Adipokines and Vascular Disease in Diabetes

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Adipokines, in particular adiponectin, have been highlighted in the pathogenesis of obesity-related illnesses, including type 2 diabetes, because of their role in the regulation of insulin sensitivity as well as vascular endothelial function. Since cardiovascular disease accounts for an overwhelming proportion of the morbidity and mortality suffered by patients with diabetes, researchers are actively seeking a better understanding of the role that adipokines play in the vasculature with the hope that the use of these agents, or activation of their signaling pathways, might help prevent micro- and macrovascular complications. This brief review highlights recent work on the vascular effects of circulating adipokines, focusing on adiponectin, and includes some recent findings with leptin and resistin. This highly active area of investigation has identified novel hormonal mechanisms by which the adipose tissue mass can influence vascular function with important consequences for cardiovascular risk.

Introduction

The prevalence of overweight has continued to increase at an alarming rate, especially in younger age groups, in which it now affects 16% to 18% of children and adolescents [1]. Obesity is frequently complicated by hypertension, dyslipidemia, and glucose intolerance, major components of the cardiovascular risk cluster that has been estimated to affect up to 27% of the overall US population [2]. Considering the morbidity and mortality due to increased cardiovascular events in this population, the disease mechanisms linking adiposity and this risk cluster are important areas of investigation.

Adipose tissue is recognized as being an active secretory organ, rather than a simple storage depot for triglycerides [3–5]. Adipocytes and their surrounding stromal tissue release a variety of factors into the bloodstream in a regulated fashion, including nonesterified free fatty acids (FFAs), tumor necrosis factor (TNF)-α, interleukin (IL)-6, resistin, leptin, complement factors, plasminogen activator inhibitor-1, and adiponectin. These proteins, synthesized and released from adipocytes, have been termed "adipokines." They function as hormones, have signaling roles at other tissues, and impact a variety of physiologic processes, including satiety, energy balance, metabolic regulation, and endothelial cell and leukocyte activation. In concert, they are believed to mediate many of the adverse systemic effects of increased adiposity. This brief article focuses on recently reported effects of adiponectin on endothelial function and atherogenesis. Recent findings with leptin and resistin are also included, although these adipokines remain less well characterized with regard to their effects in the cardiovascular system.

Adiponectin

Adiponectin is an approximately 30-kDa protein specifically secreted from adipose tissue that circulates as oligomeric complexes in healthy human subjects at relatively high concentrations (~ 2–10 μg/mL). This protein has provided a novel link between obesity, insulin resistance, and vascular disease [6•,7••,8••,9•]. As the visceral adipose mass expands, the circulating levels of a number of metabolites and adipokines that affect endothelial function are increased, including FFAs, TNF-α, resistin, and leptin, while at the same time plasma adiponectin levels are decreased. Numerous publications have consistently shown that adiponectin levels are negatively correlated with important cardiovascular risk factors as well as overt atherosclerosis [9•]. More recent work in vitro and in vivo has progressed to discover some of the mechanisms of the vascular actions of adiponectin (Fig. 1).

Adiponectin exists in the systemic circulation as an array of complexes of the full-length protein (fAd), consisting of trimers, hexamers, and high molecular weight (HMW) forms containing 12 to 18 subunits [7••]. A putative proteolytic cleavage fragment consisting of the globular C-terminal domain lacking the collagenous stalk (gAd) has also been described [10]. Whether the globular

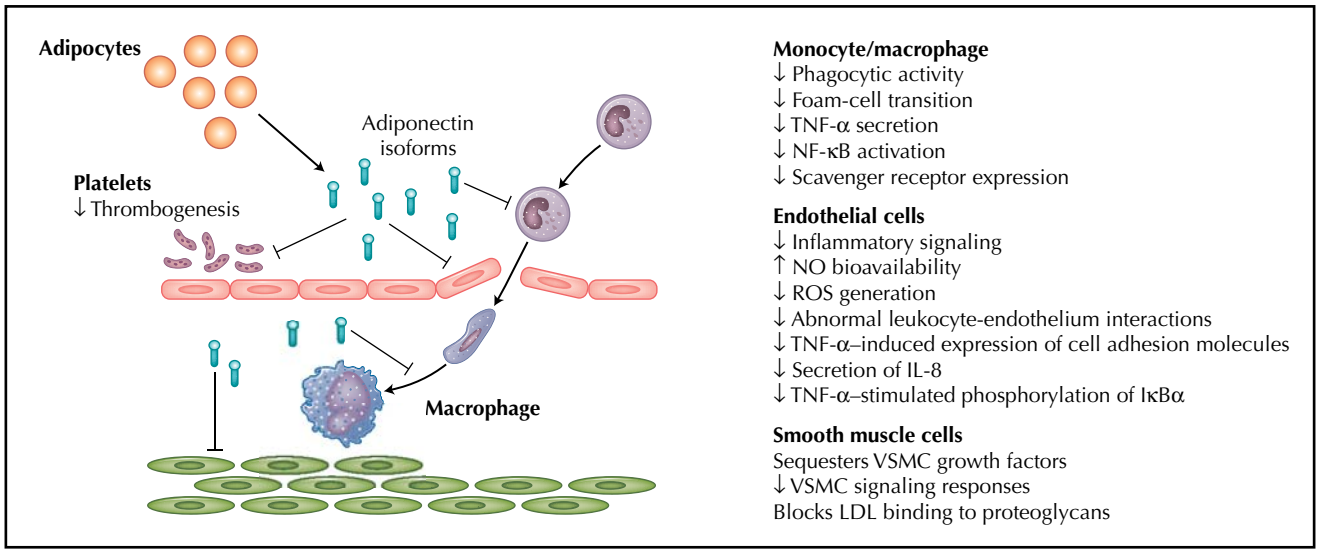

Figure 1. Multiple sites of adiponectin action on the function and signaling by vascular cells and atherogenesis. This schematic depicts the reported effects of adiponectin isoforms at many levels, including monocyte/macrophage function and differentiation, endothelial cell activation and responsiveness, vascular smooth muscle cell (VSMC) proliferation, low-density lipoprotein (LDL) binding, and platelet activation in thrombogenesis. IL-8—interleukin-8; NF-κB—nuclear factor-κB; NO—nitric oxide; ROS—reactive oxygen species; TNF-α—tumor necrosis factor-α.

fragment circulates in vivo or is generated at tissue signaling sites remains unclear. Nevertheless, many studies have now demonstrated that the recombinant gAd fragment exerts potent pharmacologic effects in vascular and in other cell types. A closer structural mimic of endogenous adiponectin is the recombinant full-length fAd. fAd can be generated recombinantly in mammalian cell expression systems where it undergoes hydroxylation and glycosylation of the collagenous stalk and it is secreted in oligomeric forms similar to the natural processing that occurs in adipocytes [11]. These post-translational modifications occur only in mammalian cells and appear to be important for some of the metabolic actions of fAd. For example, insulin action is enhanced by mammalian fAd at the liver with decreased hepatic glucose production in diabetes [12]. In contrast, gAd or recombinant fAd from bacterial sources is relatively inactive at the liver due to the absence of post-translational modifications and oligomeric assembly [7••,8••].

Adiponectin receptors

Two adiponectin receptor isoforms that differ in their tissue distribution have been identified and termed AdipoR1 and AdipoR2 [13]. AdipoR1 has a preferential affinity for gAd, whereas AdipoR2 binds both gAd and fAd [8••]. Aortic endothelial cells express both adiponectin receptor isoforms, but appear to preferentially express mRNA for AdipoR1, also supporting a signaling role for gAd in this cell type [14•,15]. T-cadherin has been identified as another putative receptor specific for the HMW oligomeric complexes of fAd [16]. T-cadherin is a glycophosphatidylinositol-anchored cell surface adhesion protein induced in a variety of pathophysiologic settings that lacks an intracellular signaling domain [17]. The exact functional role of these adiponectin receptors as well as the identification of downstream signaling pathway(s) to which they are coupled remains poorly understood.

Effects of adiponectin on vascular structure and function

Association studies in numerous populations have supported a role for adiponectin in cardiovascular protection [18•]. Adiponectin levels are increased by insulin-sensitizing maneuvers, including weight loss, in most [19] but not all recent studies [20]. Adiponectin levels, especially the HMW complexes, have been shown to increase following administration of thiazolidinedione insulin sensitizers in rodents and humans [20–22]. Very recent studies in rodents have confirmed that adiponectin mediates a significant part of the in vivo metabolic responses to thiazolidinediones [23,24]. The extent to which the effects of thiazolidinediones on vascular signaling are also mediated by adiponectin is not yet known.

Recent work has provided further understanding that the different structural forms of adiponectin correlate to varying degrees with selective clinical features of the metabolic syndrome. In particular, enhanced insulin sensitivity and a reduced cardiovascular risk profile have been attributed to the HMW complexes of adiponectin [25,26].

Potential role of adiponectin vascular effects in hyperglycemic states

In addition to the visceral obesity-associated cardiovascular risk factors discussed above, hyperglycemia itself is another well-known cause of micro- and macrovascular complications. In fact, hyperglycemia is responsible for enhanced oxidative stress with subsequent endothelial dysfunction

[27]. Interestingly, in patients with type 1 diabetes, a significant negative association has been prospectively shown between adiponectin levels and coronary artery disease, suggesting that it may protect against the adverse vascular effects of hyperglycemia [28]. This is consistent with our recent finding that adiponectin reduces reactive oxygen species (ROS) generation induced by high glucose in cultured endothelial cells, discussed below [29•].

In vivo studies of adiponectin vascular actions

Adiponectin knockout mice have provided an important experimental tool to decipher the role of adiponectin in lipid and glucose metabolism as well as in vascular homeostasis [7••,8••,30]. Mice lacking adiponectin show neointimal thickening and increased proliferation of vascular smooth muscle cells (VSMCs) in mechanically injured arteries. Conversely, adenoviral fAd replenishing therapy attenuates vascular inflammation in adiponectin-deficient mice. Adiponectin also suppresses the development of atherosclerosis in susceptible mouse models, including apolipoprotein E–deficient mice, in which it downregulates expression of vascular cell adhesion molecule (VCAM)-1 and class A scavenger receptors, in addition to reducing levels of TNF-α. Two studies have shown effects of adiponectin (fAd) on small vessel angiogenesis, although they are conflicting in their conclusions [31,32].

Endothelial cells

Studies in vitro have provided insight into some of the direct effects of adiponectin on the function of endothelial and inflammatory cells. These actions include preventing endothelial cell dysfunction in response to TNF-α and elevated ambient glucose. Overall, adiponectin vascular signaling has been considered "anti-inflammatory" because it suppresses a characteristic pleiotropic activation response pattern in endothelial cells that includes stimulation of the nuclear factor-κB (NF-κB) pathway, upregulation of cell adhesion molecules, and diminished endothelial nitric oxide (NO) generation and bioavailability. Both endothelial cells and circulating leukocytes are likely to be primary targets of the anti-inflammatory vascular protective properties of adiponectin. Our laboratory has recently demonstrated that loss of endogenous adiponectin is associated with abnormal leukocyte-endothelium interactions in the microcirculation (Ouedraogo et al., Unpublished data). Adhesion of circulating leukocytes to the vascular endothelium causes endothelial dysfunction by exposing endothelial cells to the damaging action of inflammatory mediators released by activated leukocytes [33]. Adiponectin also preserves endothelial NO by directly shifting the balance between NO and ROS generation in a direction favorable to NO availability. A negative relationship between systemic oxidative stress and circulating adiponectin levels has been shown in rodent models of obesity and the metabolic syndrome [34] and in several [35,36], but not all [37], recent studies in human subjects.

Adiponectin has been shown to have several important signaling effects in the endothelium. In vitro, fAd generated in *Escherichia coli* inhibited TNF-α–induced expression of the adhesion molecules VCAM-1, E-selectin, and intercellular adhesion molecule-1 on the surface of endothelial cells [38]. TNF-α–induced adhesion of monocytic THP-1 cells to cultured endothelial cells is also inhibited by fAd [38]. fAd suppresses TNF-α–induced inflammatory changes in endothelial cells by blocking IκB phosphorylation and NF-κB activation without affecting TNF-α–mediated activation of JNK, p38, and Akt [39].

In aortic endothelial cells stimulated with TNF-α, adiponectin decreased abundance of mRNA and secretion of IL-8, a proinflammatory chemokine [40]. Phosphorylation of IκB-α, which leads to NF-κB activation, was decreased by adiponectin, but phosphorylation of ERK, SAPK/JNK, and p38 mitogen-activated protein kinase (MAPK) were unaffected.

Vascular ROS are generated in response to a variety of agonists and induce proliferation or apoptosis of endothelial cells in a dose-response manner [41]. The concept is now emerging that the adverse vascular effects of glucose as well as other mediators, including FFAs and cytokines, are mediated by the chronic or intermittent production of ROS, which activates an inflammatory signaling cascade in various endothelial cell beds [42]. Using aortic endothelial cells, we recently reported that gAd inhibited cell proliferation induced by oxidized low-density lipoprotein (LDL), as well as release of superoxide and p42/p44 MAPK activation induced by oxidized LDL [14•]. Interestingly, adiponectin has been shown to suppress superoxide generation in other systems by induction of antioxidant enzymes. Apoptosis in neuroblastoma cells induced by an inhibitor of complex I that increases cellular superoxide levels was suppressed by adiponectin via induction of both superoxide dismutase and catalase [43]. Ongoing research will help determine whether a similar process is occurring in vascular cells in response to circulating adiponectin and how this may influence the balance of cellular oxidative molecule generation.

Smooth muscle cells

The adherence of adiponectin to the lining of catheter-injured vessels but not intact vascular walls and the direct binding to adiponectin of circulating growth factors that stimulate VSMC proliferation to adiponectin may play an important role in the overall vascular protective action of this adipokine [9•]. Adiponectin treatment of VSMC in culture attenuated proliferation induced by a variety of growth factors, migration induced by heparin-binding epidermal growth factor-like growth factor (HB-EGF) or platelet-derived growth factor (PDGF)-BB, and suppressed PDGF-induced ERK phosphorylation and PDGF receptor autophosphorylation, possibly by binding to PDGF-BB and inhibiting its cellular association [44]. Adiponectin isoforms bind PDGF-BB, basic fibroblast growth factor

(FGF), and HB-EGF with distinct affinities. PDGF-BB bound to high and middle molecular weight adiponectin complexes, but not the low molecular weight form. FGF preferentially interacted with HMW adiponectin, whereas HB-EGF bound to all three forms with comparable affinities. The interactions of adiponectin with PDGF-BB, basic FGF, and HB-EGF precluded binding to their respective membrane receptors and attenuated the DNA synthesis and cell proliferation induced by these growth factors [45]. Another protein interaction of adiponectin involves blocking the binding of LDL to proteoglycans. Both fAd and gAd inhibited LDL binding to biglycan in a dose-dependent manner [46]. This effect may confer additional antiatherogenic properties to circulating adiponectin.

Monocytes and macrophages

Additional anti-inflammatory effects of adiponectin that impact on vascular function are mediated by direct effects on circulating inflammatory cells. Adiponectin (fAd) suppressed leukocytic colony formation, and reduced phagocytic activity, foam-cell transition, and TNF-α secretion from macrophages [47,48]. Because inflammatory signaling in leukocytes is at least in part mediated by toll-like receptors (TLRs), it is interesting that gAd was recently shown to bind to AdipoR1 receptors and suppress TLR-mediated activation of the NF-κB cascade in a cultured mouse macrophage cell line [49]. In the THP-1 monocytic cell line, adiponectin induced apoptosis and reduced macrophage scavenger receptor A (MSRA) mRNA expression, and the low molecular weight adiponectin isoform suppressed NF-κB activation by lipopolysaccharide [50].

Adiponectin signaling mechanisms

The structures of the cloned adiponectin receptors AdipoR1 and AdipoR2 are predicted to be related to the seven-transmembrane domain G-protein-coupled receptor family but are topologically unique (Fig. 2) [8••]. Recently, a potentially important molecular link in AdipoR1 and AdipoR2 signaling has been ascribed to APPL1 (adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain, and leucine zipper motif) [51•]. Protein interactions between adiponectin receptors and APPL1 are stimulated by adiponectin in mammalian cells, and this interaction can modulate adiponectin downstream effects, including lipid oxidation and glucose transporter translocation, which also involves the small GTPase Rab5. These novel findings provide the first potential molecular context for the mechanism of the insulin-sensitizing function of adiponectin, but whether APPL1 is involved in adiponectin effects in vascular cells is not known.

AMPK

Studies on downstream signaling in metabolically responsive liver, skeletal muscle, and adipose cells have highlighted the integral role of the pleiotropic enzyme 5' adenosine monophosphate–activated protein kinase (AMPK) in the signaling effects of adiponectin [7••,8••,52]. AMPK is also activated by adiponectin in endothelial cells [31,53,54]. The enhancement of endothelial NO availability by adiponectin is linked to AMPK activation as well as signaling through the phosphatidylinositol 3 (PI3)-kinase activity [53]. Effects of adiponectin on angiogenesis were also found to be dependent on adiponectin-stimulated phosphorylation of both AMPK and Akt [31]. AMPK appears to be upstream of Akt, because disrupting AMPK activation inhibited adiponectin-induced Akt phosphorylation [31].

A potential mechanism for gAd stimulation of endothelial NO synthase (eNOS) activity was shown in a recent study to involve eNOS-Hsp90-Akt complex formation [55]. Inhibition of PI3-kinase activity suppressed endothelial NO release stimulated by gAd, indicating a role for the PI3-kinase-Akt pathway in gAd-induced eNOS phosphorylation and NO production, which also enhanced endothelium-dependent vasorelaxation in aortic rings studied ex vivo.

cAMP signaling

In addition to AMPK, a cyclic AMP (cAMP)/protein kinase A (PKA)–linked pathway has been implicated in adiponectin endothelial signaling. Ouchi et al. [39] initially reported that the inhibitory effect of adiponectin on TNF-α signaling in endothelial cells was accompanied by cAMP accumulation and blocked by an inhibitor of either adenylate cyclase or PKA. Recently, the inhibitory effect of adiponectin on TNF-α–induced IL-8 synthesis in endothelial cells was shown to be associated with increased intracellular cAMP levels and PKA activity, and blocked by PKA inhibition [40]. Adiponectin also enhanced Akt phosphorylation and the inhibitory effect of adiponectin on TNF-α–induced IL-8 synthesis was abrogated in part by pretreatment with a PI3-kinase inhibitor, which prevents Akt activation [40].

We have recently reported that both recombinant bacterial gAd and eukaryotically expressed fAd suppress ROS production induced by high glucose in endothelial cells [29•]. In these studies, gAd increased cellular cAMP content and inhibition of PKA blocked the effect of either gAd or fAd to suppress ROS generation. The suppression of glucose-induced ROS was also mimicked by increasing cellular cAMP with the adenylyl cyclase activator forskolin or with the cAMP mimetic, dibutyryl cAMP. These findings demonstrate that adiponectin suppression of ROS production in high glucose is mediated by a cAMP/PKA–dependent pathway. In murine peritoneal macrophages, gAd was also recently shown to increase cAMP and PKA activity and reduce leptin-induced TNF-α production by blocking ERK1/2 and p38 MAPK phosphorylation [56]. Together with the studies noted above, these data establish the cAMP/PKA pathway as a major signaling system mediating the beneficial actions of adiponectin to counter

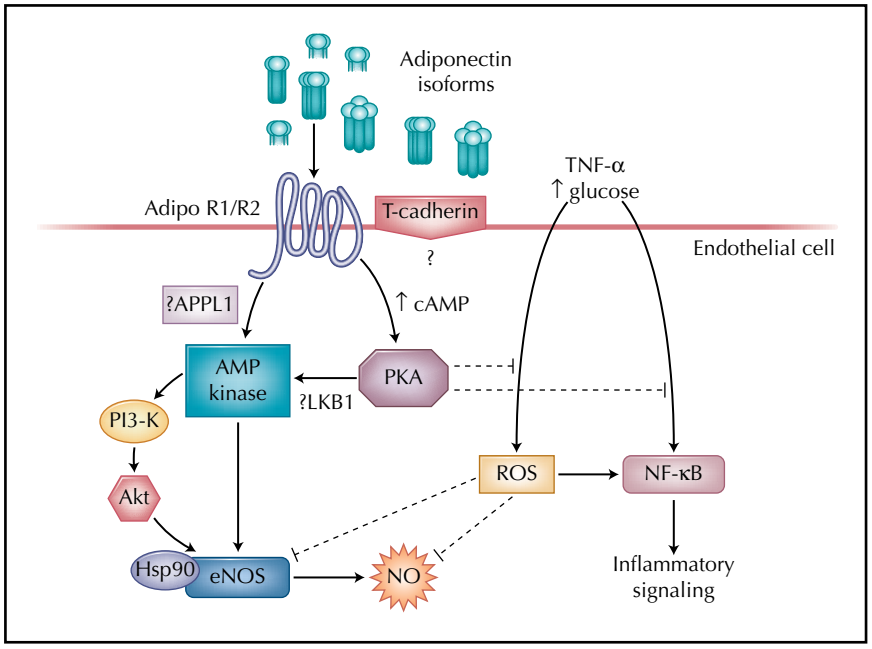

Figure 2. Simplified view of potential signaling pathways for adiponectin in endothelial cells. Adiponectin activates 5′-adenosine monophosphate-activated protein (AMP) kinase, which in turn stimulates endothelial nitric oxide synthase (eNOS) via a pathway dependent on Akt activation. Adiponectin also increases cyclic AMP (cAMP) and activates protein kinase A (PKA), which plays a key role in the suppression of reactive oxygen species (ROS) generation by multiple inputs, including tumor necrosis factor-α (TNF-α), high glucose, and oxidized low-density lipoprotein (LDL). Reduced ROS may facilitate suppression of inflammatory signaling via the nuclear factor-κB (NF-κB) cascade. The *solid arrows* and *dotted lines* reflect stimulatory and inhibitory effects, respectively. NO—nitric oxide; PI3-K— phosphatidylinositol 3-kinase.

the adverse effects of TNF-α or high glucose in endothelial and potentially other vascular cells.

Cross-talk in adiponectin signaling pathways: AMPK and cAMP/PKA

The finding that both AMPK and cAMP/PKA pathways are implicated in the vascular action of adiponectin suggested that there may be signaling cross-talk between these pathways. In fact, it has been shown recently that LKB1, an upstream kinase that phosphorylates AMPK, can be phosphorylated by PKA [57]. In other systems, PKA is also upstream of AMPK [58], and signaling cross-talk has been reported between cAMP and AMPK for maximal activation of lipolysis in cultured adipocytes [59]. In endothelial cells exposed to high glucose conditions, we also found an interaction between AMPK and PKA signaling. Pharmacologic activation of AMPK with 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside only partially diminished glucose-induced ROS production, and the effect of submaximal elevation of cellular cAMP content was further enhanced by activation of AMPK. However, inhibition of AMPK did not block the action of gAd to suppress glucose-induced ROS production, suggesting that this effect of gAd was largely independent of AMPK [29•]. Also, increasing cAMP levels or blocking PKA activity in endothelial cells did not affect the ability of gAd to activate AMPK.

Adiponectin isoform-specific vascular signaling

The array of adiponectin multimeric isoforms in the circulation and their differential association with cardiovascular risk factors have suggested that they may differ in their signaling properties. Unfortunately, the vascular actions of fAd and gAd have generally been studied separately in published papers, and only a few reports in the literature have directly compared their signaling properties in the same experimental system [29•,50]. We recently showed that both recombinant gAd from *E. coli* and fAd purified from a eukaryotic expression system equally suppressed ROS generation in endothelial cells induced by high glucose or by treatment with oxidized LDL [29•].

In undifferentiated THP-1 cells, both low molecular weight (trimeric) adiponectin from a baculovirus system and HMW adiponectin from mammalian cells induced apoptosis, reduced MSRA mRNA expression, and stimulated phosphorylation of AMPK [50]. However, in this study the low molecular weight form reduced lipopolysaccharide-mediated IL-6 release by suppression of NF-κB activation, whereas HMW adiponectin induced secretion of IL-6 in human monocytes and THP-1 cells but did not suppress lipopolysaccharide-induced IL-6 secretion. Apoptosis, activation of AMPK, and the reduction of MSRA mRNA were found to be mediated by all adiponectin isoforms, but only the low molecular weight form displayed anti-inflammatory properties. Additional work directly comparing dose responses and signaling properties of various adiponectin isoforms is needed.

Additional effects of adiponectin that impact on atherogenesis

Adiponectin has recently been shown to serve as an endogenous antithrombotic factor [60•]. Adiponectin knockout mice have normal platelet counts and coagulation parameters, but after carotid artery injury the knockout mice showed accelerated thrombus formation, which was attenuated by replenishing circulating adiponectin levels by adenovirus transduction. Moreover, recombinant adiponectin inhibited the enhanced aggregation of platelets from adiponectin knockout mice that was observed in vitro [60•].

Adiponectin and lipids

Consistent with the reported correlation in human subjects between adiponectin, insulin resistance, and clinical features of the metabolic syndrome, the potential effects of adiponectin on lipid metabolism have been an active area of research. Adiponectin was found to be strongly correlated with various lipoproteins and apolipoproteins; in particular with high-density lipoprotein (HDL) cholesterol, and to a lesser degree with markers of inflammation such as C-reactive protein, IL-6, or markers of coagulation or fibrinolysis [61]. In both male and female patients, the mean circulating adiponectin levels were significantly lower in patients with a familial combined hypercholesterolemia phenotype [62].

Hepatic lipase, a key enzyme involved in the dyslipidemia of insulin resistance in the metabolic syndrome (high triglycerides and low HDL cholesterol), was inversely correlated with adiponectin, independent of age, gender, body mass index, plasma triglycerides, insulin, HDL cholesterol, and high-sensitivity C-reactive protein [63]. Thus, a suppressive effect of adiponectin on hepatic lipase activity may help to explain the association of adiponectin with elevated HDL cholesterol levels.

Studies using in vivo kinetic techniques showed a strong negative correlation between adiponectin and the degradation of apolipoprotein (apo) A-I [64]. In multivariate analyses, adiponectin and the HDL triglyceride/cholesterol ratio explained 62% of the variance of the apo A-I fractional catabolic rate. Adiponectin alone explained 43%, suggesting that adiponectin may have a direct role on HDL catabolism.

Adiponectin and leptin levels were directly and inversely correlated, respectively, with very low density lipoprotein (VLDL) apo B catabolism and HDL cholesterol concentration, whereas resistin, IL-6, and TNF-α were not significantly associated with any of these variables [65]. In this study, adiponectin was the most significant predictor of plasma VLDL apo B concentration and was an independent predictor of VLDL apo B catabolism, whereas leptin was not associated with the kinetics of VLDL apo B.

Adiponectin and myocardial ischemia-reperfusion injury

Beyond the scope of this brief review of vascular effects of adiponectin, but worth mentioning here, are the exciting findings published during the past year demonstrating a protective effect of adiponectin on myocardial ischemia-reperfusion injury. Refer to the paper by Ouchi et al. [66•] for an updated review of this rapidly progressing aspect of adiponectin action.

Leptin and Resistin

Vascular effects of leptin and resistin

In addition to the key role that leptin plays in the regulation of body weight and energy balance, it has been shown to broadly affect a variety of systems in the body, including reproductive function, regulation of the hypothalamic-pituitary-adrenal axis, and vascular function. The effects of leptin and resistin in the vasculature have recently been carefully reviewed [67•–69•]. Leptin exerts several potentially atherogenic effects, such as impairment of endothelial function, stimulation of inflammatory signaling pathways, increased oxidative stress, decreased paraoxonase activity, platelet aggregation, and migration, hypertrophy, and proliferation of VSMCs. Leptin-deficient and leptin receptor–deficient mice are protected from arterial thrombosis and neointimal hyperplasia in response to arterial wall injury. Several clinical studies have demonstrated that high leptin levels predict acute cardiovascular events, restenosis after coronary angioplasty, and cerebral stroke independently of traditional risk factors. In addition, plasma leptin correlates with markers of subclinical atherosclerosis, such as carotid artery intima-media thickness and coronary artery calcifications. These findings have led to the notion that inhibition of leptin signaling may help slow the progression of atherosclerosis in hyperleptinemic obese subjects. Some investigators have suggested that the leptin:adiponectin ratio might be a useful index for carotid intima-media thickness and may be a suitable marker for atherosclerosis in patients with type 2 diabetes, because the ratio would magnify the difference in these adipokines among individuals [70].

Endothelial cells express leptin receptors and previous work showing enhanced endothelial ROS in response to leptin stimulation has implicated this adipokine in the pathogenesis of vascular inflammatory signaling and atherogenesis [67•–69•]. Leptin caused significant endothelial dysfunction and attenuated coronary dilation and relaxation to acetylcholine in nondiabetic dogs and rodents [71]. Using high-fat-fed dogs as a hyperleptinemic model of prediabetes, coronary vessels were rendered insensitive to the vascular effects of leptin, suggesting an early protective adaptation against endothelial dysfunction [72]. The available data are conflicting on whether leptin acts on the endothelial system to decrease arterial pressure or whether it has a neutral effect on arterial blood pressure [69•]. In recent work, leptin exhibited vasoactive responses in human vessels ex vivo, but its action was not dependent on endothelial NO generation [73].

Resistin contributes to dysglycemia in obesity and promotes endothelial cell impairment [74]. In a large human cohort, resistin levels were recently shown to be positively associated with levels of circulating inflammatory markers as well as with increasing coronary artery calcification after adjustment for age, gender, and established risk factors [75]. Resistin protein was found in both murine and human atherosclerotic lesions, suggesting a direct role in atherogenesis [76].

Resistin caused endothelial dysfunction of porcine coronary arteries by increasing cellular ROS and downregulation of NO availability [77]. Resistin impaired

endothelium-dependent dilation to bradykinin but not acetylcholine in the coronary circulation in anesthetized dogs and in isolated coronary artery rings, acting at a site at the bradykinin receptor or proximal to NOS or cyclooxygenase [78]. In endothelial cells in vitro, resistin increased endothelin-1 mRNA expression and secretion; resistin-treated cells also showed increased expression of VCAM-1 and monocyte chemoattractant protein-1 [76,79]. In endothelial cells, resistin inhibited insulin signaling and eNOS activation via upregulation of the abundance of PTEN (phosphatase and tensin homologue deleted on chromosome 10) through a mechanism involving p38 MAPK activation of sites in the PTEN promoter [80]. Increased PTEN blocks signaling through the PI3-kinase/Akt pathway and suppresses activation of eNOS.

Proliferation of VSMCs was recently shown to be stimulated by resistin via both ERK1/2 and Akt signaling pathways [81]. Interestingly, cross-talk between adiponectin and resistin was shown by adiponectin inhibition of cell adhesion molecule expression induced by resistin in endothelial cells, which affected leukocyte adherence and the activation of vascular inflammatory signaling [82•]. Although the metabolic effects of adiponectin have been extensively studied in leptin-deficient mouse models [7••,8••], no reports have yet appeared examining the potential interactions of adiponectin and leptin on endothelial cells or vascular function.

Conclusions and future directions

The developed world is currently experiencing an epidemic of cardiovascular disease that is associated with an ever-increasing prevalence of insulin resistance with obesity due to excess caloric intake and sedentary lifestyles. Key contributors to the increased vascular risk in the setting of insulin resistance and the "metabolic syndrome" are the impairment of vascular endothelial and smooth muscle cell function, which are important components of the initiation of the complex process of atherogenesis. Adipokines, including adiponectin, leptin, and resistin, have been shown to have important effects in the vasculature, as well as in insulin signaling, glucose metabolism, and energy balance. Active work in this area will continue to provide novel insights into the multiple ways that adiposity can affect vascular health and identify new targets for intervention to prevent the widespread morbidity and mortality associated with obesity and insulin-resistant states, including the metabolic syndrome and type 2 diabetes mellitus.

Acknowledgments

Because of space considerations, primary references for many of the papers published prior to 2005 can be found in the cited review articles. Work on adiponectin in the authors' laboratories is supported by NIH grants RO1-DK63018 and DK71360. The grants already mentioned are to Dr. Goldstein. Dr. Scalia is supported by NIH Grant DK064344.

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