the regulation of glucose metabolism improving insulin sensitivity.

## 4. Dysfunctional PVAT and its implication in CVDs and T2DM

Under physiological conditions PVAT exerts its protective role on vascular and glucose homeostasis by releasing a wide range of bioactive molecules with anti-contractile and anti-inflammatory effects and by secreting adipocytokines involved in the regulation of insulin sensitivity, thus preventing the development of atherosclerosis and IR [16,19,80,81]. In contrast, under pathological conditions, such as obesity, PVAT becomes dysfunctional, contributing to the development of ED, the hallmark of atherosclerosis and CVDs, and of IR, the hallmark of T2DM [32,82,83].

*4.1. Dysfunctional PVAT in the pathogenesis of atherosclerosis*

The development of PVAT dysfunction in obesity is related to the "obesity triad", characterized by hypoxia, oxidative stress and inflammation [84]. Hypoxia seems to be a direct consequence of adipocyte hypertrophy, which in turn is responsible for a higher distance between adipocytes and blood vessels leading to reduced oxygen diffusion within tissues and to enhanced production of ROS and inflammatory cytokines [39,85,86]. ROS, such as superoxide anion and $H_2O_2$, primarily derived from Nox family of NADPH oxidase and eNOS uncoupling, contribute to the development of ED by reducing the bioavailability of NO [87,88]. Moreover, superoxide anion stimulates endothelial production of ET-1 [89], which in turn reduces NO bioavailability by an up-regulation of caveolin-1 expression, leading to eNOS inhibition, or by increasing NO degradation, dependent on eNOS uncoupling [90]. In addition, ROS may induce the expression of TNF-$\alpha$ [84,91], which is also secreted in large amount by dysfunctional PVAT [81]. TNF-$\alpha$ contributes to the development of ED by stimulating ROS production through the activation of NADPH oxidase [58,91], by inducing the up-regulation of the c-Jun N-terminal kinase pathway (JNK) unbalancing ET1/NO system in favour of ET-1 [57] and by reducing adiponectin release [92], leading to a reduced endothelial NO bioavailability [57,58,91]. At last, PVAT dysfunction is also characterized by hyperleptinemia, which leads to endothelial leptin resistance with a loss of balance between ET-1/NO system contributing to decreased NO bioavailability in the vasculature [84,93].

Thus, taking together, these data suggest that dysfunctional PVAT, similar to dysfunctional endothelium, is characterized by a decreased secretion of protective factors, like adiponectin and NO, by an increased production of ROS and by the development of a low grade of inflammation, which contributes to a detrimental release of adipokines and cytokines, such as IL-6 and TNF-$\alpha$ [87,94]. Such changes in the secretory pattern of dysfunctional PVAT contribute to the development of ED, which in turn leads to the development of atherosclerotic lesion by increasing the endothelial expression of adhesion molecules, like monocyte chemoattractant protein-1 (MCP-1), vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1), involved in the adherence and migration of monocytes into subendothelial layer of the intima where they become macrophages and secrete pro-inflammatory cytokines inducing low-density lipoprotein (LDL)-oxidation leading to the development and progression of atherosclerotic plaque [10,31,82,95], and thus contributing to the pathogenesis of CVDs [19,95].

*4.2. Dysfunctional PVAT inducing insulin resistance*

Similarly to its contribution to the pathogenesis of CVDs, as previously described, PVAT dysfunction is tightly related to the development of vascular IR [16,19], characterized by the loss of insulin-mediated NO production and consequently by impaired skeletal muscle perfusion contributing to a decreased glucose disposal [96]. Indeed, the enhanced production of pro-inflammatory cytokines, particularly of TNF-$\alpha$, that inactivates the Akt/PI3-K/eNOS signalling pathway by increasing the enzyme phosphatase and tensin homologue (PTEN) phosphorylation [97] and by activating JNK pathway which induces an increased ET-1 production [98], leads to a decreased glucose uptake by impairing insulin-mediated vasodilation and reducing GLUT-4 translocation in skeletal muscle [18,21,76,82,99].

Furthermore, it has been shown that PVAT-derived adiponectin modulates insulin-dependent muscle perfusion and glucose uptake [83]. Thus, the decreased release of adiponectin by dysfunctional PVAT contributes to the development of vascular IR by impairing of insulin-dependent Akt signalling pathway [75].

Accordingly, these data suggest that PVAT play a central role in the pathogenesis of T2DM by regulating muscle perfusion, insulin sensitivity and glucose disposal.

**5. PVAT: new therapeutic target?**

Dysfunctional PVAT is common in obesity and contributes to the development of ED and IR, which represent the hallmark of CVDs and T2DM respectively, as described above. Thus, given the

association between PVAT dysfunction and the occurrence of cardiometabolic diseases, restoring PVAT function could contribute to reduce the onset and the progression of these diseases, mainly in obesity.

Several studies have been shown that weight loss leads to restore PVAT function in obesity [100,101]. Particularly, in a previous study was demonstrated that PVAT function was restored six months after bariatric surgery, leading to improvement in local adiponectin and NO bioavailability [100]. However, bariatric surgery is not always the first line treatment for obesity, thus subsequent studies analyse the effects of caloric restriction and physical activity on PVAT function in obese mice and demonstrate that diet-induced sustained weight loss and exercise also contribute to improve PVAT function by reducing PVAT inflammation and increasing PVAT-adiponectin availability and PVAT-eNOS activity [101,102], suggesting that restoring PVAT function is associated with improvement in insulin sensitivity and endothelial function. Although further investigations are need to confirm these results in human obesity, we can speculate that PVAT could be a new therapeutic target and restoring its function could improve cardiometabolic risk. According with this hypothesis, several studies have analysed the effects of some drugs, such as statins and antidiabetic agents, on PVAT function.

*5.1. Statins*

Despite their beneficial effects in reducing cardiovascular risk through the inhibition of hydroxyl-methyl-glutaryl coenzyme A reductase (HMG-CoA-reductase) which induces LDL reduction [103], it has been described that statins have several pleiotropic effects, some of which are responsible of the endothelial modulation of the Akt pathway and caveolin-1 expression, leading to an increased NO production and thus improving ED [104,105], without affecting IR [106,107]. Consistent with these data, it could be hypothesized that statins may mediate PVAT secretion of bioactive molecules involved in the regulation of vascular homeostasis. Accordingly, some studies have been demonstrated that atorvastatin could restore PVAT function in rat model by increasing the release of PVRFs [108], like $H_2S$ [109]]. To date, further investigations are needed to prove the effects of statins in human PVAT.

*5.2. Antidiabetic drugs*

Similarly to statins, in addition to their glucose-lowering effects, new antidiabetic drugs, especially GLP-1 receptor agonists (GLP-1 RAs) and sodium-glucose cotransporter-2 (SGLT-2) inhibitors, directly reduce cardiovascular risk [110,111], by improving endothelial cell function and reducing inflammation and oxidative stress, and thereby preventing the development and progression of atherosclerosis [112–114].

Since PVAT appears to play a central role in the development of atherosclerosis, by contributing to the onset of ED, as previously mentioned, several studies have evaluated the effects of GLP-1 RAs and SGLT-2 inhibitors on dysfunctional PVAT. In this context, it has been demonstrated that the GLP-1 RA liraglutide reduces PVAT inflammation through the inhibition of nuclear factor (NF)-κ B signalling pathway in rat models [115] and improves ED through the activation of PVAT-AMPK/eNOS pathway and through the enhancement of PVAT-derived adiponectin bioavalability in mice [116]. In view of these findings, it may be speculated that GLP-1 RAs contribute to restore the bioavailability of adiponectin and NO in PVAT, leading to reduce cardiovascular risk.

Furthermore, treating apolipoprotein E-deficient (ApoE$^{-/-}$) mice with the SGLT-2 inhibitor empagliflozin was associated with a decreased expression of pro-inflammatory cytokines, including adhesion molecules, and with a reduced activity of NADPH oxidase in PVAT [117]. Therefore, as well as in animal models, also in humans empagliflozin could prevent ED and atherosclerosis by reducing PVAT inflammation and oxidative stress, but further studies are needed.

## 6. Discussion

PVAT is a metabolically active endocrine/paracrine organ involved in the regulation of vascular homeostasis and impact on the insulin sensitivity. The loss of balance between anti-contractile/anti-inflammatory and pro-contractile/pro-inflammatory adipocytokines, as observed in obesity, characterizes PVAT dysfunction, which contributes to the pathogenesis of both CVDs and T2DM. Therefore, restoring PVAT function could improve cardiometabolic risk, leading to a decreased onset of ED and IR. Although caloric restriction and physical activity contribute to restore PVAT function, several studies have investigated the role of statins and antidiabetic drugs on dysfunctional PVAT in animal models, showing that they could modulate several intracellular signalling pathway and regulate PVAT secretion of adipocytokines, thus contributing to prevent ED and atherosclerosis. As regard to their effects on insulin-mediated vasodilation, which also depends on PVAT function, the literature evidences are scarce.

In conclusion, dysfunctional PVAT could be a new therapeutic target for treatment and prevention of CVDs and potentially of T2DM.

## References

1. Budreviciute, A.; Damiati S. Management and prevention strategies for non-communicable diseases (NCDs) and their risk factors. *Front Public Health* **2020**, *8*, 574111.
2. Huijts, T.; Stornes, P. Prevalence of physical and mental non-communicable diseases in Europe: findings from the European Social Survey (2014) special module on the social determinants of health. *Eur J Public Health* **2017**, *27 (suppl. 1)*, 8-13.
3. World Health Organization. Noncommunicable Diseases (NCD). 2019. Available online at: https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases (accessed June 29, 2023).
4. Noncommunicable diseases country profiles 2018. Geneva: World Health Organization; 2018. Licence: CC BY-NC-SA 3.0 IGO.
5. Monitoring noncommunicable disease commitments in Europe 2021: are we on track to reach targets 10 years after the Moscow Declaration and First United Nations High-Level Meeting? Copenhagen: WHO Regional Office for Europe; 2021. Licence: CC BY-NC-SA 3.0 IGO.
6. Piepoli, M.F.; Hoes, A.W. ESC Scientific Document Group. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: the sixth joint Task Force of the European Society of Cardiology and other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J* **2016**, *37 (29)*, 2315-2381.
7. Roth, G.A.; Mensah, G.A. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 Study *J Am Coll Cardiol*, **2020**, *76 (25)*, 2982-3021.
8. Schinzari, F.; Iantorno, M. Vasodilator responses and endothelin-dependent vasoconstriction in metabolically healthy obesity and the metabolic syndrome. *Am J Physiol Endocrinol Metab* **2015**, *309 (9)*, E787-E792.
9. Li, M.; Qian, M. Adipose tissue-endothelial cell interactions in obesity-induced endothelial dysfunction. *Front Cardiovasc Med* **2021**, *8*, 681581.

10.  Xu, S.; Ilyas, I. Endothelial dysfunction in atherosclerotic cardiovascular diseases and beyond: from mechanism to pharmacotherapies. *Pharmacol Rev* **2021**, *73 (3)*, 924-967.

11.  Little, P.J.; Askew, C.D. Endothelial dysfunction and cardiovascular disease: history and analysis of the clinical utility of the relationship. *Biomedicines* **2021**, *9 (6)*, 699.

12.  Widlansky, M.E.; Gokce, N. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol* **2003**, *42 (7)*, 1149-1160.

13.  Bonetti, P.O.; Lerman, L.O. Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol* **2003**, *23 (2)*, 168-175.Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range.

14.  Gimbrone, M.A. Jr; García-Cardeña, G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. *Circ Res* **2016**, *118 (4)*, 620-636.

15.  Münzel, T.; Sinning, C. Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. *Ann Med* **2008**, *40 (3)*, 180-196.

16.  Houben, A.J.; Eringa, E.C. Perivascular fat and the microcirculation: relevance to insulin resistance, diabetes, and cardiovascular disease. *Curr Cardiovasc Risk Rep* **2012**, *6 (1)*, 80-90.

17.  Eringa, E.C.; Bakker, W. Paracrine regulation of vascular tone, inflammation and insulin sensitivity by perivascular adipose tissue. *Vascul Pharmacol* **2012**, *56 (5-6)*, 204-209.

18.  Meijer, R.I.; Serne, E.H. Perivascular adipose tissue and its role in type 2 diabetes and cardiovascular disease. *Curr Diab Rep* **2011**, *11 (3)*, 211-217.

19.  Lastra, G.; Manrique, C. Perivascular adipose tissue, inflammation and insulin resistance: link to vascular dysfunction and cardiovascular disease. *Horm Mol Biol Clin Investig* **2015**, *22 (1)*, 19-26.

20.  Szasz, T.; Bomfim, G.F. The influence of perivascular adipose tissue on vascular homeostasis. *Vasc Health Risk Manag* **2013**, *9*, 105-116.

21.  Yudkin, J.S.; Eringa, E. "Vasocrine" signalling from perivascular fat: a mechanism linking insulin resistance to vascular disease. *Lancet* **2005**, *365 (9473)*, 1817-1820.

22.  Eelen, G.; de Zeeuw, P. Endothelial cell metabolism in normal and diseased vasculature. *Circ Res* **2015**, *116 (7)*, 1231-1244.

23.  Poredos, P.; Poredos, A. V. Endothelial dysfunction and its clinical implications. *Angiology* **2021**, *72 (7)*, 604-615.

24.  Michiels, C. Endothelial cell functions. *J Cell Physiol* **2003**, *196 (3)*, 430-443.

25.  Godo, S.; Shimokawa, H. Endothelial Functions. *Arterioscler Thromb Vasc Biol* **2017**, *37 (9)*, e108-e114.

26.  Deanfield, J.E.; Halcox, J.P. Endothelial function and dysfunction: testing and clinical relevance. *Circulation* **2007**, *115 (10)*, 1285-1295.

27.  Tousoulis, D.; Kampoli, A.M. The role of nitric oxide on endothelial function. *Curr Vasc Pharmacol* **2012**, *10 (1)*, 4-18.

28.  Kwaifa, I.K.; Bahari, H. Endothelial dysfunction in obesity-induced inflammation: molecular mechanisms and clinical implications. *Biomolecules* **2020**, *10 (2)*, 291.

29.  Vanhoutte, P.M.; Shimokawa, H. Endothelial dysfunction and vascular disease - a 30th anniversary update. *Acta Physiol (Oxf)* **2017**, *219 (1)*, 22-96.

30.  Higashi, Y.; Maruhashi, T. Oxidative stress and endothelial dysfunction: clinical evidence and therapeutic implications. *Trends Cardiovasc Med* **2014**, *24 (4)*, 165-169.

31.  Incalza, M.A.; D'Oria, R. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vascul Pharmacol* **2018**, *100*, 1-19.

32.  Brown, N.K.; Zhou, Z. Perivascular adipose tissue in vascular function and disease: a review of current research and animal models. *Arterioscler Thromb Vasc Biol* **2014**, *34 (8)*, 1621-1630.

33.  Zaborska, K.E.; Wareing, M. Comparisons between perivascular adipose tissue and the endothelium in their modulation of vascular tone. *Br J Pharmacol* **2017**, *174 (20)*, 3388-3397.

34.  Adachi, Y.; Ueda, K. Perivascular adipose tissue in vascular pathologies-a novel therapeutic target for atherosclerotic disease? *Front Cardiovasc Med* **2023**, *10*, 1151717.

35.  Deja, M.A.; Malinowski, M. Perivascular tissue mediated relaxation - a novel player in human vascular tone regulation. *J Physiol Pharmacol* **2015**, *66 (6)*, 841-846.

36.  Li, X.; Ma, Z. Regional heterogeneity of perivascular adipose tissue: morphology, origin, and secretome. *Front Pharmacol* **2021**, *12*, 697720.

37.  Szasz, T.; Webb, R.C. Perivascular adipose tissue: more than just structural support. *Clin Sci (Lond)* **2012**, *122 (1)*, 1-12.

38.  Gil-Ortega, M.; Somoza, B. Regional differences in perivascular adipose tissue impacting vascular homeostasis. *Trends Endocrinol Metab* **2015**, *26 (7)*, 367-375.

39.  Cheng, C.K.; Ding, H. Perivascular adipose tissue: fine-tuner of vascular redox status and inflammation. *Redox Biol* **2023**, *62*, 102683.

40.  Rajsheker, S.; Manka, D. Crosstalk between perivascular adipose tissue and blood vessels. *Curr Opin Pharmacol* **2010**, *10 (2)*, 191-196.

41. Ahmed, A.; Bibi, A. Perivascular adipose tissue and vascular smooth muscle tone: friends or foes? *Cells* **2023**, *12 (8)*, 1196.

42. Soltis, E.E.; Cassis, L.A. Influence of perivascular adipose tissue on rat aortic smooth muscle responsiveness. *Clin Exp Hypertens A* **1991**, *13 (2)*, 277-296.

43. Löhn, M.; Dubrovska, G. Periadventitial fat releases a vascular relaxing factor. *FASEB J* **2002**, *16 (9)*, 1057-1063.

44. Gollasch, M.; Dubrovska, G. Paracrine role for periadventitial adipose tissue in the regulation of arterial tone. *Trends Pharmacol Sci* **2004**, *25 (12)*, 647-653.

45. Gollasch, M. Vasodilator signals from perivascular adipose tissue. *Br J Pharmacol* **2012**, *165 (3)*, 633-642.

46. Gollasch, M. Adipose-vascular coupling and potential therapeutics. *Annu Rev Pharmacol Toxicol* **2017**, *57*, 417-436.

47. Bełtowski, J. Endogenous hydrogen sulfide in perivascular adipose tissue: role in the regulation of vascular tone in physiology and pathology. *Can J Physiol Pharmacol* **2013**, 91 *(11)*, 889-898.

48. Man, A.W.C.; Zhou, Y. Endothelial nitric oxide synthase in the perivascular adipose tissue. *Biomedicines* **2022**, *10 (7)*, 1754.

49. Gao, Y.J.; Lu, C. Modulation of vascular function by perivascular adipose tissue: the role of endothelium and hydrogen peroxide. *Br J Pharmacol* **2007**, *151 (3)*, 323-331.

50. Chang, L.; Garcia-Barrio, M.T. Perivascular adipose tissue regulates vascular function by targeting vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* **2020**, *40 (5)*, 1094-1109.

51. Lee, R. M.; Lu, C. Endothelium-dependent relaxation factor released by perivascular adipose tissue. *J Hypertens* **2009**, *27 (4)*, 782-790.

52. Cheng, C.K.; Bakar, H.A. Perivascular adipose tissue: the sixth man of the cardiovascular system. *Cardiovasc Drugs Ther* **2018**, *32 (5)*, 481-502.

53. Sowka, A.; Dobrzyn, P. Role of perivascular adipose tissue-derived adiponectin in vascular homeostasis. *Cells* **2021**, *10 (6)*, 1485.

54. Cheng, K.K.; Lam, K.S. Adiponectin-induced endothelial nitric oxide synthase activation and nitric oxide production are mediated by APPL1 in endothelial cells. *Diabetes* **2007**, *56 (5)*, 1387-1394.

55. Bełtowski J. Leptin and the regulation of endothelial function in physiological and pathological conditions. *Clin Exp Pharmacol Physiol* **2012**, *39 (2)*, 168-178.

56. Procopio, C.; Andreozzi, F. Leptin-stimulated endothelial nitric-oxide synthase via an adenosine 5'-monophosphate-activated protein kinase/Akt signaling pathway is attenuated by interaction with C-reactive protein. *Endocrinology* **2009**, *150 (8)*, 3584-3593.

57. Virdis, A.; Duranti, E. Tumour necrosis factor-alpha participates on the endothelin-1/nitric oxide imbalance in small arteries from obese patients: role of perivascular adipose tissue. *Eur Heart J* **2015**, *36 (13)*, 784-794.

58. Virdis, A.; Colucci, R. Microvascular endothelial dysfunction in human obesity: role of TNF-$\alpha$. *J Clin Endocrinol Metab* **2019**, *104 (2)*, 341-348.

59. Virdis, A.; Santini, F. Vascular generation of tumor necrosis factor-$\alpha$ reduces nitric oxide availability in small arteries from visceral fat of obese patients. *J Am Coll Cardiol* **2011**, *58 (3)*, 238-247.

60. Saura, M.; Zaragoza, C. Stat3 mediates interleukin-6 [correction of interelukin-6] inhibition of human endothelial nitric-oxide synthase expression. *J Biol Chem* **2006**, *281 (40)*, 30057-30062.

61. Hung, M.J.; Cherng, W.J. Interleukin-6 inhibits endothelial nitric oxide synthase activation and increases endothelial nitric oxide synthase binding to stabilized caveolin-1 in human vascular endothelial cells. *J Hypertens* **2010**, *28 (5)*, 940-951.

62. Didion, S.P. Cellular and oxidative mechanisms associated with interleukin-6 signaling in the vasculature. *Int J Mol Sci* **2017**, *18 (12)*, 2563.

63. Rami, A.Z.A.; Hamid, A.A. Exploring the relationship of perivascular adipose tissue inflammation and the development of vascular pathologies. *Mediators Inflamm* **2022**, *2022*, 2734321.

64. Petersen, M.C.; Shulman, G.I. Mechanisms of insulin action and insulin resistance. *Physiol Rev* **2018**, *98 (4)*, 2133-2223.

65. DeFronzo, R.A.; Gunnarsson, R. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. *J Clin Invest* **1985**, *76 (1)*, 149-155.

66. Keske, M.A.; Premilovac, D. Muscle microvascular blood flow responses in insulin resistance and ageing. *J Physiol* **2016**, *594 (8)*, 2223-2231.

67. Pepe, G.J.; Albrecht, E.D. Microvascular skeletal-muscle crosstalk in health and disease. *Int J Mol Sci* **2023**, *24 (13)*, 10425.

68. Eringa, E.C.; Bakker, W. Regulation of vascular function and insulin sensitivity by adipose tissue: focus on perivascular adipose tissue. *Microcirculation* **2007**, *14 (4-5)*, 389-402.

69. Zhang, H.; Zhang, C. Adipose "talks" to distant organs to regulate insulin sensitivity and vascular function. *Obesity (Silver Spring)* **2010**, *18 (11)*, 2071-2076.

70. Ruan, H.; Dong, L. Q. Adiponectin signaling and function in insulin target tissues. *J Mol Cell Biol* **2016**, *8 (2)*, 101-109.

71.   Zhao, L.; Fu, Z. Adiponectin and insulin cross talk: the microvascular connection. *Trends Cardiovasc Med* **2014**, *24 (8)*, 319-324.

72.   Taniguchi, C.M.; Emanuelli, B. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol* **2006**, *7 (2)*, 85-96.

73.   Zhao, L.; Chai, W. Globular adiponectin enhances muscle insulin action via microvascular recruitment and increased insulin delivery. *Circ Res* **2013**, *112 (9)*, 1263-1271.

74.   Zhao, L.; Fu, Z. Globular adiponectin ameliorates metabolic insulin resistance via AMPK-mediated restoration of microvascular insulin responses. *J Physiol* **2015**, *593 (17)*, 4067-4979.

75.   Meijer, R.I.; Bakker, W. Perivascular adipose tissue control of insulin-induced vasoreactivity in muscle is impaired in db/db mice. *Diabetes* **2013**, *62 (2)*, 590-598.

76.   Turaihi, A.H.; Serné, E.H. Perivascular adipose tissue controls insulin-stimulated perfusion, mitochondrial protein expression, and glucose uptake in muscle through adipomuscular arterioles. *Diabetes* **2020**, *69 (4)*, 603-613.

77.   Dominici, F.P.; Burghi, V. Modulation of the action of insulin by angiotensin-(1-7). *Clin Sci (Lond)* **2014**, *126 (9)*, 613-630.

78.   Fu, Z.; Zhao, L. Angiotensin-(1-7) recruits muscle microvasculature and enhances insulin's metabolic action via mas receptor. *Hypertension* **2014**, *63 (6)*, 1219-1227.

79.   Schinzari, F.; Tesauro, M. Favorable vascular actions of angiotensin-(1-7) in human obesity. *Hypertension* **2018**, *71 (1)*, 185-191.

80.   Chen, Y.; Qin, Z. Role of inflammation in vascular disease-related perivascular adipose tissue dysfunction. *Front Endocrinol (Lausanne)* **2021**, *12*, 710842.

81.   Hillock-Watling, C.; Gotlieb, A.I. The pathobiology of perivascular adipose tissue (PVAT), the fourth layer of the blood vessel wall. *Cardiovasc Pathol* **2022**, *61*, 107459.

82.   Qi, X.Y.; Qu, S.L. Perivascular adipose tissue (PVAT) in atherosclerosis: a double-edged sword. *Cardiovasc Diabetol* **2018**, *17 (1)*, 134.

83.   Saxton, S.N.; Clark, B.J. Mechanistic links between obesity, diabetes, and blood pressure: role of perivascular adipose tissue. *Physiol Rev* **2019**, *99 (4)*, 1701-1763.

84.   Xia, N.; Li, H. The role of perivascular adipose tissue in obesity-induced vascular dysfunction. *Br J Pharmacol* **2017**, *174 (20)*, 3425-3442.

85.   Trayhurn, P.; Wang, B. Hypoxia in adipose tissue: a basis for the dysregulation of tissue function in obesity? *Br J Nutr* **2008**, *100 (2)*, 227-235.

86.   Greenstein, A.S.; Khavandi, K. Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. *Circulation* **2009**, *119 (12)*, 1661-1670.

87.   Victorio, J.A.; Davel, A.P. Perivascular adipose tissue oxidative stress on the pathophysiology of cardiometabolic diseases. *Curr Hypertens Rev* **2020**, *16 (3)*, 192-200.

88.   Man, A.W.C.; Zhou, Y. Perivascular adipose tissue as a target for antioxidant therapy for cardiovascular complications. *Antioxidants (Basel)* **2020**, *9 (7)*, 574.

89.   Münzel, T.; Gori, T. Is oxidative stress a therapeutic target in cardiovascular disease? *Eur Heart J* **2010**, *31 (22)*, 2741-2748.

90.   Iglarz, M.; Clozel, M. Mechanisms of ET-1-induced endothelial dysfunction. *J Cardiovasc Pharmacol* **2007**, *50 (6)*, 621-628.

91.   Zhang, H.; Park, Y. Role of TNF-alpha in vascular dysfunction. *Clin Sci (Lond)* **2009**, *116 (3)*, 219-230.

92.   Wang, B.; Jenkins, J.R. Expression and secretion of inflammation-related adipokines by human adipocytes differentiated in culture: integrated response to TNF-alpha. *Am J Physiol Endocrinol Metab* **2005**, *288 (4)*, E731-E740.

93.   Schinzari, F.; Tesauro, M. Leptin stimulates both endothelin-1 and nitric oxide activity in lean subjects but not in patients with obesity-related metabolic syndrome. *J Clin Endocrinol Metab* **2013**, *98 (3)*,1235-1241.

94.   Guzik, T.J.; Skiba, D.S. The role of infiltrating immune cells in dysfunctional adipose tissue. *Cardiovasc Res* **2017**, *113 (9)*, 1009-1023.

95.   Libby, P.; Buring, J.E. Atherosclerosis. *Nat Rev Dis Primers* **2019**, *5 (1)*, 56.

96.   Schulman, I.H.; Zhou, M.S. Vascular insulin resistance: a potential link between cardiovascular and metabolic diseases. *Curr Hypertens Rep* **2009**, *11 (1)*, 48-55.

97.   da Costa, R.M.; Neves, K.B. TNF-$\alpha$ induces vascular insulin resistance via positive modulation of PTEN and decreased Akt/eNOS/NO signaling in high fat diet-fed mice. *Cardiovasc Diabetol* **2016**, *15 (1)*, 119.

98.   Eringa, E.C., Stehouwer, C.D. Physiological concentrations of insulin induce endothelin-dependent vasoconstriction of skeletal muscle resistance arteries in the presence of tumor necrosis factor-alpha dependence on c-Jun N-terminal kinase. *Arterioscler Thromb Vasc Biol* **2006**, *26 (2)*, 274-280.

99.   Shemyakin, A.; Salehzadeh, F. Endothelin-1 reduces glucose uptake in human skeletal muscle in vivo and in vitro. *Diabetes* **2011**, *60 (8)*, 2061-2067.

100. Aghamohammadzadeh, R.; Greenstein, A.S. Effects of bariatric surgery on human small artery function: evidence for reduction in perivascular adipocyte inflammation, and the restoration of normal anticontractile activity despite persistent obesity. *J Am Coll Cardiol* **2013**, *62 (2)*, 128-135.

101. Bussey, C.E.; Withers, S.B. Obesity-related perivascular adipose tissue damage is reversed by sustained weight loss in the rat. *Arterioscler Thromb Vasc Biol* **2016**, *36 (7)*, 1377-1385.

102. Saxton, S.N.; Toms, L.K. Restoring perivascular adipose tissue function in obesity using exercise. *Cardiovasc Drugs Ther* **2021**, *35 (6)*, 1291-1304.

103. Antoniades, C.; Channon, K.M. Statins: pleiotropic regulators of cardiovascular redox state. *Antioxid Redox Signal* **2014**, *20 (8)*, 1195-1197.

104. Antonopoulos, A.S.; Margaritis, M. Statins as anti-inflammatory agents in atherogenesis: molecular mechanisms and lessons from the recent clinical trials. *Curr Pharm Des* **2012**, *18 (11)*, 1519-1530.

105. Davignon, J. Beneficial cardiovascular pleiotropic effects of statins. *Circulation* **2004**, *109 (23 Suppl 1)*, III39-III43.

106. Bellia, A.; Rizza, S. Early vascular and metabolic effects of rosuvastatin compared with simvastatin in patients with type 2 diabetes. *Atherosclerosis* **2010**, *210 (1)*, 199-201.

107. Bellia, A; Rizza, S. Deterioration of glucose homeostasis in type 2 diabetic patients one year after beginning of statins therapy. *Atherosclerosis* **2012**, *223 (1)*, 197-203.

108. Zeng, Z.H.; Zhang, Z.H. The functional changes of the perivascular adipose tissue in spontaneously hypertensive rats and the effects of atorvastatin therapy. *Clin Exp Hypertens* **2009**, *31 (4)*, 355-363.

109. Bełtowski, J.; Jamroz-Wiśniewska, A. Modulation of $H_2S$ metabolism by statins: a new aspect of cardiovascular pharmacology. *Antioxid Redox Signal* **2012**, *17 (1)*, 81-94.

110. Ussher, J.R.; Drucker, D.J. Cardiovascular biology of the incretin system. *Endocr Rev* **2012**, 33 (2), 187-215.

111. Kaneto, H.; Obata, A. Unexpected pleiotropic effects of SGLT2 inhibitors: pearls and pitfalls of this novel antidiabetic class. *Int J Mol Sci* **2021**, *22 (6)*, 3062.

112. Xu, J.; Hirai, T. Effects of SGLT2 inhibitors on atherosclerosis: lessons from cardiovascular clinical outcomes in type 2 diabetic patients and basic researches. *J Clin Med* **2021**, *11 (1)*, 137.

113. Ma, X.; Liu, Z. GLP-1 receptor agonists (GLP-1RAs): cardiovascular actions and therapeutic potential. *Int J Biol Sci* **2021,** *17 (8)*, 2050-2068.

114. Elrakaybi, A.; Laubner, K. Cardiovascular protection by SGLT2 inhibitors - Do anti-inflammatory mechanisms play a role? *Mol Metab* **2022**, *64*, 101549.

115. Chen, X.; Huang, Q. GLP-1 alleviates NLRP3 inflammasome-dependent inflammation in perivascular adipose tissue by inhibiting the NF-κB signalling pathway. *J Int Med Res* **2021**, *49 (2)*, 300060521992981.

116. Han, F.; Hou, N. Liraglutide improves vascular dysfunction by regulating a cAMP-independent PKA-AMPK pathway in perivascular adipose tissue in obese mice. *Biomed Pharmacother* **2019**, *120*, 109537.

117. Ganbaatar, B.; Fukuda, D. Empagliflozin ameliorates endothelial dysfunction and suppresses atherogenesis in diabetic apolipoprotein E-deficient mice. *Eur J Pharmacol* **2020**, *875*, 173040.