

# **The interplay between adipose tissue and the cardiovascular system: is fat always bad?**

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## **Abstract**

Obesity is a risk factor for cardiovascular disease (CVD). However, clinical research has revealed a paradoxically protective role for obesity in patients with chronic diseases including CVD, suggesting that the biological “quality” of adipose tissue (AT) may be more important than overall AT mass or body weight. Importantly, AT is recognized as a dynamic organ secreting a wide range of biologically active adipokines, microRNAs, gaseous messengers and other metabolites that affect the cardiovascular system in both endocrine and paracrine ways. Despite being able to mediate normal cardiovascular function under physiological conditions, AT undergoes a phenotypic shift characterised by acquisition of pro-oxidant and pro-inflammatory properties in cases of CVD. Crucially, recent evidence suggests that AT depots such as perivascular AT and epicardial AT are able to modify their phenotype in response to local signals of vascular and myocardial origin respectively. Utilisation of this unique property of certain AT depots to dynamically track cardiovascular biology may reveal novel diagnostic and prognostic tools against CVD. Better understanding of the mechanisms controlling the “quality” of AT secretome, as well as the communication links between AT and the cardiovascular system, is required for the efficient management of CVD.

**Keywords:** obesity; perivascular fat; epicardial fat; vascular redox state; myocardial redox state

## 1. Introduction

Obesity is an established cardiovascular risk factor, conventionally defined as a body mass index  $>30\text{kg/m}^2$ , that predisposes to metabolic abnormalities and cardiovascular disease (CVD).<sup>1</sup> Recently, it has become evident that the anatomical distribution of adipose tissue (AT) is crucial for the biological implications of obesity. Distinct AT depots (i.e., subcutaneous, visceral, perivascular etc.), are differentially linked to CVD. Abdominal fat (male-type obesity), for example, is associated with increased cardiometabolic risk, while gluteal fat deposition (female-type obesity) may be protective against CVD.<sup>2</sup> For this reason, the World Health Organisation (WHO) has redefined obesity based on the waist and hip circumferences.<sup>3</sup>

AT has been revealed as an endocrine organ, secreting a variety of cytokines as well as hormones called adipokines, which elicit a variety of local and systemic responses,<sup>4</sup> influencing lipid and glucose metabolism and regulating systemic inflammation. Secreted AT products may derive from adipocytes (e.g. adiponectin) or stromal cells within AT (e.g. interleukin 6 (IL-6)) (Table 1).<sup>5</sup> AT depots distant to the large vessels and the myocardium contribute to the circulating pool of adipocytokines, affecting cardiovascular biology via the endocrine route; on the contrary, AT depots in anatomical proximity to the cardiovascular system (e.g. perivascular AT (PVAT) and epicardial AT (EpAT)) interact directly with the vasculature and the myocardium via the paracrine route (figure 1).<sup>6</sup>

The complexity of the role of AT in CVD is highlighted by the “obesity paradox”, namely the fact that, in cases of advanced CVD and type 2 diabetes mellitus (T2DM), moderately obese patients (defined mainly by BMI) have lower cardiovascular morbidity compared to lean individuals.<sup>7</sup> Although several alternative theories have been proposed to explain this observation, it is generally accepted that a major component of the obesity paradox may originate from the biological heterogeneity of AT and the inability of BMI (the classical marker of obesity) to account for variability in body fat distribution and function.<sup>7</sup> Thus, changes in

AT biology and secreted AT products, rather than whole body or AT mass, determine the risk for cardiovascular complications in overweight and obesity.

## **2. The crosstalk between AT and the cardiovascular system: endocrine versus paracrine effects**

### **2.1. Cross-talk of AT with the vascular wall**

Obesity has been linked to vascular disease progression.<sup>8</sup> Visceral AT mass, in particular, is an independent predictor of clinical outcome in patients with coronary artery disease (CAD).<sup>9</sup> Importantly, AT of obese individuals has a pro-inflammatory phenotype characterized by M1 macrophage infiltration and pro-inflammatory cytokine secretion.<sup>10</sup> As a result, obesity is accompanied by AT inflammation that induces a systemic pro-inflammatory environment, potentiating metabolic dysfunction and facilitating vascular disease development via endocrine effects.

#### ***2.1.1. PVAT: a unique AT depot with vascular effects***

PVAT is able to regulate vascular biology in paracrine ways due to its anatomical proximity to the vascular wall.<sup>11</sup> PVAT displays structural and functional heterogeneity depending on its anatomical location. From a structural point of view, PVAT is clearly separated from the vascular wall of large arteries, but is essentially integrated into the wall of small vessels.<sup>12</sup> Furthermore, PVAT may have brown AT (BAT)-like characteristics depending on species and anatomical location, displaying thermogenic capabilities and increasing triglyceride clearance after exposure to cold.<sup>13</sup> Indeed, murine PVAT around the thoracic aorta contains brown (or rather beige) adipocytes, whereas PVAT at more peripheral anatomical sites (e.g. in the legs) resembles white AT (WAT).<sup>12</sup>

PVAT directly influences vascular biology. Firstly, PVAT regulates vascular tone by secreting adventitium-derived relaxing factors (ADRFs) that cause vascular smooth muscle cell

(VSMC) relaxation.<sup>14</sup> Vascular potassium channels such as the K<sub>v</sub>7 (KCQN) subfamily of voltage-dependent potassium (K<sub>v</sub>) channels are putative targets of ADRFs.<sup>15</sup> Furthermore, K<sub>ATP</sub> channels are believed to comprise major mediators of the vasodilatory effects of H<sub>2</sub>S, a candidate ADRF.<sup>16</sup> Notably, K<sub>v</sub>7 channel activators such as flupiritine have demonstrated blood pressure-lowering *in vivo* effects in animal models,<sup>17</sup> confirming the potential role of K<sub>v</sub>7 channels in the regulation of vascular tone. The presence of PVAT also increases NO bioavailability in human and rat arterial preparations *ex vivo*.<sup>18</sup>

Interestingly, PVAT is also involved in a novel type of signaling referred to as “vasocrine” (figure 1).<sup>19</sup> In detail, PVAT-derived adipokines can penetrate the underlying vascular wall and reach the vascular lumen, thus being able to circulate in downstream microcirculation.<sup>19</sup> This offers PVAT the potential to orchestrate the homeostasis of entire vascular beds.<sup>19</sup> Importantly, PVAT facilitates insulin-mediated vasoreactivity and glucose uptake in mouse skeletal muscle by this vasocrine type of signaling,<sup>20</sup> an effect that is lost in obese mice.<sup>20, 21</sup> Considering that endothelial insulin signaling is important for capillary recruitment,<sup>22</sup> the ability of PVAT to control the effect of insulin on microcirculatory networks in organs such as the skeletal muscle may be crucial for the regulation of systemic insulin sensitivity.

### **2.1.2. Phenotypic shift of PVAT in CVD**

PVAT secretes an impressive repertoire of biologically active substances that are able to influence vascular biology in both protective and detrimental ways.<sup>16</sup> Under normal conditions, PVAT presumably has a net vasoprotective role by secreting vasorelaxing and anti-oxidant molecules such as adiponectin, NO and other ADRFs, and by modulating local inflammation.<sup>23</sup> On the contrary, in conditions such as hypertension and atherosclerosis, PVAT secretes high levels of pro-inflammatory and pro-oxidant adipokines, consequently acquiring a detrimental role.<sup>24</sup> Vascular disease is thus associated with a phenotypic shift in PVAT, which ultimately

becomes an active source of pro-oxidant and pro-inflammatory mediators that propagate vascular disease.<sup>23</sup>

Inflammation of PVAT, a common feature of vascular diseases such as atherosclerosis, hypertension and aneurysm formation, is the main cause of the aforementioned shift in PVAT phenotype.<sup>23</sup> Perivascular inflammation may be initiated by vascular signals such as reactive oxygen species (ROS) and pro-inflammatory cytokines released by the dysfunctional endothelium, VSMC and vascular macrophages.<sup>23</sup> Consequently, vascular inflammation resulting from endothelial dysfunction, an early step in vascular disease, is able to stimulate PVAT inflammation.<sup>23</sup>

PVAT phenotype is also influenced by the presence of obesity.<sup>16</sup> Indeed, PVAT's anti-contractile vascular properties are abolished in obesity.<sup>24</sup> Peri-aortic fat mass has also been positively associated with hypertension in humans.<sup>25</sup> The phenotypic alterations of PVAT in obesity are attributed to adipocyte hypertrophy, hypoxia, oxidative stress and inflammation,<sup>26</sup> ultimately leading to a detrimental change in PVAT's secretome.<sup>16</sup> Interestingly, cellular insulin resistance at the level of adipocyte has been associated with impaired suppression of lipolysis in AT, resulting in the secretion of free fatty acids (FFA), which abolish the physiological anti-contractile effect of PVAT.<sup>27</sup> Furthermore, T2DM is associated with decreased expression of adiponectin and increased expression of pro-inflammatory molecules such as MCP-1 in human EpAT.<sup>28</sup> Considering that obesity is frequently associated with insulin resistance and T2DM, it is difficult to distinguish the separate effects of obesity and cellular insulin resistance on AT phenotype.

Changes in EpAT biology have been associated with CAD. EpAT thickness positively correlates with CAD onset and progression.<sup>29</sup> Consistently, EpAT density as evaluated by computational tomography (CT) is associated with CAD progression.<sup>30</sup> Additionally, peri-coronary AT is characterized by increased M1 macrophage polarization compared to EpAT

regions distal to the coronaries, suggesting that peri-coronary EpAT inflammation influences vascular biology.<sup>31</sup> Moreover, post-mortem histological evaluation of peri-coronary AT volume and macrophage infiltration has revealed close links with the presence and size of atherosclerotic lesions in humans.<sup>30</sup> Recently, it has been revealed that ischaemic myocardial injury is followed by EpAT formation via differentiation of mesothelial progenitors, an event mediated by insulin-like growth factor 1 receptor (IGF1R) signalling.<sup>32</sup> However, the implications of this observation are uncertain.

## **2.2. Cross-talk of EpAT with the heart**

EpAT lies between the myocardium and the visceral pericardium. Therefore, it may have paracrine interactions with the myocardium in both physiological and pathophysiological states. Additionally, EpAT surrounds the coronary arteries, and part of it is thus regarded as PVAT.<sup>33</sup> This dual interaction of EpAT with the myocardium and the coronaries identifies this unique AT depot as a potentially critical regulator of CVD pathogenesis.

EpAT is able to regulate cardiac metabolism. FFA, normally reaching the myocardium via the coronary circulation, are a major source of energy in the heart.<sup>34</sup> In this setting, EpAT may act as an energy buffer, potentially protecting the myocardium from FFA overload.<sup>35</sup> Conversely, EpAT may act as an excess source of FFA potentially associated with myocardial steatosis and dysfunction in cases of systemic insulin resistance and T2DM.<sup>36</sup> Furthermore, EpAT of patients with heart failure (HF) is characterized by impaired glucose uptake and lipolysis and by increased content of monounsaturated FFA and palmitoleic acid.<sup>37</sup> Consequently, EpAT may be a critical regulator of cardiac metabolism in health and disease.

EpAT may be involved in the pathogenesis of cardiac arrhythmias such as atrial fibrillation (AF). AF is a common arrhythmia characterized by dysregulation of the electrical properties of atrial cardiomyocytes as well as by atrial remodeling.<sup>38</sup> Atrial fibrosis, in particular, is a

critical step in the generation of the AF substrate.<sup>38</sup> Clinical evidence has shown that EpAT volume is an independent predictor of AF occurrence.<sup>39</sup> Accordingly, certain adipokines secreted by EpAT, including activin A and matrix metalloproteinases such as MMP8, were recently found to have fibrotic effects on the myocardium, being alternatively called “fibrokines”.<sup>40</sup>

### **2.3. AT as a sensor of cardiovascular stress: A novel paracrine “inside-to-outside” signalling concept**

Recently, it has been revealed that the vascular wall may directly influence AT biology. For example, overexpression of p22<sup>phox</sup> (a critical subunit of the NOX1, NOX2 and NOX4 isoforms of NADPH-oxidases) in VSMC leads to exaggerated obesity in high-fat fed mice; conversely p22<sup>phox</sup> deletion in VSMC prevents weight gain induced by a similar diet.<sup>41</sup> This work proposes a novel role for vascular ROS as causal regulators of obesity and systemic insulin resistance.<sup>41</sup> In addition, wire endovascular injury of mice aortae has been shown to upregulate inflammatory marker expression while downregulating adiponectin gene expression in peri-aortic AT, an effect that was attributed to injury-stimulated vascular inflammation.<sup>42</sup> Interestingly, PVAT surrounding the large arteries contains its own vasculature, namely the vasa vasorum, which are believed to contribute to the bi-directional crosstalk between PVAT and the downstream vasculature.<sup>43</sup> For example, hypoxic PVAT stimulates the formation of vasa vasorum in the context of obesity;<sup>11</sup> the neovascularised vasa vasorum, in turn, transport macrophages from the systemic circulation to EpAT. Macrophages may then obtain oxidized cholesterol in EpAT and transfer it to the intima.<sup>44</sup> These findings suggest that AT phenotype could be dynamically modified by biological signals of vascular origin, in an inside-to-outside way.



The relationship of adiponectin with CVD is not straightforward, and regulation of adiponectin's secretion is a prime example of the reciprocal interactions between AT and the cardiovascular system. Indeed, circulating adiponectin is decreased in individuals with high risk for CVD, but it is significantly increased in advanced disease states (e.g. in HF).<sup>45</sup> Furthermore, genetic variability in the expression of adiponectin and its receptors influences adiponectin's integrated relationship with CVD.<sup>46</sup> Work from our group has revealed that expression of *ADIPOQ*, the gene encoding for adiponectin, in AT is downregulated by low-grade inflammation in non-CAD individuals, while upregulated in advanced stages of CAD in parallel with an increase in circulating BNP levels.<sup>45</sup> We have also demonstrated that BNP is a strong stimulus for PPAR- $\gamma$  activation in AT, resulting in increased secretion of adiponectin.<sup>45</sup> This suggests that the regulation of adiponectin release depends on the underlying CVD status. This may explain why decreased plasma adiponectin predicts the early onset of inflammation-associated CVD, while increased plasma adiponectin, driven by BNP, predicts adverse cardiovascular outcomes in patients with advanced disease status such as HF<sup>47</sup> and/or renal failure.<sup>45</sup> This has been called the “adiponectin paradox”, which parallels the “obesity paradox” discussed earlier.<sup>48</sup>

We have recently shown that high circulating adiponectin levels are associated with reduced vascular oxidative stress and improved endothelial function in CAD patients; however, high expression and release of adiponectin from PVAT was associated with higher superoxide generation from neighboring vessels.<sup>49</sup> This indicates that local, redox-sensitive signals may drive the release of adiponectin in PVAT as opposed to other AT depots remote to the vasculature. On the ground of these findings, we have demonstrated that, in the presence of increased vascular oxidative stress in humans, products of peroxidation produced from the vascular wall (one of which is 4-HNE) trigger adiponectin expression in PVAT via a PPAR- $\gamma$ -mediated mechanism (Figure 2A).<sup>49</sup>

Interestingly, we observed a similar crosstalk between EpAT and the myocardium in humans. In particular, we noticed that *ADIPOQ* expression in EpAT was positively associated with myocardial oxidative stress, in contrast with other AT depots.<sup>50</sup> We then showed that increased myocardial oxidative stress results in the production of transferable factors such as 4-HNE that upregulate adiponectin expression in EpAT via PPAR $\gamma$  (Figure 2B).<sup>50</sup> Other work has also revealed that the atrial myocardium is able to secrete a number of products, including BNP, which are highly adipogenic and upregulated in patients with AF.<sup>51</sup> Consequently, myocardial signals induce local adipogenesis from mesothelial progenitors and result in EpAT accumulation in patients with AF, further linking EpAT with the pathogenesis of the AF substrate.<sup>51</sup> Further elucidation of the complex crosstalk loops that may exist between the cardiovascular system and its proximal AT may reveal novel therapeutic targets.

### **3. The dynamic AT secretome and its cardiovascular effects**

Recent evidence has partially elucidated AT's secretome, identifying several adipokines and attempting to characterise their cardiovascular effects.<sup>10</sup> Adiponectin is perhaps the most extensively investigated adipokine with established protective effects against CVD,<sup>6</sup> while apelin and omentin are novel adipokines suggested to also have beneficial roles.<sup>52, 53</sup> On the other hand, leptin is another well-characterised adipokine adversely associated with CVD;<sup>54</sup> resistin is also secreted by AT and has revealed detrimental cardiovascular actions,<sup>55</sup> as has the novel adipokine visfatin.<sup>56</sup> Despite this knowledge, there is still a long way to go in order to unravel the full range of secreted products secreted by AT. Furthermore, the discrepancy of AT biology between species compromises translational research on this topic. For example, mouse PVAT resembles BAT much more so than human PVAT, suggesting that this AT depot may behave differently in terms of biology among species.<sup>57</sup> Furthermore, in contrast with humans,

rodents do not have distinct EpAT other than the pericardial fat that surrounds the heart externally to the pericardium.<sup>31</sup>

In this section, we concisely recapitulate the main cardiovascular roles of some of the most studied adipokines, while also evaluating the role of novel, non-hormonal AT products such as NO, hydrogen sulfide (H<sub>2</sub>S), methyl palmitate and microRNAs (miRs).

### **3.1. Adipokines with overall “protective” potential**

#### ***3.1.1. Adiponectin***

Adiponectin is mainly produced by adipocytes and exerts its effects via its membrane receptors, AdipoR1 and AdipoR2.<sup>58</sup> Adiponectin exhibits multiple beneficial effects in the heart and the vasculature (figure 3, table 2). More specifically, adiponectin has anti-inflammatory properties by inhibiting NFκB signaling and endothelial adhesion molecule expression.<sup>59</sup> In addition, adiponectin increases AMPK-mediated eNOS phosphorylation in cell culture models,<sup>60</sup> while also improving eNOS coupling and NO bioavailability in the human vasculature.<sup>49</sup> Adiponectin also inhibits NADPH-oxidases activity in humans.<sup>61</sup> Furthermore, adiponectin decreases ischaemia/reperfusion injury and pathological myocardial remodeling following AMI.<sup>62</sup>

Despite its vaso- and cardio-protective roles, adiponectin as a circulating biomarker has failed to translate into clinical practice successfully.<sup>6</sup> Systemic adiponectin levels are regulated by various factors such as the underlying inflammation status and BNP levels, compromising its value as a biomarker.<sup>45</sup>

#### ***3.1.2. Omentin***

Omentin is secreted mainly by visceral AT and PVAT<sup>63</sup> and its clinical significance in CVD has been suggested by observational studies in humans. Obesity and T2DM are associated with reduced secretion of omentin by AT.<sup>64</sup> Reduced circulating omentin is also independently associated with arterial calcification, endothelial dysfunction and poor prognosis in patients with HF.<sup>53</sup>

The cellular and molecular effects of omentin involve reduction of inflammation and oxidative stress in the vasculature as well as stimulation of NO production.<sup>65</sup> Omentin also inhibits neo-intima formation.<sup>66</sup> Omentin may also prevent myocardial injury via inhibition of mitochondrial oxidative stress.<sup>67</sup> Overall, omentin may be a promising target in CVD (figure 3, table 2).

### **3.1.3. Apelin**

Apelin is an adipokine that is variably linked to CVD *in vivo*, being upregulated within atherosclerotic lesions in humans.<sup>68</sup> On the other hand, plasma apelin is reduced following AMI.<sup>69</sup> Apelin has vasodilating and antioxidant properties *ex vivo*<sup>70</sup> and anti-atherogenic effects in *in vivo* animal models.<sup>52</sup> Apelin also has positive inotropic effects on the myocardium.<sup>71</sup> Conversely, APJ<sup>-/-</sup>ApoE<sup>-/-</sup> mice have demonstrated less atheromatous disease, lower NADPH-oxidases activity and reduced VSMC proliferation compared to APJ<sup>+/+</sup>ApoE<sup>-/-</sup> mice.<sup>72</sup> Overall, the cardiovascular effects of apelin are miscellaneous (figure 3, table 2).

## **3.2. Adipokines with overall “pathogenic” potential**

### **3.2.1. Leptin**

Leptin originates mainly from subcutaneous AT.<sup>73</sup> Its effects are mediated by various mediators including the JAK/STAT, SOCS (suppressors of cytokine signaling, which inhibit leptin receptor activation), PI3K/Akt and MAPK pathways.<sup>74</sup> While most studies identify detrimental cardiovascular roles for leptin, its range of effects is controversial (Figure 3, table 2). Leptin reportedly stimulates eNOS expression;<sup>75</sup> in fact, leptin has displayed a synergistic effect with insulin on NO production in rat vascular segments.<sup>76</sup> However, leptin also increases cardiovascular oxidative stress.<sup>75</sup> Moderately elevated plasma leptin levels, as observed in obesity, are associated with impaired endothelial function *ex vivo*.<sup>77</sup> While leptin signaling may

have cardioprotective effects following ischaemia,<sup>78</sup> several studies have identified leptin as a fibrotic agent in the heart.<sup>79</sup>

Circulating leptin levels have been positively correlated with obesity in clinical studies,<sup>80</sup> while leptin expression in EpAT of patients suffering from CAD is increased compared to healthy individuals.<sup>81</sup> Interestingly, serum leptin levels are negatively correlated with endothelial function in “healthy” obese individuals,<sup>82</sup> whereas no such correlation exists in non-obese healthy subjects.<sup>83</sup> These findings suggest that obesity may determine the observational association of circulating leptin levels with CVD.

### **3.2.2. Resistin**

Resistin was first described in rodents, where adipocytes comprise its main source.<sup>84</sup> However, monocytes/macrophages are the major sources of resistin in humans.<sup>85</sup> This between-species functional variability compromises the translational potential of animal models exploring resistin’s biological roles.

Resistin has detrimental cardiovascular effects (figure 3, table 2). Indeed, circulating resistin is an independent predictor of cardiovascular events,<sup>86</sup> while mechanistic studies have confirmed its ability to decrease NO production, suppress catalase activity and stimulate ROS generation.<sup>87</sup> Resistin is secreted by macrophages in atheromatous plaques in humans,<sup>88</sup> while EpAT is a significant source of resistin in cardiovascular conditions like CAD.<sup>89</sup> In addition, resistin is involved in ischaemia/reperfusion injury.<sup>90</sup>

### **3.2.3. Visfatin**

Visfatin originates from stromal cells,<sup>91</sup> and is believed to share common downstream targets with insulin, such as MAPK and Akt.<sup>92</sup> The cardiovascular effects of visfatin appear to be rather harmful (figure 3, table 2). Visfatin may increase NO production in the vasculature<sup>93</sup>, while also having cardioprotective properties.<sup>94</sup> Conversely, several studies have proposed that

visfatin promotes oxidative stress,<sup>95</sup> atherosclerotic plaque destabilisation in mice<sup>96</sup> and myocardial recruitment of neutrophils after AMI.<sup>97</sup> Elevated visfatin expression in PVAT is also linked to atherosclerosis in humans.<sup>98</sup>

Clinical studies have further highlighted the involvement of visfatin in CVD. Serum visfatin is elevated in obesity and T2DM,<sup>99</sup> and may be a biomarker of atherosclerotic plaque development, possibly reflecting the chronic inflammation that underlies atherosclerosis.<sup>100</sup> Recently, increased circulating visfatin has been revealed as a predictor of major cardiovascular events following AMI.<sup>101</sup>

### **3.3. Beyond adipokines: novel mediators of the AT-cardiovascular system interaction**

Recent evidence suggests that AT is able to interact with the cardiovascular system in novel ways beyond adipokine secretion. Indeed, AT also secretes miR molecules<sup>102</sup>, gaseous messengers such as NO and hydrogen sulfide (H<sub>2</sub>S)<sup>16</sup> and metabolites such as methyl palmitate,<sup>24</sup> which may all influence cardiovascular biology. With the exception of NO, little is known about the clinical relevance of these factors as mediators of the interplay between AT and CVD.

MiRs are small, non-coding RNA molecules that bind complementary mRNA sequences, thus inhibiting their translation.<sup>103</sup> By regulating gene silencing, miRs influence protein synthesis and multiple cellular functions.<sup>103</sup> MiRs exist in the intracellular compartment of most tissues, but can also be secreted extracellularly and in the plasma, mainly incorporated in exosomes, i.e., vesicles of endocytic origin that serve as messengers between cells.<sup>102</sup> A variety of miRs have been associated with risk factors for CVD and mechanistically linked to CVD pathogenesis.<sup>104</sup> Importantly, a wide variety of miRs exist in AT, where they are differentially regulated in metabolic disease.<sup>105</sup> In fact, it has recently been shown that AT in mice contributes to the circulating miR pool and is able to regulate expression of proteins such as FGF21 in

remote tissues in miR-dependent ways.<sup>102</sup> Moreover, recent work has revealed that the miR expression profile of EpAT is dysregulated in individuals with CAD, promoting local inflammation.<sup>106</sup> In specific, miR-103-3b is upregulated in EpAT of patients with CAD and has been identified as a potential modulator of the pro-inflammatory chemokine CCL13.<sup>106</sup> Importantly, obesity, insulin resistance and T2DM are characterised by changes in AT regarding the expression of miRs such as miR-29a and miR-143, which regulate AT browning and inflammation.<sup>107</sup> Interestingly, miR-29a has been associated with myocardial fibrosis<sup>108</sup> while miR-143 is a relatively specific biomarker of VSMC activation that has been linked to atherosclerosis and hypertension.<sup>109</sup> Thus, miRs may comprise novel mediators of the crosstalk between AT and the cardiovascular system, and the spectrum of miRs secreted by AT in health and disease needs to be further investigated.

Vast literature has established the beneficial effects of NO for cardiovascular biology.<sup>110</sup> AT expresses NOSs<sup>111</sup> and is able to produce NO that may originate from both adipocytes and tissue macrophages and is secreted locally or in the circulation.<sup>112</sup> Interestingly, NO stimulates lipolysis<sup>113</sup> and has direct anti-inflammatory effects on AT,<sup>114</sup> thus identifying NO of either endothelial or adipose source as a potential target in obesity and metabolic disease. In addition, NO is able to modulate AT perfusion, with implications for modulation of AT hypoxia.<sup>115</sup> Importantly, NO is produced by PVAT and is regarded as an ADRF and a paracrine regulator of vascular inflammation.<sup>24</sup> Crucially, dysfunctional PVAT, as observed in cases of obesity, is associated with decreased secretion of NO and thus with locally increased inflammation, vasoconstriction and oxidative stress.<sup>116</sup>

H<sub>2</sub>S is another endogenously produced gaseous molecule that has attracted interest as a potential mediator of CVD. H<sub>2</sub>S is mainly synthesized by three enzymes, i.e., cystathionine- $\beta$ -synthase (CBS), cystathionine- $\gamma$ -lyase (CSE) and 3-mercaptosulfurtransferase (3-MST), which are expressed in both the cardiovascular system and AT.<sup>16</sup> Following its production, H<sub>2</sub>S may

diffuse locally or enter the circulation, thus exerting both paracrine and endocrine effects.<sup>16</sup> H<sub>2</sub>S has longer half-life than NO, its effects thus being more prolonged than those of NO.<sup>117</sup> H<sub>2</sub>S has displayed anti-oxidant, anti-atherogenic and anti-inflammatory properties in cell culture and animal models.<sup>16</sup> H<sub>2</sub>S also induces vasorelaxation through the activation of ATP-regulated potassium channels (K<sub>ATP</sub>)<sup>118</sup> or by interfering with NO synthesis<sup>16</sup>, while it may also convey protective effects in the heart during ischaemia/reperfusion injury via rescuing mitochondrial function and by reducing mitochondrial ROS.<sup>119</sup> Importantly, H<sub>2</sub>S is secreted by AT, where its synthesis is dysregulated in cases of obesity.<sup>120</sup> However, the contribution of AT to the systemic levels of H<sub>2</sub>S and the role of PVAT-derived H<sub>2</sub>S are not clarified at present. Furthermore, the complex kinetics of H<sub>2</sub>S and its numerous interactions with other endogenous molecules challenge the investigation of its *in vivo* effects.<sup>119</sup>

AT secretes fatty acid metabolites such as palmitic acid methyl ester (PAME), which is an ADRF.<sup>121</sup> Indeed, PAME is able to induce vasorelaxation via activation of K<sub>v</sub> potassium channels,<sup>24</sup> and may also have anti-inflammatory effects.<sup>122</sup> Due to its small molecular weight and its hydrophobic nature, PAME is believed to be especially important for the paracrine crosstalk of PVAT with the vasculature.<sup>121</sup>

## 4. Conclusion

Recent evidence has revealed that AT is a dynamic endocrine organ secreting a wide range of products with diverse cardiovascular roles. These products may interact with the cardiovascular system via the endocrine route, exerting beneficial (e.g., adiponectin), detrimental (e.g., resistin) or even contrasting, context-dependent effects. Such effects include regulation of systemic and local inflammation, oxidative stress, cell proliferation and apoptosis. Anatomically distinct AT depots have unique secretory profiles, underlying the importance of defining obesity based on regional AT distribution, rather than by simple measurement of body



mass index. Importantly, hypertension, atherosclerosis, obesity and T2DM are variably associated with dysregulation of circulating adipokines and other AT products.

AT depots that are anatomically attached to the heart and vessels (i.e., EpAT and PVAT respectively) are able to interact with their neighbouring organs directly in a paracrine way. Hypertension, atherosclerosis, obesity and T2DM are associated with a phenotypic switch in these unique AT depots, resulting in local propagation of inflammation, oxidative stress and impaired vasorelaxation. Interestingly, EpAT and PVAT are able to “sense” biological signals from the heart and vessels, modifying their secretome accordingly, which indicates that the crosstalk between AT and the cardiovascular system is mutual. Similar endocrine bi-directional communication loops also exist between the heart and remote AT depots (e.g. subcutaneous AT), the interaction between myocardial-derived BNP and AT-derived adiponectin in advanced disease states being a typical example. Further understanding of such paracrine or endocrine bi-directional loops between AT and the cardiovascular system may lead to the discovery of new therapeutic targets or diagnostic biomarkers, allowing us to improve clinical practise in the future.

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**Table 1:** Key adipokines secreted by AT, their biological role and their clinical relevance

| Adipocytokine | Major cell source        | Biological roles  | Clinical relevance   |
|---------------|--------------------------|---|--|
| Adiponectin   | Adipocytes               | Ameliorates IR; has anti-oxidant and anti-inflammatory roles  | Low plasma adiponectin in seemingly healthy subjects predicts CAD onset; in advance disease status, plasma adiponectin predicts detrimental outcomes, reflecting BNP levels; polymorphisms in adiponectin and its receptors have been associated with CVD risk |
| Leptin        | Adipocytes               | Regulates energy storage; leptin resistance is associated with IR and hypertension                                | Elevated plasma omentin is associated with CVD risk; polymorphisms in the gene of leptin and its receptors are linked to obesity and CVD; leptin resistance may be crucial for CVD   |
| Omentin       | Adipocytes               | Increases insulin sensitivity; has anti-oxidant and anti-inflammatory effects                                     | Novel adipokine the clinical potential of which is not fully explored yet; multiple studies have linked circulating omentin to favorable CVD outcome in humans   |
| Visfatin      | Stromal cells            | Unclear roles; believed to be pre-atherogenic   | Visfatin is associated with multiple CVD endpoints in humans such as atherosclerosis, hypertension and HF; Its production by AT is disturbed in obesity, providing a link between metabolic disease and CVD  |
| Apelin        | Adipocytes               | Poorly understood roles; may regulate cardiac contractility and cardiovascular redox state                        | Novel adipokine that may be linked to CVD; its clinical relevance has been suggested by animal models and observational studies in humans  |
| Resistin      | Monocytes/macrophages    | Induces IR; has pro-oxidant, pro-inflammatory, pro-atherogenic properties   | Recent work has unraveled the detrimental effects of resistin on multiple aspects of cardiovascular biology, and its circulating levels are consistently linked with CVD prognosis; therefore, it may be a promising target in CVD                             |
| TNF           | Monocytes/macrophages    | Promotes inflammation, oxidative stress and IR  | TNF is of questionable translational potential since it is a universal mediator of immunity; however, its over-secretion by inflamed AT is pivotal to the pathogenesis of metabolic disease and CVD  |
| IL6           | Monocytes/macrophages    | Promotes inflammation, oxidative stress and IR  | Similarly to TNF, IL6 is crucial to the orchestration of inflammation in obesity and CVD.  |
| IGF-I         | All AT cell types        | Regulates adipocyte and VSMC differentiation; interferes with insulin signaling; has unclear cardiovascular roles | The cardiovascular roles of IGF-I are poorly explored. However, due to its pleiotropic effects and its similarity to insulin, it may comprise a novel target linking metabolic and cardiovascular disease  |
| <b>ET-1</b>   | <b>All AT cell types</b> | <b>Has pro-oxidant, pro-inflammatory roles</b>  | <b>Targeting of ET-1 has been proven to be challenging; nonetheless, its implication in the pathogenesis of CVD is established and the fact that it is dynamically secreted by AT may reveal novel roles for this molecule in obesity-related CVD</b>          |

IR: Insulin resistance; TNF: Tumor necrosis factor; IL6: Interleukin 6; IGF-I: Insulin-like Growth Factor I; AT: Adipose tissue; VSMC: Vascular smooth muscle cells; ET-1: Endothelin 1; CAD: Coronary artery disease; CVD: Cardiovascular disease; HF: Heart failure

**Table 2:** Pathophysiological effects of important adipokines on myocardial biology

| Adipokine          | Effects on myocardial biology |                              |             |             |              |
|--------------------|-------------------------------|------------------------------|-------------|-------------|--------------|
|                    | Oxidative stress              | Ischaemia/reperfusion injury | Hypertrophy | Remodelling | Inflammation |
| <b>Adiponectin</b> | ↓                             | ↓                            | ↓           | ↓           | ↓            |
| <b>Visfatin</b>    | ↑                             | ↑                            | ↓           | ↓           | ↑            |
| <b>Apelin</b>      | ↓                             | ↓                            | U           | U           | ↓            |
| <b>Leptin</b>      | ↑                             | ↓                            | ↑           | ↑           | ↑            |
| <b>Resistin</b>    | ↑                             | ↑                            | ↑           | ↑           | ↑            |
| <b>Omentin</b>     | ↓                             | ↓                            | U           | U           | ↓            |

↑ indicates a stimulatory effect; ↓ indicates an inhibitory effect; U indicates an undetermined/controversial effect

## Figure legends

**Figure 1:** Overview of the crosstalk routes between AT and the vascular wall. AT depots remote to the vascular wall secrete adipokines that reach the vascular wall via the circulation, exerting endocrine effects on both endothelial cells and VSMC. Due to its anatomical proximity to the vascular wall, PVAT in particular can secrete adipokines originating from either adipocytes or stromal cells (such as macrophages, lymphocytes and fibroblasts) which diffuse to the vascular wall to exert paracrine effects. PVAT may hypothetically also participate in “vasocrine” signalling, whereby its secreted adipokines diffuse into the underlying vascular lumen and propagate local signals to adjacent vascular beds of downstream circulation.

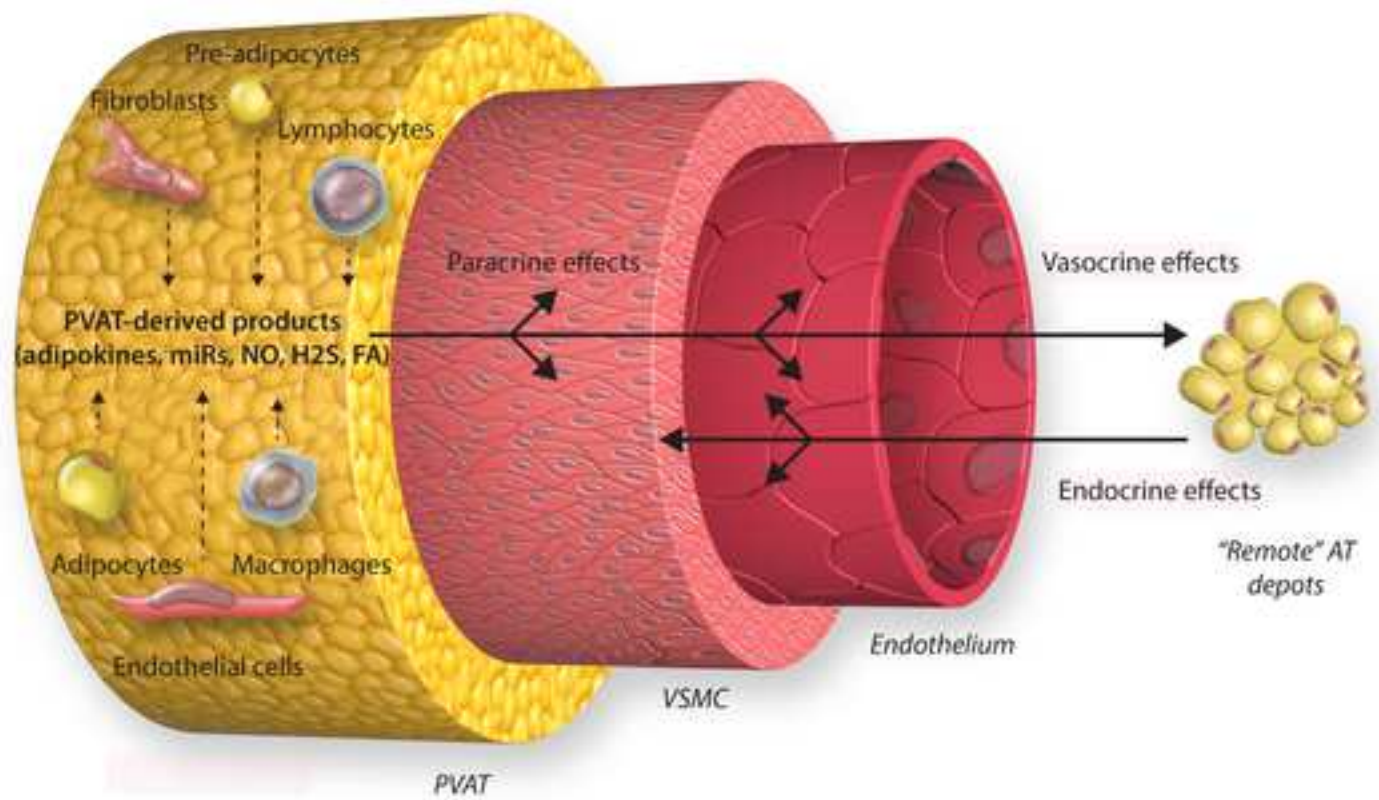
AT: Adipose tissue; VSMC: Vascular smooth muscle cells; PVAT: Perivascular adipose tissue

**Figure 2:** The adiponectin paradigm of the novel inside-out crosstalk between the cardiovascular system and AT. Adiponectin, secreted by PVAT or remote AT depots, has anti-oxidant roles in the vasculature by inhibiting the activity of NADPH-oxidases as well as through AMPK- and Akt-mediated stimulation of eNOS activity and coupling, resulting in increased production of NO. Increased vascular  $O_2^-$  production, on the other hand, increases local production of lipid peroxidation products such as 4HNE, which are able to diffuse to PVAT and upregulate PPAR $\gamma$ -mediated *ADIPOQ* expression and adiponectin secretion in PVAT, potentially as a protective defence mechanism against oxidative stress (panel A). Similarly, adiponectin has anti-oxidant effects in the myocardium via AMPK-regulated inhibition of NADPH-oxidases activity. Furthermore, increased  $O_2^-$  production results in increased lipid peroxidation products such as 4HNE, which diffuses to the EpAT to locally upregulate adiponectin expression. Pro-inflammatory cytokines and BNP associated with myocardial disease exert opposing endocrine effects on adiponectin secretion by AT, influencing its potential as a biomarker (panel B).

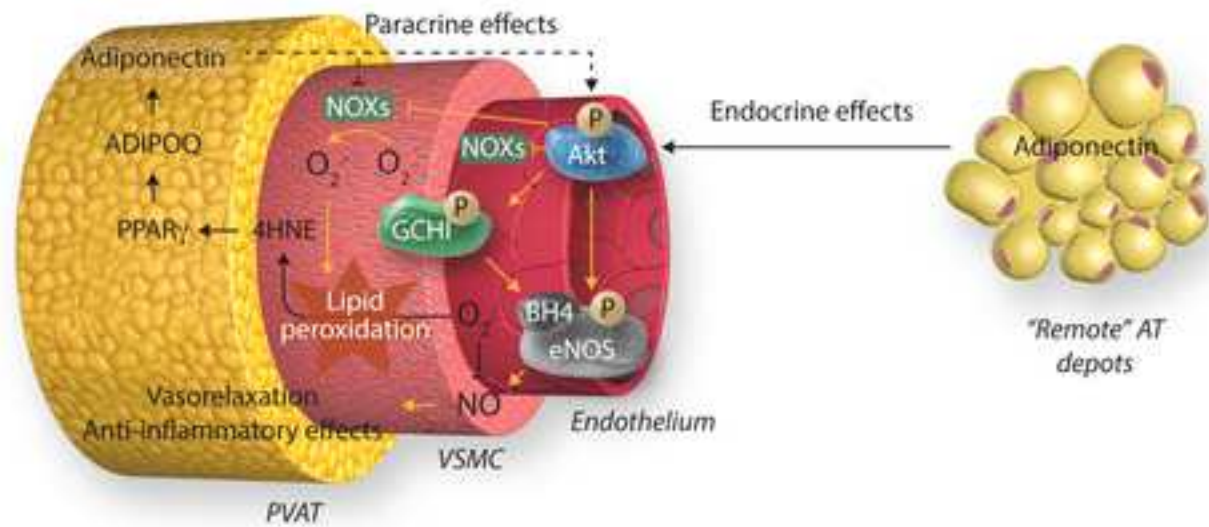
AT: Adipose tissue; PVAT: Perivascular adipose tissue; NADPH: Nicotinamide adenine dinucleotide phosphate; eNOS: Endothelial nitric oxide synthase; GCHI: guanosine triphosphate cyclohydrolase I; BH4: Tetrahydrobiopterin; BH2: Dihydrobiopterin; NO: Nitric oxide; O<sub>2</sub><sup>-</sup>: superoxide; 4HNE: 4-hydroxynonenal; PPAR $\gamma$ : Peroxisome proliferator-activated receptor gamma; ADIPOQ: Adiponectin, C1Q and Collagen Domain Containing locus; AMPK: Adenine monophosphate-activated protein kinase; EpAT: Epicardial adipose tissue; BNP: Brain natriuretic peptide; PI3K: Phosphoinositide 3 kinase; iNOS: Inducible nitric oxide synthase; PTEN: Phosphatase and tensin homolog; ATF: Activating transcription factor 2

**Figure 3:** Effects of important adipokines on the vascular wall. As illustrated in the figure, adipokines can reach the endothelium from both sides of endothelial surface, namely the luminal and the interstitial surface. Adiponectin has a number of anti-inflammatory and antioxidant effects via activation of AMPK and Akt and by inhibition of NF-kB and TNF $\alpha$ , resulting in reduced oxidative stress and in increased NO bioavailability. Similarly, omentin exerts vasoprotective effects by reducing VSMC migration while decreasing oxidative stress and increasing NO bioavailability. On the other hand, resistin and visfatin are presumably detrimental to the vascular wall via induction of oxidative stress and inflammatory responses. Leptin, especially in the context of leptin resistance, is also considered detrimental, by inducing ONOO<sup>-</sup> formation and NADPH-oxidases activity. Apelin is a poorly studied adipocytokine that may stimulate eNOS activation, but is associated with increased atherosclerosis risk in some animal models, therefore having unclear roles in the vasculature.

AMPK: Adenine monophosphate-activated protein kinase; NF-kB: Nuclear factor kappa beta; TNF $\alpha$ : Tumour necrosis factor alpha; NO: Nitric oxide; VSMC: Vascular smooth muscle cells; ONOO<sup>-</sup>: Peroxynitrite; NADPH: Nicotinamide adenine dinucleotide phosphate; eNOS: Endothelial nitric oxide synthase



A



B

