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The role of the adipocyte hormone adiponectin in cardiovascular disease

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Adiponectin, a novel hormone made by fat tissue, regulates energy metabolism and endothelial activation. Serum levels of adiponectin are reduced in conditions that are associated with an increased risk of cardiovascular disease, such as diabetes and the metabolic syndrome. Adiponectin trimers assemble into higher-order oligomers, which have different signaling properties. Adiponectin trimers and a C-terminal globular domain activate AMP-activated protein kinase, whereas hexamer and high-molecular weight isoforms activate nuclear factor- κ B signaling pathways. Exogenous adiponectin corrects metabolic defects that are associated with insulin resistance, and might protect the endothelium from the progression of cardiovascular disease. Receptors for adiponectin have been described and might provide future therapeutic targets for the treatment of cardiovascular disease.

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Introduction

Adiponectin, first described as Acrp30 (adipocyte complement related protein of 30 kDa; [1]) and also termed AdipoQ, Apm1 and GBP-28, is an adipokine that is secreted specifically from differentiated adipocytes. Adiponectin expression is upregulated during the differentiation of preadipocytes into mature adipocytes [1]. The primary sequence (Figure 1) of the polypeptide contains a signal sequence (cleaved in the mature protein) and a non-conserved N-terminal domain, followed by 22 collagen repeats and a C-terminal globular domain that has structural similarities to tumor necrosis factor α (TNF- α) [2]. Adiponectin is abundant in serum, and is found at

concentrations of up to 10 μ g/ml. Like all collagen-domain-containing proteins, the polypeptide forms the basic unit of a trimer. The trimer self-associates through a conserved N-terminal cysteine residue to form disulfide-linked dimers of trimers (also termed hexamers), which further assemble into high-molecular weight (HMW) forms that consist of multiple oligomers of the basic trimer. These species have been fractionated and visualized using electron microscopy, as shown in Figure 2 [3]. A mutant lacking the N-terminal cysteine residue will only form trimers [3]. A proteolytic form of the globular C-terminal domain, although postulated to exist *in vivo* [4,5], has only been studied with recombinant material. Obese mice injected with globular adiponectin demonstrate increased β -oxidation of fatty acids and weight loss [4].

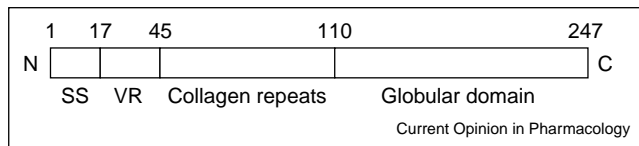
Adiponectin, an abundant serum adipokine, is increasingly recognized to be both a potential biomarker for the metabolic syndrome and a possible therapeutic target for the treatment of cardiovascular disease. We will review the recently described association of adiponectin with cardiovascular and metabolic disease, the known activities of adiponectin as determined using *in vivo* and *in vitro* models, and conclude with the recent description of several adiponectin receptors.

Clinical correlations of reduced adiponectin levels

Adiponectin is reduced in the serum of both type 2 diabetic and obese individuals, and is further decreased in patients with cardiovascular disease [6,7]. Correction of body weight in multiple studies, as well as improvement of glycemic control with diet, weight loss and hypoglycaemic agents, has resulted in normalization of adiponectin levels, suggesting an intimate connection between this adipokine and metabolic control. The ratio of HMW to low molecular weight forms is increased following treatment with drugs of the thiazolidinedione class of antidiabetic agents [8*,9], indicating that the complex distribution of adiponectin isoforms has functional relevance, as discussed below.

The metabolic syndrome, also termed the 'deadly quartet' by Kaplan [10,11], consists of abdominal obesity, insulin resistance, dyslipidemia and hypertension. Progression and development of the metabolic syndrome has been linked to polymorphisms at the genomic locus of adiponectin, suggesting that altered expression or activity of adiponectin is causative in the development of this syndrome [12]. The hypothesis that adiponectin is related

Figure 1



Schematic of the primary sequence of adiponectin, with amino acid residues numbered above and domain regions indicated below. The signal sequence (SS) is cleaved during secretion from the adipocyte, and is followed by the non-homologous variable region (VR), which contains the N-terminal cysteine residue 22 that is responsible for higher-order oligomerization (see text for details). 22 collagen repeats precede the globular domain. Trypsin cleaves adiponectin at residue 104 to generate the globular domain preparation used in initial *in vivo* studies [4].

to development of the metabolic syndrome was first put forth by Matsuzawa and co-workers [13]. Several of the individual aspects of this syndrome have been described in association with reduced levels of adiponectin [6,7,13]. Recently, an adiponectin haplotype in the coding sequence (designated I164T) has been described [14]. The salient points of this work are that this mutation is associated with decreasing serum adiponectin levels to approximately half that of the wild-type, and that these individuals had an increase in risk factors for the metabolic syndrome and cardiovascular disease relative to control individuals lacking this mutation. Interestingly, serum adiponectin levels associated with this haplotype were found to be independent of the body mass index, whereas control subjects in this study replicated the inverse association of body mass index and adiponectin levels described by other investigators. Other researchers have also detected association of adiponectin haplotypes with coronary artery disease and diabetes [15]. A large study that examined adiponectin levels at baseline in males free from diagnosed coronary artery disease and that followed these subjects for six years has demonstrated that individuals with adiponectin concentrations in the highest quintile compared with the lowest quintile had a decreased risk for myocardial infarction, suggesting

a protective effect of elevated serum adiponectin levels [16].

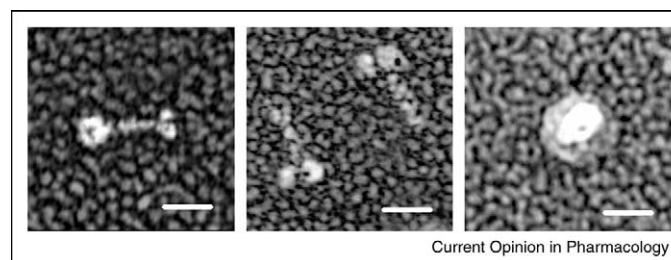
Several conditions are associated with elevated adiponectin levels, and might indicate further the role of adipokines in metabolic and inflammatory processes. Patients with chronic renal failure have increased markers for inflammation, including elevated C-reactive protein and markers for endothelial activation. These patients also have elevated adiponectin levels, all the more notable given the concordance of diabetes and renal failure [17]. This is thought to represent a compensatory protective increase in adiponectin levels, although it is not known whether the mechanism by which this occurs involves increased adiponectin synthesis or reduced clearance [18].

A mouse model in which the collagen domain of adiponectin is truncated and overexpressed *in vivo* under the control of an adipocyte-specific promoter has led, in one founder line, to an approximately threefold increase in serum levels of adiponectin, as well as abnormal fat distribution including proptosis and increased interscapular fat pads in female but not in male mice [19]. The female mice were insulin sensitive and displayed lower than normal serum triglycerides and free fatty acids, compared to male mice. Hinting at an association with thyroid homeostasis, the proptosis in these animals is reminiscent of the clinical features of patients with Graves disease; such patients have elevated adiponectin levels and increased orbital adipogenesis [20,21].

In vitro activities of adiponectin

The *in vitro* biochemical activities of adiponectin depend upon the structure and the oligomeric state of the hormone, and also might be influenced by the method of production and purification of adiponectin. Fruebis *et al.* [4] used unfractionated bacterial-expressed full-length and globular domain (produced by cleavage at residue 104 by trypsin) adiponectin to demonstrate an increased β -oxidation of fatty acids in differentiated myotubes, an effect that was greatest with the globular domain. Using

Figure 2



Recombinant adiponectin purified from 293T cells was fractionated into each of the three oligomeric species and visualized in the electron microscope by freeze-etch rotary shadowing. Shown are the trimer (left panel), hexamer (middle panel) and HMW (right panel) oligomers. Bar, 14.2 nm. Experimental details are as described in [3].

bacterial-expressed full-length and globular domain adiponectin, and eukaryotic-expressed full-length adiponectin, Tsao *et al.* [22] demonstrated that different molecular weight pools were present, corresponding to the trimer and hexamer species observed in serum. Notably, however, only eukaryotic-derived material was capable of forming the HMW species found in native mouse serum; bacterial-derived adiponectin only formed the trimeric and hexameric species. One explanation for this result is that the collagen domain does not fold properly in prokaryotic-expressed proteins; alternatively, other post-translational modifications, such as glycosylation, might be required for proper structure formation and oligomerization [23,24,25].

The cellular activation of adiponectin signaling pathways has been explored by adding purified and size-fractionated recombinant adiponectin to tissue culture cells [22]. Using a series of reporter constructs, in which transcriptional control elements direct the expression of luciferase, we determined that both the hexamer and the HMW adiponectin species activated nuclear factor (NF)- κ B-mediated gene expression driven from the E-selectin promoter. This effect was seen using both bacterial- and eukaryotic-derived adiponectin. In this assay, bacterial-expressed globular and trimer preparations derived from both eukaryotic and bacterial sources were inactive. Other researchers have demonstrated that adiponectin modulates the NF- κ B signaling pathway, albeit using different methods and obtaining different magnitudes of response. By pre-treatment of tissue culture cells with adiponectin followed by stimulation with TNF- α , Matsuzawa and co-workers [26] demonstrated that bacterial-derived adiponectin inhibited subsequent NF- κ B activation. The discordance of these results might, in part, be explained by the differences in cells and experimental techniques. Other activities of the HMW isoforms include inhibition of endothelial apoptosis [27]; however, the bacterial-derived preparations used in these experiments might not contain native HMW forms.

A major signaling pathway that is activated by full-length trimeric adiponectin as well as by the isolated globular domain is that of the AMP-activated protein kinase (AMPK) [3,28,29]. This enzyme is activated in response to many types of cellular stress, in particular to elevated ratios of AMP to ATP, such as those that occur during muscle contraction and increased metabolic demand (reviewed in [30–32]). Activation of AMPK leads to alterations in downstream pathways that are involved in metabolism and energy utilization, with increased activation of ATP-generating pathways and the concomitant inhibition of ATP-consuming pathways. Multiple downstream pathways are activated; however, only two have been well characterized in the context of cardiovascular disease. These are the increase in mitochondrial β -oxidation, which is regulated by the phosphorylation

and inactivation of acetyl CoA carboxylase, and the phosphorylation and activation of malonyl CoA decarboxylase, which results in the reduction of malonyl CoA levels. The end result is an increase in the β -oxidation of fatty acids leading to the replenishment of ATP levels. The finding that metformin, an anti-hypoglycemic agent, acts through this enzyme system suggests that AMPK is intimately related to diabetes and the metabolic syndrome [33].

***In vivo* activities of adiponectin**

However, the association of endothelial dysfunction with the metabolic syndrome, as well as with low levels of adiponectin [34], raises the possibility that adiponectin could have either direct or indirect actions on the vascular endothelium, and that these actions might be mediated by AMPK or NF- κ B signaling. Quon and co-workers have shown that both insulin [35] and adiponectin [36[•]] stimulate nitric oxide production in aortic endothelial cells. This effect is hypothesized to lead to vasodilatation, increased blood flow and increased glucose disposal. The effects of adiponectin are blocked by inhibition of phosphoinositide 3-kinase and by a dominant-negative AMPK mutant [36[•]], implying that adiponectin might synergize with insulin in the endothelium as it does in the liver [37^{••}].

The disruption of the gene for adiponectin has yielded slightly varying results, with similar phenotypes being seen by two groups. A third group [38] found that a deletion of the adiponectin gene increased β -oxidation but had no other metabolic effects. Kubota *et al.* [39] observed moderate insulin resistance in mice that were heterozygous for the adiponectin gene; this effect was more severe in mice lacking both copies of the gene. These two types of mice had similar body weights to wild-type mice. When the femoral artery in homozygous mice was injured with a balloon cuff, there was a twofold increase in neointimal thickening. This result suggests a role for adiponectin in maintaining the integrity of the vessel wall [40].

A similar mouse strain was generated by Maeda *et al.* [41]. In this study, homozygous adiponectin-deficient mice were not hyperglycemic when maintained on a normal diet; however, they did have reduced clearance of serum free fatty acids, and when supported on a high-fat, high-sucrose diet they exhibited severe insulin resistance and increased weight gain relative to control animals. TNF- α was increased in serum and in adipose tissue of the homozygous mice at baseline, and there was reduced skeletal muscle expression of fatty acid transport protein-1 (FATP-1), a transmembrane protein involved in the cellular uptake of serum fatty acids [42,43]. These biochemical alterations, as well as the abnormal neointimal proliferation following vascular injury, were corrected by infecting the mice with an adenoviral vector expres-

sing adiponectin [44]. These data suggest that adiponectin and TNF- α act in counter-regulatory pathways and that the net balance of their actions results in the proper homeostasis of glucose and fatty acid metabolism. Under conditions of metabolic stress, such as diet-induced obesity in adiponectin-deficient mice, unopposed TNF- α activity might lead to a shift towards insulin resistance. Recently, Wong *et al.* have described paralogs of adiponectin that might function in metabolic regulation, and might partially compensate for the loss of adiponectin in the knockout mice [45 \bullet].

Receptors and binding proteins for adiponectin

Receptors for many of the adipokines are well characterized, yet identifying the adiponectin receptor has proven elusive. Binding of adiponectin to collagens has been described *in vitro* [46]; binding to endothelial cells was first described by Ouchi *et al.* [26]. Several confounding factors influence studies of full-length adiponectin. For example, the collagen domain undergoes prolyl-hydroxylation, common among this protein structure. This reaction, important in maintaining the integrity of collagen fibrils and possibly affecting the tertiary structure of collagen domains, does not occur in bacteria, the source of the recombinant adiponectin used in many studies. Mutation of conserved lysine residues that undergo glycosylation reduces the ability of adiponectin to inhibit hepatic glucose production [24], consistent with the inability of bacterial-produced full-length protein to inhibit glucose secretion in isolated hepatocytes, an activity that was seen for the full-length eukaryotic-produced protein [37].

The situation with the globular domain of the protein is different; no post-translational modifications have been described and several groups have demonstrated the activation of AMPK [28,29]. Recently, putative receptors termed AdipoR1 and AdipoR2 have been described [47 \bullet]. These proteins, distantly related to the seven-transmembrane spanning receptor family, were expression-cloned using FACS enrichment of Ba/F3 cells infected with a skeletal muscle cDNA retroviral library. The ligand, bacterial-expressed biotinylated globular adiponectin, was bound to infected cells and then detected with multiple fluorescent labels. Unusual aspects of the AdipoR1 and AdipoR2 molecules include an inverted membrane topology with an intracellular N-terminus, which is different from other seven-transmembrane spanning receptors, and a small extracellular C-terminal domain of approximately 25 amino acids [47 \bullet]. Another seven-transmembrane spanning receptor, the follicle-stimulating hormone receptor, binds its glycoprotein hormone ligand, follicle-stimulating hormone, through two 330 residue extracellular domains [48]. Each of these domains is almost the size of the entire AdipoR1 (375 amino acids) or AdipoR2 (311 amino acids). There is precedent for

small receptors to bind to protein hormones, such as the TNF- α superfamily member ligand TNF-like weak inducer of apoptosis (TWEAK) and its receptor, the 102 amino acid TWEAK-R [49]. No confirmation of adiponectin binding to AdipoR1 and AdipoR2 has been reported; however, there might be other molecules that are capable of binding to adiponectin.

We used expression cloning on immobilized eukaryotic-produced adiponectin of BaF3 cells that were transduced with a retroviral cDNA expression library derived from undifferentiated C2C12 myocytes. This identified the glycosylphosphatidylinositol-linked cell-surface molecule T-cadherin ('truncated'; also known as H-cadherin and CDH13) to be a receptor for the hexameric and HMW forms of eukaryotic-produced adiponectin [50 \bullet]. Neither the trimeric globular nor the bacterial-produced adiponectin bound to T-cadherin, suggesting that there might be another signaling receptor that is as yet unidentified. T-cadherin is expressed in the intima of the vascular endothelium as well as in heart, muscle and the nervous system [51,52] at sites that position it to interact with adiponectin. Interestingly, T-cadherin is upregulated in models of intimal damage and neointimal formation, suggesting a role in controlling cellular proliferation [53], reminiscent of the findings in adiponectin-knockout animals.

How might T-cadherin and adiponectin function in cardiovascular physiology and pathology? First, T-cadherin might act as a storage depot for adiponectin. As the two molecules are present in the same tissue compartments, such an activity might regulate the levels of adiponectin in the serum, maintaining high levels within the luminal side of the vasculature, leading to increased adiponectin activity. An alternative role of T-cadherin might be to sequester adiponectin. As T-cadherin preferentially binds to the hexamer and HMW forms of adiponectin, such binding might reduce the circulating levels of these isoforms. Over-expression of T-cadherin might then lead to progressive hyperglycemia and worsening atherosclerosis, whereas deletion of T-cadherin might lead to increased insulin sensitivity by promoting increased adiponectin serum concentration. A third possibility is that T-cadherin acts as a co-receptor to present adiponectin to a signaling receptor, possibly in conjunction with a reduction or cleavage event to generate trimeric or globular domain adiponectin from hexameric and HMW forms, or by itself acting as a receptor to signal directly to downstream pathways. In this case, overexpression of T-cadherin might lead to increased insulin sensitivity and endothelial protection, whereas a reduction in the levels of T-cadherin might lead to diabetes and vascular disease. Although there is a dearth of information concerning the *in vivo* activity of T-cadherin, expression of T-cadherin is increased in the non-atherogenic internal mammary artery compared with the atherogenic-prone

coronary artery [54] and might protect the endothelium from damage through association with adiponectin.

Conclusions

Adiponectin, a recently described adipokine, is involved in the control of metabolism and the regulation of the cardiovascular endothelium, in which it might play a central role in the progression of cardiovascular disease. Thus, it is increasingly recognized as a biomarker for cardiovascular disease and a possible therapeutic target. However, the mechanisms by which adiponectin transmits biological signals are not fully understood and will be the focus of further investigation. Future research will clarify the role of several proposed adiponectin receptors and could possibly generate novel therapeutic targets for cardiovascular disease.

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