Adiponectin

Adiponectin is a protein hormone excreted into the bloodstream by fatty tissue and it plays a role in the suppression of inflammation-associated metabolic disorders that may result in type 2 diabetes, obesity, and atherosclerosis. In the *Journal of Clinical Investigation*, Rosario Scalia and colleagues from Thomas Jefferson University studied the vascular protective actions of adiponectin in mice. The authors found that adiponectin-deficient (Ad-/-) mice possessed high levels of nitric oxide and a 5-fold increase in the adhesion of leukocytes to the blood vessel wall. This effect could be blocked and reversed by the addition of the recombinant globular adiponectin domain (gAd). Importantly, prior administration of gAd was also shown to protect healthy mice against the induction of leukocyte-endothelium interactions. The study demonstrates a clear role for normal levels of adiponectin in the regulation of leukocyte-endothelium interactions in mice. The data also suggest that gAd may serve as a potential pharmacological treatment of abnormal endothelial dysfunction occurring in pathological conditions associated with adiponectin deficiency.

Low levels of serum adiponectin are associated with increased risk and aggressiveness of obesity-related cancer. The purpose of the study reported here was to investigate the association between serum adiponectin levels and clinicopathological parameters of renal cell carcinoma.

There were no statistically significant associations between total adiponectin and HMW adiponectin and pathological stage, regional lymph node involvement, histological grade, histological type (clear cell carcinoma versus other types) or presence of venous invasion. Total and HMW adiponectin levels in patients with metastasis, however, were significantly lower than in patients without metastasis ($P = 0.044$ for total adiponectin and $P = 0.041$ for HMW adiponectin). Low total and HMW adiponectin levels were significantly associated with metastasis in patients with a normal BMI

(<25 kg/m^2) (P = 0.034 for total adiponectin and P = 0.028 for HMW adiponectin) but not in overweight and obese patients (P = 0.652 for total adiponectin and P = 0.489 for HMW adiponectin). Multivariate logistic regression analysis showed that total adiponectin level was an independent predictor of metastasis of renal cell carcinoma in all patients (P = 0.024, 95% CI = 1.031–1.560) and in patients with a normal BMI (P = 0.040, 95% CI = 1.043–6.534). Conclusions: Serum total and HMW adiponectin levels were decreased in patients with metastatic renal cell carcinoma. Adiponectin might be a molecular link between obesity and the progression of renal cell carcinoma.

The prevalence of obesity has increased dramatically in recent years and become a serious problem. Obesity is a significant risk factor not only for the development of cardiovascular disorder and diabetes but also for carcinogenesis. Between 2 and 3% of all adult cancers are renal cell carcinoma and obesity is a well-known risk factor for the development of renal cell carcinoma. The mechanism underlying this relationship remains to be elucidated.

Adipose tissue has been shown to be not only a site of energy storage but also an important endocrine organ that produces a variety of cytokines. One of them, adiponectin, has recently been identified as an insulin sensitizer. Adiponectin has a variety of functions including anti-atherogenic, anti-diabetogenic and anti-inflammatory actions protecting against the development of obesity-related disorders such as cardiovascular disease and diabetes. Adiponectin exists in the circulation in a wide range of multimer complexes and combines via its collagen domain to create three major oligomeric forms: a low-molecular-weight (LMW) trimer, a middle-molecular-weight (MMW) hexamer and high-molecular-weight (HMW) 12- to 18-mer adiponectins that are the most active forms of the protein. Serum adiponectin levels are reduced in obese humans, particularly those with visceral obesity, and are inversely correlated with insulin resistance.

Adiponectin has emerged as a possible link between obesity and cancer. It has been suggested that decreased levels of serum adiponectin are associated with an increased risk for obesity-related cancers such as colon, breast, endometrial and prostate cancer. Furthermore, serum adiponectin in patients with gastric cancer has been found to be inversely correlated with pathological findings such as tumor size, depth of invasion and tumor stage in patients with gastric cancer. Because renal cell carcinoma is a well-known obesity-related cancer, we thought that adiponectin might play an important role in the development or progression of renal cell carcinoma. The purposes of the study reported here were to evaluate serum levels of total adiponectin and HMW adiponectin in patients with renal cell carcinoma and to investigate the association between these levels and clinicopathological parameters.

Role of adiponectin in CHD not clear-cut

Contrary to what may have been expected, raised blood levels of adiponectin, the major protein secreted by fat cells, appear to indicate a higher risk of coronary heart disease (CHD) in older adults, a new study suggests.

Adiponectin has long been thought to be protective against both diabetes and CHD. It is known to be an insulin sensitizer, and clinical studies have confirmed animal and laboratory findings that it protects against the development of diabetes. But its role in CHD is more uncertain, and although raised levels of adiponectin are associated with leanness, better cholesterol fractions, and lower levels of blood glucose and inflammatory markers, studies of its relation to actual CHD events have produced contradictory results.

While a study published in the *Journal of the American Medical Association* in 2004 suggested a protective effect of adiponectin against CHD in initially healthy middle-aged men, this has not been easily replicated, with subsequent studies in patients with established heart disease suggesting an opposite association.

"When we first planned this study we thought high levels of adiponectin would be protective, but we actually found the opposite—that people with the highest levels had a higher risk of heart disease than those with lower levels. Two other studies have also recently been reported showing an association between higher levels of adiponectin and all-cause mortality and cardiovascular mortality. Our findings are in line with these studies and suggest that higher adiponectin levels do not necessarily signal a healthier population, as we initially thought."

The current nested case-control study focused on 3857 participants free of cardiovascular disease at the 1992-1993 examination in the **Cardiovascular Health Study**, a population-based longitudinal survey of community-dwelling adults aged 65 to 100. Cases consisted of 604 men and women who experienced a CHD event through June 2001. Of these events, 255 were nonfatal MI, 115 were fatal CHD, and the remainder nonfatal CHD. Controls were participants free of incident CHD who were matched to cases in terms of age, sex, race/ethnicity, subclinical disease status, and center.

Results showed that serum adiponectin concentration did not differ significantly among participants with and without baseline subclinical cardiovascular disease. As has been shown before, adiponectin exhibited significant negative correlations with baseline adiposity, insulin resistance, dyslipidemia, inflammatory markers, and leptin. However, after researchers controlled for matching factors, adjustment for waist/hip ratio, hypertension, smoking, alcohol, LDL cholesterol, creatinine, and leptin, individuals in the highest adiponectin quintile had a modestly increased risk of incident CHD than those in the lower four quintiles. This association was stronger when the outcome was limited to nonfatal MI and fatal CHD.

The findings were not influenced by additional adjustment for weight change, health status, or cystatin C or by adjustment for potential mediators such as diabetes, cholesterol, and inflammatory markers.

The authors write: "Our findings, which are based on the largest number of first incident CHD events in an elderly cohort to date, are at odds with prior reports of a protective association with CHD, whether overall or limited to nonfatal events. They also contrast with previous null results regarding fatal and nonfatal CHD, while failing to confirm a previously noted interaction by race."

These results confirm that the adiponectin story is more complicated than first thought. "This peptide does not seem to be associated with a straightforward protective effect, as we first thought it would be. There has been much enthusiasm about this hormone, resulting in many published studies, but each study just seems to complicate the picture more. While it does seem to have a beneficial effect in diabetes, its associations in CHD are all over the place. The best summary is perhaps that, in middle-aged individuals free of heart disease, adiponectin may be modestly protective against developing CHD, while in people already affected with heart disease and in older persons, it appears to be a marker of harm."

Adiponectin may have both beneficial and detrimental actions, some of which have not been elucidated yet. Discussing this in the paper, the authors say: "This leaves the possibility that, in addition to its salutary actions, adiponectin has direct harmful effects, which could be more operative in the elderly. Indeed, adiponectin has been shown to increase energy expenditure through direct actions in the central nervous system in mice, an effect that, if present in humans, could be particularly deleterious in older adults by potentially accelerating sarcopenia."

It was initially hoped that high levels of adiponectin would be protective against both diabetes and CHD. Then one could set about looking for drugs to increase it. But now we are realizing that we have to know more about the full range of actions of this hormone before we can think about manipulating it with drugs. Future research will focus on individual multimers of adiponectin as well as

more investigation into the effect of altered body composition, particularly in the elderly.

Adiponectin Reduces Plasma Triglyceride by Increasing VLDL Triglyceride Catabolism

Adiponectin is an adipocyte derived hormone that plays an important role in glucose and lipid metabolism. The main aims of this study are to investigate the effects of adiponectin on VLDL triglyceride (VLDL-TG) metabolism and the underlying mechanism.

Three days after Ad-mACRP30 adenovirus injection, plasma adiponectin protein levels were increased 12-fold. All three main multimeric adiponectin molecules were proportionally elevated. Fasting plasma TG levels were significantly decreased (~40%) in the mice with elevated adiponectin in circulation, as were the plasma levels of large and medium VLDL subclasses. Although apolipoprotein B mRNA levels were robustly suppressed in the livers of adiponectin-over expressing mice and in cultured HepG2 cells treated with recombinant human adiponectin, hepatic VLDL-TG secretion rates were not altered by elevated plasma adiponectin. However, Ad-mACRP30–treated mice exhibited a significant increase of post heparin plasma lipoprotein lipase (LPL) activity compared with mice that received control viral vector. Skeletal muscle LPL activity and mRNA levels of LPL and VLDL receptor (VLDLr) were also increased in Ad-mACRP30–treated mice. Recombinant human adiponectin treatment increased LPL and VLDLr mRNA levels in differentiated C1C12 myotubes. These results suggest that adiponectin decreases plasma TG levels by increasing skeletal muscle LPL and VLDLr expression and consequently VLDL-TG catabolism.

Linking adiponectin to proteinuria

Obesity and kidney disease

The obesity epidemic has been linked to rising incidences of type 2 diabetes, cardiovascular disease, nonalcoholic fatty liver disease, sleep apnea, and cancer. Studies also indicate that obesity increases the risk of kidney disease independently of diabetes and hypertension. Renal blood flow and glomerular filtration rate are elevated in obesity and are related to increased levels of the protein albumin in the urine (albuminuria). Epidemiological studies suggest that microalbuminuria, defined as a urine albumin/creatinine ratio of 30–300 μg/mg, increases cardiovascular morbidity. Furthermore, an albumin/creatinine ratio considered to be within the normal range (10–30 μg/mg) is associated with higher cardiovascular risk. Insulin resistance, oxidative stress, and inflammation have all been implicated in albuminuria and declining kidney function, but the underlying mechanisms are unclear.

Hypoadiponectinemia is related to albuminuria

In the current issue of the *JCI*, Sharma et al. describe a role of adiponectin in the pathogenesis of albuminuria. Adiponectin is secreted exclusively by adipocytes. The monomer is a 30-kDa protein with 3 distinct domains: N-terminal hyper variable region, collagenous stalk, and C-terminal globular domain. Adiponectin circulates in plasma as various complexes: high–molecular weight (HMW; 12- to 36-mer), low–molecular weight (hexamer), and trimeric forms. Total and HMW adiponectin levels are more abundant in females and decline in obesity. Low adiponectin levels are related to higher prevalence of type 2 diabetes, inflammation, and atherosclerosis and these abnormalities are reversed by adiponectin treatment. The insulin-sensitizing effect of thiazolidinediones is related to increased HMW adiponectin levels.

Previously, adiponectin receptors AdipoR1 and AdipoR2 — containing 7-transmembrane domains that are structurally and functionally distinct from G protein–coupled receptors — have been described. AdipoR1 is more widely expressed and enriched in muscle, while AdipoR2 is abundant in liver. Binding of adiponectin to

AdipoR1 and AdipoR2 increases AMPK activation and PPARα signaling, resulting in suppression of gluconeogenesis, stimulation of fatty acid oxidation, and amelioration of diabetes. AdipoR1- and AdipoR2-mediated signal transduction has been implicated in steatosis, inflammation, and oxidative stress, all key abnormalities associated with obesity and the metabolic syndrome.

Sharma et al. found that plasma adiponectin concentration was inversely related to urinary albumin excretion in obese African Americans, a group prone to obesity and chronic kidney disease. Conversely, BMI, blood pressure, lipid levels, and plasma levels of IL-6 and plasminogen activator inhibitor–1 (PAI-1) were not associated with albuminuria in this group. To establish a role of adiponectin in the pathogenesis of albuminuria, Sharma et al. compared wild-type mice with adiponectin-deficient mice. Blood pressure, glucose levels, and lipid levels were not affected in $Ad^{-/-}$ mice fed a regular rodent diet; however, albuminuria was significantly higher, worsened with age, and was exacerbated by diabetes. Hydrogen peroxide levels increased in the urine of $Ad^{-/-}$ mice, consistent with oxidative stress. Electron microscopic examination revealed segmental fusion of the feet processes of podocytes (interdigitated cells that closely invest the glomerular capillary network in the kidney, and act in part as a filter for large macromolecules) in $Ad^{-/-}$ mice, although the thickness of the glomerular basement membrane and the structures of endothelial and mesangial cells were not altered by adiponectin deficiency. Albumin permeability was increased in a monolayer culture of podocytes from $Ad^{-/-}$ mice, consistent with albuminuria in this model.

The authors showed that AdipoR1 was highly expressed in podocytes (7). AMPK phosphorylation was increased in podocytes by adiponectin or the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-d-ribonucleoside (AICAR; Figure 2A). The distribution of the tight junction protein zonula occludens–1 (ZO-1) was disrupted in podocytes from $Ad^{-/-}$ mice and restored by adiponectin or AICAR treatment. Although the involvement of AdipoR1 in albuminuria and how this is coupled to AMPK signaling requires further study, these results demonstrate major effects of adiponectin on the kidney.

Adiponectin decreases oxidative stress and albuminuria

To determine whether adiponectin is causally related to kidney disease, the authors assessed effects of adiponectin treatment on urine albumin and hydrogen peroxide excretion (7). These parameters were related to podocyte structure, activity of AMPK, and expression of oxidative enzymes. $Ad^{-/-}$ mice were treated with the full-length or globular forms of adiponectin. Restoration of plasma adiponectin to levels measured in wild-type mice blunted albuminuria in nondiabetic as well as diabetic $Ad^{-/-}$ mice. Remarkably, adiponectin reversed the fusion of podocyte foot processes in $Ad^{-/-}$ mice. AMPK activity was attenuated in podocytes and glomeruli, and the expression of the NADPH oxidase Nox4, but not Nox1 or Nox2, was enhanced in the absence of adiponectin in $Ad^{-/-}$ mice. As predicted, adiponectin treatment restored AMPK activity and decreased Nox4 expression in parallel with improvements in urinary albuminuria and hydrogen peroxide levels.

Adiponectin as a biomarker of kidney disease: ready for prime time?

These carefully conducted studies demonstrate an important link between adiponectin and albuminuria (Figure 2) (7). The experiments in rodents and cell culture suggest that adiponectin signaling via AMPK regulates oxidative stress, segmental fusion of podocyte foot processes, and albuminuria. The capacity of adiponectin to reverse these abnormalities in $Ad^{-/-}$ mice is independent of glucose. These results are in agreement with exacerbation of albuminuria following surgical resection of kidneys in $Ad^{-/-}$ mice (12), which resulted in glomerular hypertrophy, podocyte injury, oxidative stress, inflammation, and fibrosis. Adiponectin decreased albuminuria, glomerular hypertrophy, and tubulointerstitial fibrosis, and these changes were associated with restoration of VCAM-1, monocyte chemoattractant protein–1, TNF-α, TGF-β1, collagen type I/III, and Nox to the levels found in wild-type mice. An antiinflammatory role for adiponectin in the kidney is in agreement with its similar actions in the vasculature, liver, and colon (13–15).

Low plasma adiponectin concentration could potentially serve as a biomarker for early detection of kidney disease. However, the inverse relationship between adiponectin and proteinuria among obese African Africans found in the current study (7) has not been consistently observed in others (16, 17). The plasma adiponectin concentration may reflect the extent of kidney damage, metabolic state, and ethnic background of the population under study (7, 15, 16). As mentioned earlier, adiponectin exists as HMW, hexamer, and trimer complexes, yet these forms of the protein were not measured in patients or mice in the current study (7).

The authors' findings in $Ad^{-/-}$ mice raise an intriguing possibility that increasing adiponectin levels or stimulating AMPK activity may blunt albuminuria and prevent the progression of kidney disease. However, there are pitfalls in the translation of these results. Recombinant adiponectin was administered in mice with total adiponectin deficiency. As is often the case in hormone replacement, $Ad^{-/-}$ mice are expected to be hyperresponsive to adiponectin treatment. Because there are no patients with total adiponectin deficiency, the question remains as to whether patients with partial adiponectin deficiency will benefit from treatments that increase adiponectin levels. Previous studies in diabetic patients suggest this may be the case: rosiglitazone increased adiponectin levels, enhanced insulin sensitivity, and suppressed albuminuria in patients with type 2 diabetes (18, 19). AMPK activation also has the potential to ameliorate kidney disease, as evidenced by the effects of metformin and AICAR to increase AMPK phosphorylation and inhibit renal hypertrophy in diabetic rats (20).

Conclusions

The article by Sharma et al. (7) advances our understanding of renal pathophysiology. Until the discovery of leptin, the prevailing view of adipose tissue was that of a passive storage site for triglycerides. It is now well established that adipocytes secrete proteins that actively control energy homeostasis, glucose and lipid metabolism, neuroendocrine and cardiovascular function, and various physiological systems. Adiponectin is a logical candidate for exploring the link between adipose tissue and kidney function. The tools for assessing the relationships among adiposity, adiponectin, metabolism, and renal pathophysiology in rodents and humans are readily available, making the clinical translation of key elements of this paper feasible. The demonstration of the validity of adiponectin as a biomarker of albuminuria in humans is a crucial step in this process and may ultimately facilitate the prevention and treatment of kidney disease.