Adipokines protecting CKD

Satoshi Miyamoto and Kumar Sharma

Correspondence and offprint requests to: Kumar Sharma; E-mail: kumarsharma@ucsd.edu

ABSTRACT

Increasing incidence of chronic kidney disease (CKD) which leads to end-stage renal disease (ESRD) is one of the major health issues in the modern world and requires novel strategies for treatment. Adipose tissue has been recognized to have endocrine function and secretes a variety of hormones called adipokines. Several adipokines have been implicated in the pathogenesis of CKD and may have a strong impact as a risk factor for renal decline. The aim of this review is to provide an overview of the role of adipokines in the progression of CKD, with focus on recent experimental and clinical advances.

INTRODUCTION

With the increasing number of diabetic and hypertensive patients, the frequency of chronic kidney disease (CKD) and end-stage renal disease (ESRD) continues to increase worldwide [1]. The estimated prevalence rate of CKD in the population in the USA and around the world is approaching 10–13% [2]. On the other hand, the presence of CKD is associated with an increase in hypertension (HTN), diabetes and cardiovascular disease (CVD) [2]. Obesity is a common risk factor in both diabetes and HTN, which also leads to the development of CKD. Recent studies in obesity research have shed light on the recognition that adipose tissue is an active organ with endocrine function. Adipose tissue produces a variety of hormones called adipokines that exert their bioactive actions systemically. The present review focuses on the effect of major adipokines, with particular attention on the roles of adiponectin, apelin, omentin, leptin, visfatin and resistin in the progression of CKD.

ADIPONECTIN

Adiponectin in clinical studies

Adiponectin is a 30-kDa (Acro30) protein predominantly produced in adipose tissue and circulates in plasma as a trimer (low-molecular weight; LMW), a hexamer generated from two trimers or a multimer consisting of 12–18 hexamers existing in a bouquet-like structure (high-molecular weight, HMW). Levels of adiponectin are decreased in obesity, coronary artery disease (CAD) and type 2 diabetes mellitus [3]. In addition, low adiponectin levels also correlate with albuminuria in obese African-American subjects [4]. In patients with CKD, adiponectin is inversely correlated with renal function and BMI, and positively correlated with albuminuria or proteinuria [5, 6]. Furthermore, high adiponectin is a predictor of CKD progression in male patients with CKD [7].

It remains controversial whether serum adiponectin levels predict future cardiovascular risk factors in CKD subjects. There are many reports indicating that high adiponectin levels are associated with less CVD risk factors [8–12]. Becker et al. [8] reported that lower plasma adiponectin levels were correlated with both past history of CVD events and new CVD events during the prospective observation period in patients with mild and moderate renal failure. Zoccali et al. [9] also showed that lower adiponectin levels were an inverse predictor of fatal and non-fatal CVD events but not for all-cause mortality among hemodialysis patients. In contrast, several studies suggested a high rather than low adiponectin level to be associated with increased CVD and all-cause mortality and a faster decline of renal function [13, 14]. Tsai et al. [15] reported the lack of relationship between adiponectin levels and CVD outcomes in hemodialysis patients. These discrepancies may result from differences in the populations of subjects, non-linear correlation between serum adiponectin and cardiovascular event [16], proportion of HMW to total adiponectin [17], differences...
in genetic background [18] and different etiologies of kidney disease [19].

The association between HTN and low adiponectin has been reported. Adamczak et al. [20] reported the negative association between blood pressure and plasma adiponectin concentration in subjects with normal kidney function. Chow et al. [21] also showed a negative correlation between serum adiponectin level and future development of HTN in their 5-year prospective study of Chinese subjects, independent of the effects of known risk factors of HTN including age, sex and baseline BMI. The pathways involved in the regulatory mechanisms of adiponectin in HTN have recently been elegantly reviewed elsewhere [22].

Adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) are the two major receptors for adiponectin. AdipoR1 is abundantly expressed in skeletal muscle and AdipoR2 is predominantly expressed in the liver [23]. AdipoR1 expression in mouse kidneys and podocytes is of a similar degree as in the liver, whereas AdipoR2 expression in kidney and podocytes is much less than in the liver [4]. In humans, there are some differences in adiponectin, AdipoR1 and AdipoR2 expression between non-diabetic and diabetic ESRD subjects. In non-diabetic ESRD subjects, but not diabetic ESRD subjects, adiponectin mRNA expression is upregulated in both visceral and subcutaneous fat tissue compared with healthy subjects [24]. This difference between non-diabetic ESRD and diabetic ESRD subjects may result from the fact that low adiponectin levels are associated with type 2 diabetes mellitus. In both non-diabetic and diabetic ESRD patients, AdipoR1 expression is increased in various tissues including adipose tissue and peripheral blood mononuclear cells compared with healthy subjects [24, 25]. In non-diabetic ESRD subjects, but not diabetic ESRD subjects, AdipoR2 expression in peripheral blood mononuclear cells is increased compared with healthy subjects [24, 25].

Adiponectin binds to these two receptors and signal via stimulation of 5’-AMP-activated protein kinase (AMPK) and potentially other intracellular pathways [26]. AMPK is a stress-activated kinase that is activated in response to depleting ATP or mounting intracellular AMP levels, and AMPK plays a central role in the effects of adiponectin. Kobayashi et al. [17] reported that only HMW adiponectin was suppressed in patients with CAD compared with normal control, and that the protective effect of adiponectin against apoptosis of vascular endothelial cells via activation of AMPK is exerted only by HMW adiponectin. This suggests HMW adiponectin is of importance in the adiponectin-AMPK pathway. In addition, purified HMW adiponectin has potential anti-inflammatory properties via NF-κB inhibition in endothelial cells [27]. Thiazolidinedione (TZD), a peroxisome proliferator-activated receptor γ (PPARγ) agonist, can strongly upregulate HMW adiponectin compared with total adiponectin or LMW adiponectin, and improvements of insulin sensitivity by TZD are closely correlated with increased levels of serum HMW adiponectin [28]. In addition, angiotensin type-1 receptor blockers (ARBs) can also increase circulating HMW adiponectin levels, and this may be mediated via PPARγ [29, 30]. Although there is not enough evidence to clearly demonstrate whether angiotensin-converting enzyme (ACE) inhibitors can increase serum HMW adiponectin, ACE inhibitors have been demonstrated to activate PPARγ and increase serum total adiponectin [31–33]. In ESRD patients, the percentage of HMW adiponectin, but not trimer or hexamer form, to total adiponectin was also correlated with future ischemic heart disease (IHD) events [34].

Adiponectin is also detected in human urine samples as an LMW form (monomer, dimer or trimer) [35]. Urinary adiponectin level is increased in patients with overt diabetic nephropathy compared with healthy subjects or diabetic patients with normo- or microalbuminuria [36]. Among type 2 diabetic patients, urinary adiponectin concentration is also correlated with common carotid artery intimamedia thickness, a strong risk factor of CVD [34]. In diabetic patients with macroalbuminuria, urinary adiponectin levels are positively correlated with urinary albumin excretion levels, but not in diabetic patients with normoalbuminuria and microalbuminuria [36]. Von Eynatten et al. [35] demonstrated that adiponectin was distributed in the glomerular and intratubular capillaries, and that the glomerular expression of adiponectin in diabetic patients was reduced compared with healthy subjects. As the positive adiponectin staining of tubular casts is observed in patients with diabetic nephropathy [35], the increased urinary adiponectin may result from the increased urinary excretion of adiponectin from glomerular capillaries; however, the exact basis is unclear.

These studies indicate, low circulating adiponectin, especially low circulating HMW adiponectin, is a strong risk factor for CVD, HTN and albuminuria. On the other hand, in patients with CKD, the association between high adiponectinemia and progression of CKD has been reported as ‘reverse causality’. With the deterioration of the eGFR, increase in albuminuria or both, the mortality risk in CKD patients increases steadily [37]. Although treatment with TZD causes a slight decrease in the GFR in patients with CKD, the beneficial effect on CVD events exceeds the reduction in the GFR. [38]. Therefore, the activation of the adiponectin-AMPK pathway may also be one of the important targets for the treatment of CKD patients.

Animal studies

We previously used adiponectin knockout mice to verify the protective roles of adiponectin in the progression of diabetic nephropathy. These mice have normal glucose tolerance, insulin sensitivity, lipid profiles, blood pressure and body weight when fed a regular rodent diet [39, 40]; however, albuminuria was twice as high as that of wild-type (C57BL/6) mice at one month of age, and worsened with age. In adiponectin-knockout mice, when compared with diabetic wild-type mice, albuminuria was significantly increased within 2 months of diabetes induction and exhibited a progressive increase at 4 months after diabetes induction. Elevated oxidant stress in non-diabetic or diabetic adiponectin-knockout mice, as measured by urinary hydrogen peroxide levels, was normalized with 5-aminoimidazole-4-carboxamide-1-β-d-ribonucleoside (AICAR), a specific activator of AMPK, or adiponectin treatment. The segmental fusion of the podocyte foot...
under high-glucose conditions, and AMPK activation by TGF-β is a ubiquitously expressed transcription factor that degrades mesangial cells and enhances albuminuria in a paracrine manner. Increased MCP-1 production may contribute to the subcellular processes of podocytes, and increases Nox4 to a similar degree as treatment with recombinant adiponectin.

In our study, the increased macrophage infiltration in the kidney of HFD mice was prevented by AICAR administration. At 12 weeks after high-fat exposure, although the significant increase in adiponectin level was no longer observed, renal AMPK activity and plasma adiponectin level were decreased within 1 week of high-fat exposure, and associated with increased urinary MCP-1 and urine hydrogen peroxide level. Elevated urinary MCP-1 and hydrogen peroxide in HFD mice were ameliorated by AICAR administration. Low-grade inflammation via macrophage infiltration into the kidney may be involved in the pathogenesis of CKD, especially in the progression of diabetic nephropathy [42, 43].

In our previous study, we also observed linear distribution of ZO-1 in podocytes with normal glucose exposure, which was further enhanced by treatment with recombinant adiponectin and markedly reduced with high glucose exposure or inhibition of AMPK. In animal models of both type 1 and type 2 diabetes, ZO-1 expression is suppressed and associated with redistribution of ZO-1 from the podocyte membrane to the cytoplasm [49]. Together with fenestrated endothelium and glomerular basement membrane (GBM), podocytes form the glomerular filtration barrier in the glomerulus. Podocytes are terminally differentiated cells and have branching foot processes that connect the podocyte to the GBM via integrins and dystroglycans [46]. The process of podocyte effacement is associated with foot process widening and effacement is associated with degree of albuminuria or proteinuria [47]. Foot process effacement has to be initiated at the actin cytoskeleton of the podocyte and results in the alteration of the cell-cell contacts at the slit diaphragm and in a mobilization of the cell-matrix contacts [46]. The slit diaphragm contains specific transmembrane proteins including nephrin, podocin, FAT, P-cadherin and NEPH1-3. The cytoplasmic components of these transmembrane proteins bind to adaptor proteins such as CD2-associating protein, synaptopodin, α-actinin, zona occludens-1 and MAGI-1/2 that attach to the actin-associated proteins and the actin network [48]. ZO-1 is a 225-kDa protein that is located along the cytoplasmic surfaces of the slit diaphragms. ZO-1 links slit diaphragm proteins through its PDZ domains to the actin cytoskeleton. In animal models of both type 1 and type 2 diabetes, ZO-1 expression is suppressed and associated with redistribution of ZO-1 from the podocyte membrane to the cytoplasm [49].

AMPK activity in cultured podocytes was decreased under a high-glucose condition and restored by AICAR or adiponectin. Treatment with adiponectin increases p-AMPK and decreases Nox4 in podocytes to a similar degree as treatment with AICAR. Our results indicate that adiponectin plays a protective role to reduce albuminuria by directly affecting podocyte function via the AMPK-Nox4 pathway (Figure 1). Consistent with our findings, Eid et al. [50] reported that podocyte apoptosis with diabetes was mediated by inactivation of AMPK, upregulation of Nox4 and an increase in NADPH oxidase-mediated ROS production. They demonstrated that inactivation of AMPK by high glucose stimulation upregulated the expression and phosphorylation of p53, and...
downstream from Nox4. A recent study demonstrated that p-AMPK was activated by hydrogen peroxide in HEK 293 cells and podocytes [51, 52]. However, in these studies, hydrogen peroxide was used at a much higher concentration (100–300 μM) than the urine concentration of diabetic animals. Therefore, in our study, activation of p-AMPK by hydrogen peroxide does not appear to be a feature in the diabetic kidney.

The adiponectin–AMPK pathway plays a crucial role in both the maintenance of podocyte function and the inhibition of ROS in the diabetic kidney.

**APELIN**

In 1998, apelin was first isolated from bovine stomach extracts as an endogenous ligand of the orphan G-protein-coupled receptor, called APJ [53]. Apelin is derived from a prepropeptide (preproapelin) consisting of 77 amino acid residues, and which is cleaved and secreted as apelin-36, -17 and -13 [53]. Of these peptides, apelin-13 has the strongest biological activities [53]. Although the APJ receptor has ~30% similarities to the angiotensin II receptor in its amino acid sequences, apelin and angiotensin II do not exert its activity in the cells expressing the APJ receptor [53]. Apelin has been recognized as an adipokine that is secreted from white adipose tissues and upregulated by insulin and obesity [54]. Apelin expression in both human and mouse adipose tissue is positively regulated by TNF-α, and injection of TNF-α in mice induces increased both apelin expression in adipose tissue and blood plasma levels of apelin [55].

Apelin is also highly expressed in the kidney tissues as well as heart, ileum, uterus and ovary [56]. 
Małyszko et al. [57] reported that the plasma apelin level was significantly lower in hemodialyzed patients with CAD compared with patients without CAD, and it was associated with cardiac function. In the kidney, APJ mRNA expression was very high in glomerulus, but less in the other nephron segments such as proximal convoluted tubule, thick ascending limb from the cortex and collecting duct from the inner medulla [58]. In glomerulus, APJ mRNA was expressed in the vascular wall of arterioles, both in endothelial and vascular smooth muscle cells [58]. Hus-Citharel et al. [58] reported that apelin administration caused not only vasorelaxation in angiotensin II-preconstricted efferent and afferent arterioles but also exerted a vasoconstrictive effect in vascular smooth muscle.

Day et al. [59] recently demonstrated the role of apelin in the progression of diabetic nephropathy using a mice model of type 1 diabetes. They showed that both renal APJ expression...
and renal apelin-13 levels were reduced in type 1 diabetic mice
but increased after treatment with apelin-13. Although en-
hanced albuminuria in diabetic mice was significantly inhib-
ited by apelin-13 treatment, the podocyte number in glomeruli
was not restored by apelin-13 administration. On the other
hand, they found that the expression of megalin, involved in
the reabsorption of the albumin in the proximal tubule, was
suppressed in the diabetic kidney and was restored by adminis-
tration of apelin-13. In addition, glomerular hypertrophy as
well as renal inflammation, including MCP-1 and vascular cel-
lar adhesion molecule-1 (VCAM-1) expression, NF-κB activ-
ation and monocyte infiltration, was inhibited by treatment
with apelin-13. Interestingly, apelin-13 reversed not only activ-
ation of NF-κB, but also its binding to the VCAM-1 promoter.

It is noteworthy that apelin exerts its biological activity via
AMPK and its downstream target acetyl CoA carboxylase
(ACC) in adipose tissue and increases glucose uptake [60].
Similarly, apelin treatment increases fatty acid oxidation, mito-
chondrial respiratory capacity and mitochondrial biogenesis
via activation of AMPK in skeletal muscle of insulin-resistant
mice [61]. In kidney tissues, there was no report about AMPK
activity by apelin treatment.

Apelin may have the potential to inhibit the progression of
diabetic nephropathy mainly via suppression of renal inflam-

**OMENTIN**

Omentin is a recently discovered adipokine with 313 amino
acids and primarily expressed in visceral adipose tissue [62, 63].
The two omentin genes, omentin-1 and omentin-2, are localized
adjacent to each other in the 1q22-q23, which has been linked to
type 2 diabetes in several populations [62]. Omentin-1 is abun-
dantly expressed in visceral adipose tissue; however, omentin-2 is
expressed in considerably lower levels in visceral adipose
tissues and in higher levels in the intestine [64]. In adipose
tissue, omentin is produced not by adipocytes but by stromal
vascular cells [62]. Omentin increases insulin-induced glucose
uptake in human adipocytes through enhanced Akt phosphoryl-
ation [62]. In humans, circulating omentin-1 level is negatively
correlated with BMI, waist-to-hip ratio, glucose, HOMA-IR and
high-sensitivity CRP (hsCRP) [65], and positively correlated
with plasma adiponectin levels [64]. Furthermore, omentin sup-
presses NF-κB activity in human microvascular endothelial cells
cultured with serum of normal subjects, serum of polycystic
ovary syndrome (PCOS) subjects, TNF-α or CRP [65]. Systemic
delivery of an adenoviral vector expressing omentin enhances
blood flow recovery and capillary density in ischemic limbs of
mice [66]. In cultured human umbilical vein endothelial cells,
administration of omentin enhances the phosphorylation of
AMPK and ACC followed by activation of the Akt-eNOS signal-
ing pathway, and increases differentiation into vascular-like
structures and decreases apoptotic activity under conditions of
serum starvation [66]. Metformin treatment significantly in-
creases serum omentin-1 levels in patients with PCOS, and the
change in omentin-1 levels are negatively associated with hsCRP
levels [65]. In patients with ESRD receiving hemodialysis,
plasma levels of omentin are ∼1.7 times higher than those of
healthy subjects [67].

Although there is no evidence for the effect of omentin in
the progression of CKD, it might play a protective role via activ-
ation of AMPK or its anti-inflammatory properties.

**LEPTIN, VISFATIN AND RESISTIN**

Leptin is an adipocyte-derived hormone that acts as a major
regulator for food intake and energy homeostasis. In normal
Wistar rats, short-term (3 days) leptin infusion shows no effect
on urinary protein excretion, whereas TGF-β1 mRNA in gloi-
meruli is upregulated and the number of proliferating cells is
increased by leptin treatment [68]. When used long term (21
days), the difference in glomerular TGF-β1 mRNA expression
is no longer observed, however, urinary protein excretion and
type IV collagen accumulation are increased in the leptin-
treated group [68]. Gunduz et al. [69] also reported that long-
term (28 days) continuous leptin infusion induced enhanced
TGF-β expression in renal tubular cells, but not in glomeruli.
In mesangial cells, leptin increases the TGF-β type II receptor
and type I collagen [70].

Visfatin is a 52-kDa adipokine that has been implicated in
the regulation of insulin receptor activity and insulin sensi-
tivity. While visfatin is mainly produced by visceral adipose
tissue, visfatin synthesis is also observed in murine podocytes,
murine proximal tubular cells and mesangial cells obtained
from rats, and is increased by high glucose exposure [71, 72].
In these renal cells, administration of visfatin induces in-
creased profibrotic and proinflammatory molecules, such as
TGF-β1, PAI-1 and type I collagen [71, 72].

Resistin was first discovered in mice in 2001, and associated
with insulin resistance, endothelial damage and inflammation
[73]. In patients with CKD, serum resistin levels are negatively
associated with GFR and positively associated with inflamma-
tory biomarkers such as hsCRP, IL-6, intracellular adhesion
molecule-1 (ICAM-1), VCAM-1 and TNF-α [74]. In cultured
human aortic endothelial cells, resistin is also able to induce
expression of ICAM-1 and VCAM-1, and interestingly this
effect was prevented by administration of adiponectin [75].

It is likely that leptin, resistin and visfatin have the potential
to exacerbate CKD.

**CONCLUDING REMARKS**

The worldwide epidemic of both CKD and obesity has in-
creased the urgency to develop a further understanding of the
physiological role of adipokines in the kidney. Over the years,
accumulating evidence in CKD research suggests that adipoi-
nectin plays a protective role via a variety of pathways in the
pathogenesis of CKD. It is likely that apelin also has the poten-
tial to prevent the progression of CKD. Interestingly, the
potentially beneficial adipokines (adiponectin, apelin and
omentin) all share the ability to increase AMPK. In the
current treatment of diabetic nephropathy, TZDs possess the
ability to increase serum adiponectin level via PPARγ, and
increased adiponectin level is significantly and independently correlated with the reduction of albuminuria [76]. Similarly, ACE inhibitors and ARBs can also raise adiponectin levels [30, 33]. In addition to current treatment approaches for CKD [77], further research in the adipokine-kidney network will lead to novel strategies for the treatment of CKD in the future.

ACKNOWLEDGEMENTS

Our projects are supported by grants from a VA MERIT Award (K.S.) and NIDDK (U01 DK060995, DP3 DK094352-01 and DK083142) awards to K.S., and JSPS international Training Program (ITP) and the Uehara Memorial Foundation awards to S.M.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

55. Daviaud D, Boucher J, Gesta S et al. TNFalpha up-regulates apelin expression in human and mouse adipose tissue. FASEB J 2006; 20: 1528–1530
64. de Souza Batista CM, Yang RZ, Lee MJ et al. Omentin plasma levels and gene expression are decreased in obesity. Diabetes 2007; 56: 1655–1661
Lipid mediators of inflammation in obesity-related glomerulopathy

Eileen Nolan1,2,3, Yvonne M. O’Meara2,3 and Catherine Godson1,2

Correspondence and offprint requests to: Eileen Nolan; E-mail: eileen.nolan.3@ucdconnect.ie

ABSTRACT

The interplay between chronic kidney disease (CKD) and obesity represents the convergence of two of the most common contemporary clinical issues, and is of particular interest and significance in the context of the burden presented by each at present, and the dismal projections associated with both of these conditions for the future. That obesity leads to CKD through its association with other risks, such as hypertension, type 2 diabetes mellitus and atherosclerosis, is well established; however, it is likely that obesity itself is an independent risk factor for the development of CKD. The