Mechanisms of Adiponectin-Associated Perivascular Function in Vascular Disease

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Abstract—The concept that fat cells could influence the circulation and indeed cardiac function has been in existence for ≥20 years and has gained a wide interest and no less excitement as evidence has accrued to suggest that such effects may be profound enough to explain disease states, such as hypertension and metabolic changes associated with obesity and type II diabetes mellitus. This ATVB in Focus intends to examine our current knowledge in this field, and suggests mechanisms that may be responsible for normal perivascular function and how they become disordered in obesity. There is the tantalizing prospect of developing new therapeutic approaches to keep obese individuals healthy and redesignating type II diabetes mellitus as a vascular disease.

Does PVAT Release Factors That Can Influence Vascular Tone?

The simplest way to address this question is to stimulate a blood vessel surrounded with PVAT using a spasmogen in vitro and then expose a preconstricted artery denuded of PVAT to the organ bath solution. If there is a reduction in vascular reactivity or sensitivity, then this must be attributable to factor(s) released from PVAT. This approach has been applied to murine and human tissues, including mesenteric and subcutaneous gluteal arteries and aorta, and consistently there is an anticontractile response. Furthermore, incubation and adrenergic stimulation of dissected PVAT alongside a denuded vessel further supports the secreted factor(s) hypothesis as opposed to physical blockade because vessels constrict significantly less than PVAT denuded counterparts.

What Is the Anticontractile Factor?

Löhn et al extended early studies and concluded that PVAT released a soluble factor that induces vasodilatation by opening smooth muscle K⁺ channels. As a result of these reports, several candidates have been suggested as possible PVAT-mediated relaxing factors and include adiponectin, angiotensin I-7, methylpalmitate, and hydrogen sulfide (H₂S). It is probable that a combination of factors is released and may depend on the stimulus applied, the vascular bed examined, and the phenotypic state of the fat. For example, in segments of healthy human subcutaneous small arteries in which the endothelium has been removed, a PVAT-mediated anticontractile effect can be observed, which would suggest that angiotensin I-7 is unlikely to be responsible in this situation.

Key Words: adiponectin  adipose tissue  blood supply
because angiotensin is released from PVAT to act on the endothelium, thereby evoking its anticontractile effect. Our data suggest that adiponectin plays a prominent role for the following reasons: first, B6.129-Adipoq<sup>+/−</sup>/J homozygous adiponectin-deficient mice demonstrated no anticontractile activity in response to stimulation of PVAT with norepinephrine<sup>10</sup>; second, exposure of PVAT to a soluble fragment of the type 1 adiponectin receptor abolished the anticontractile effect in murine and human vessels.<sup>10,18</sup> This result has been confirmed recently by Meijer et al.<sup>19</sup> Others have disputed these findings: Fésüs et al<sup>20</sup> reported that the PVAT from homozygous adiponectin-deficient mice produced a normal anticontractile effect; the PVAT and artery were stimulated with serotonin. However, although adiponectin may be the relaxing adipokine released by sympathomimetic stimulation, our recent data using serotonin suggest that this constrictor hormone releases a factor that causes relaxation that cannot be blocked using the type 1 adiponectin receptor fragment, indicating that the process may be agonist specific (Figure 1) and we agree that there may be many factors at work.

**How Is Adiponectin Released and How Does It Act?**

The presence of β<sub>1</sub>-adrenoreceptors on adipocytes provides a potential link between autonomic stimulation and adiponectin release. Weston et al<sup>9</sup> have provided evidence that under basal, noncontracted conditions, β<sub>1</sub>-stimulation releases an adipocyte-derived hyperpolarizing factor that is probably adiponectin. The intriguing question is how does a factor released from cells on the adventitial surface of an artery induce a reduction of tone at the level of the myocyte and the endothelium? What is not in doubt is that the exogenous application of adiponectin to a preconstricted small arterial segment denuded of fat does indeed reduce tone, suggesting that the adipokine can pass rapidly through the wall by mechanisms yet to be defined<sup>17</sup> or activate downstream signaling events to evoke its relaxation. Our and others most recent data suggest that norepinephrine produces an adiponectin-induced anticontractile effect, which is nitric oxide (NO) dependent.<sup>17</sup>

This is not to say that the effect is wholly dependent on an endothelium-mediated final action because it is clear that the adipocyte itself will produce NO when appropriately stimulated. This would explain the reported anticontractile effect of norepinephrine-stimulated human small arteries surrounded by PVAT but denuded of endothelium,<sup>21</sup> which is attenuated by incubation with LNMMA. Detailed pharmacological studies and the use of knockout mouse models implicate roles for large calcium sensitive potassium channel (BK<sub>Ca</sub>) channels on adipocytes and vascular smooth muscle cells, as well as cGMP-dependent protein kinase (PKG) again both within adipocytes and downstream. Lynch et al<sup>10</sup> reported that norepinephrine stimulation of white PVAT around wild-type mouse small arteries produced the characteristic adiponectin-mediated anticontractile effect, but in homozygous mice without BK<sub>Ca</sub> channels this was not seen. Detailed studies implicated these channels both in adipocytes and in smooth muscle cells. Our conclusion is that adiponectin elicits its effect by activating BK<sub>Ca</sub> channels on adipocytes and smooth muscle cells, as well in combination with additional endothelial mechanisms and, therefore, it is important to emphasize that PVAT release of adiponectin can elicit a reduction in tone even in a de-endothelialized vessel in consequence. In addition, our recent work has demonstrated that PKG is important in mediating PVAT function both in regard to the secretion of adiponectin and in mediating its vasoconstrictor function. In mice homozgyously lacking PKG, there is a loss of PVAT anticontractility, increased oxidative stress, and reduced adiponectin expression.<sup>22</sup> Several recent studies have demonstrated the presence of NO signaling components, including PKG within adipocytes themselves, suggesting that fat cells may contribute to vascular relaxation in concert with the endothelium. The overall impression is that the data support previous findings in human and mouse small arteries where NO synthase inhibition inhibits PVAT function by a direct effect on adipocytes and downstream anticontractile activity. Furthermore, the presence of PKG in the adipocyte is important because it is known that the BK<sub>Ca</sub> channel is a phosphorylation target for this enzyme.

Therefore, our current scheme for the release and actions of adiponectin is shown in Figure 2. Sympathomimetic stimulation of β<sub>1</sub>-adrenoreceptors on adipocytes leads to the activation of PKG and an increase in the bioavailability of adiponectin. This has 2 roles: first, it activates myocyte AMP-activated protein kinase to open BK<sub>Ca</sub> channels; indeed, myocyte AMP-activated protein kinase has been shown as a potent regulator of these channels<sup>23</sup>; and second, it liberates NO both in adipocytes and in the endothelium; through both these processes there is a reduced vascular tone.

**Figure 1.** The effect of applying a soluble fragment of the type 1 adiponectin receptor (ABP) on the response of rat mesenteric arteries to 5-HT. Perivascular adipose tissue (PVAT) in the presence of 5-HT induced an anticontractile effect (*P=0.0096; n=5; 2-way ANOVA), which was not influenced by ABP (*P=0.5055; n=4; 2-way ANOVA), suggesting that the pathway of the 5-HT–induced PVAT anticontractile effect is independent of adiponectin. Data are normalized to maximum high potassium (60 mmol/L; KPSS) constriction, which is not significantly different in the absence and presence of PVAT.
Figure 2. A, Proposed pathway for adrenergic-mediated anticontractile capacity of perivascular fat (PVAT): stimulation of β3 adrenergic receptors results in the release of adipocyte-derived nitric oxide (NO) via endothelial NO synthase (eNOS)–dependent and potentially independent mechanisms, which may modulate the release of adiponectin by influencing mitochondrial function via cGMP-dependent protein kinase (PKG)–dependent mechanisms. Adiponectin secreted from the adipocyte subsequently activates smooth muscle adiponectin receptors, leading to downstream signaling events, including myocyte AMP-activated protein kinase (AMPK) activation, eNOS phosphorylation, and NO signaling. Downstream PKG-mediated phosphorylation events, including activation of large calcium sensitive potassium channel (BKCa) channels, result in a net vasodilation of the artery in the presence of PVAT, which is at least, in part, dependent on a functional endothelium. B, Obesity and associated oxidative stress is associated with dysregulated PVAT function, including β3 adrenergic receptor desensitization, reduced expression of PKG, adiponectin, and AMPK; the net effect of these changes is the loss of PVAT-induced vasodilation.
Obesity
It is estimated that there are 475 million obese adults world-wide and that the condition contributes to ≥2.5 million deaths annually. Prevalence rates have tripled in the past 30 years and causing various physical disabilities and psychological problems, obesity increases the risk of developing hypertension, diabetes mellitus, and cancer. The condition is accompanied by insulin resistance in skeletal muscle, liver, and blood vessels. Studies of adipocyte morphology in patients with obesity and the metabolic syndrome show that individual fat cells undergo hypertrophy and there is clear evidence of local inflammation, 18 as well as a loss of PVAT-mediated anticontractile function, which is because of reduced bioavailability of adiponectin. 19 Yudkin et al. 20 suggested that low-grade inflammation dependent on the production of proinflammatory cytokines by PVAT might provide the link between arterial function and insulin resistance and make type II diabetes mellitus a vascular disease. Why should this occur in obesity? Grant 21 has suggested that there is a logical phylogenetic basis for this: historically, it would have made sense for humans to develop insulin resistance in times of food availability thereby preserving energy stores for periods of starvation when a switch to increased sensitivity would be favorable. He cites the metabolism of animals, such as the brown bear, which undergoes dramatic changes during hibernation to increase resistance to hypothermia, infection, and muscle disuse. Before hibernating such species deposit fat and there is evidence of increased insulin resistance, decreased glucose use, and leptin resistance. After hibernation, animals have lost weight and wake lean and healthy. The contrast with contemporary human life is a constant state of caloric excess but no periods of starvation or hibernation. The result will be protracted insulin resistance with its inevitable sequelae.

A change in hemodynamics in obesity would provide a direct and logical explanation for all of the consequences of this phenotype, and a loss of normal anticontractile PVAT function is the potential link. Without this one would anticipate an increase in peripheral vascular resistance, which is the hallmark of hypertension. In addition, vasoconstriction will decrease glucose uptake into skeletal muscle and insulin resistance will follow, concomitant with a decrease in availability of circulating lipoprotein lipase resulting in dyslipidaemia, 25 the other features of the constellation described as the metabolic syndrome. Therefore, the hypothesis of Yudkin et al. 20 becomes attractive: vascular dysfunction causes metabolic syndrome and type II diabetes mellitus.

PVAT Environment in Obesity and the Metabolic Syndrome
Studies of human PVAT from obese individuals and adipocytes from animal models of obesity and diabetes mellitus consistently show evidence of low-grade inflammation and hypertrophy. 4 Functionally, the anticontractile activity of PVAT is lost in small arteries preconstricted with norepinephrine. 17, 18 It can be fully restored in vitro using a combination of catalase and dismutase, suggesting that increased oxidative stress reduces the bioavailability of adipokines, such as adiponectin. Also there is evidence of reduced NO bioavailability and uncoupling of NO synthase. 20 Likewise, antagonism of tumor necrosis factor-α using preincubation with infliximab in the organ bath can restore normal PVAT anticontractile function as can the aldosterone antagonist spironolactone and eplerone. 18 Interestingly, experimental induction of the hypoxic environment also results in the loss of anticontractile capacity of PVAT in healthy vessels; this too is restored by aldosterone antagonists thereby supporting the contributing role of the hypoxic environment and subsequent oxidative stress in the loss of PVAT function. 27

Within the inflamed PVAT environment are increased numbers of classically activated or M1 macrophages (CAMs). 27 These produce the proinflammatory cytokines, such as tumor necrosis factor-α, detailed above. In the white adipose tissue of lean, healthy animals, macrophages constitute 10% to 15% of stromal cells and express markers that link them with the phenotype of alternatively activated macrophages, which are critical for maintaining insulin sensitivity in adipocytes partly through the production of interleukin-10. In obesity, Ly6c + monocytes are recruited, which increases macrophage content to 46% to 60%, and the CAM inflammatory phenotype that promotes insulin resistance is observed. 28, 29 Adipocyte hypertrophy releases chemokines, such as CCL2, CCCL5, and CCL8, to recruit additional Ly6c + monocytes that exacerbate the process. 28

The central role of the CAM in the loss of PVAT anticontractile function was demonstrated in a mouse model of inducible macrophage ablation. In mice deficient of macrophages CD11b-diptheria toxin receptor, there was no loss of anticontractile activity when proinflammatory stimuli, such as hypoxia, were applied to small arteries surrounded by PVAT. 27 Recently, it has been shown that interleukin-4 and interleukin-13 are vital for macrophages to develop into healthy protective alternatively activated macrophages. Wynn et al. 30 searched adipose tissue for the major source of these cytokines and confirmed it to be eosinophils that migrate into adipose tissue by an integrin-dependent process. Eosinophilic mice were lean and insulin sensitive; animals without eosinophils were obese and insulin resistant. Subsequently, these workers infected fat-fed mice with parasitic worms and induced an eosinophilia in fat depots with a resulting decline in adipose tissue mass and reduction in glucose intolerance. 31

Effects of Weight Reduction
Long-term follow-up studies of weight-reducing surgical management of patients with morbidly obese have demonstrated maintained falls in blood pressure, improved glucose tolerance, and reduced cardiovascular mortality with halving of cancer rates. Recently, we have had the opportunity to investigate PVAT structure and function before and 6 months after bariatric surgery. Before intervention, these patients had evidence of adipocyte hypertrophy and inflammation with increased CAMs and a complete loss of PVAT-mediated anticontractile function in small gluteal arteries preconstricted with norepinephrine. 21 Six months after surgery, there was a significant fall in blood pressure and insulin resistance, and although body mass index was also reduced, the patients remained morbidly obese. However, adipocyte hypertrophy had been completely reversed with the disappearance of
CAMs and inflammation. PVAT anticontractile activity was restored as a result of improved bioavailability of adiponectin because it could be blocked using the introduction of the fragment of the type 1 adiponectin receptor. Not only do these data provide support to the Yudkin proposal that type II diabetes mellitus is a vascular condition but also in restoring normal PVAT function there is the possibility that obese individuals might be kept healthy. Indeed not all obese subjects become diabetic or hypertensive; perhaps, these preserve a normal PVAT environment. Additional studies are required in this area.

**New Therapeutic Approaches in Obesity?**

Although only weight-reducing surgery has been demonstrated to improve survival in patients with obesity, the cost is prohibitive as a universal panacea. However, if PVAT function is indeed crucial to maintain arterial integrity, any intervention that can prevent or rescue inflammation in the obese adipocyte environment would be attractive. In this context, trials of cytokine antagonists might seem attractive but single agents, such as infliximab, have proved disappointing although targeting a single cytokine is probably insufficient. Perhaps more promising would be the use of aldosterone antagonists, such as spironolactone, a drug that is known to have anti-inflammatory properties and against a hormone known to be increased in inflamed adipocytes.

The autonomic nervous system has a role in the regulation of long- and short-term energy balance, and its dysregulation is implicated in the pathogenesis of obesity and type II diabetes mellitus. There is clear evidence that obesity and its early complications (insulin resistance and impaired fasting glucose) are associated with overactivity of the sympathetic nervous system and decreased tone of the parasympathetic nervous system. Long-term complications of type II diabetes mellitus are associated with chronic sympathetic nervous system overactivation, furthermore, autonomic dysfunction is seen in patients preceding the onset of type II diabetes mellitus, indicating that the development of autonomic imbalance parallels the development of obesity, hyperinsulinemia, and insulin resistance or has a pathogenic role in the development of diabetes mellitus. Once type II diabetes mellitus has developed chronic hyperglycemia and a persistent increase in sympathetic nervous system activity will downregulate peripheral β-adrenergic receptors resulting in an inability of the sympathetic nervous system to enhance energy expenditure. Downregulation of β receptors would lead to a loss of adiponectin secretion. Therefore, any intervention that reduced sympathetic neural drive might be advantageous. In this context, it is of interest that renal denervation has been shown to improve glucose tolerance ≥3 months of follow-up. Maybe this treatment modality will find a role in preventing diabetes mellitus in obesity by preserving PVAT function. Perhaps most exciting is a recent report identifying orally active synthetic small-molecule adiponectin receptor agonists, which have been shown to have similar effects to adiponectin in muscle and liver with regard to activation of myocyte AMP-activated protein kinase and PPAR-α pathways and ameliorated insulin resistance and glucose intolerance in mice fed a high-fat diet. The area is alive with new possibilities of thwarting the consequences of the obesity epidemic.

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None

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