

# Adiponectin, Metabolic Risk Factors, and Cardiovascular Events among Patients with End-Stage Renal Disease

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**Abstract.** Adiponectin (ADPN), which is a secretory protein of adipose tissue, attenuates endothelial inflammatory responses *in vitro*. Among human subjects, plasma ADPN concentrations are reduced among patients with atherosclerotic complications but are substantially increased among patients with advanced renal failure. The clinical and biochemical correlates of plasma ADPN levels were investigated and the predictive power of ADPN levels with respect to survival rates and cardiovascular events was prospectively tested in a cohort of 227 hemodialysis patients, who were monitored for  $31 \pm 13$  mo. Plasma ADPN levels were 2.5 times higher ( $P < 0.0001$ ) among dialysis patients ( $15.0 \pm 7.7 \mu\text{g/ml}$ ) than among healthy subjects ( $6.3 \pm 2.0 \mu\text{g/ml}$ ), were independent of age, and were higher ( $P = 0.03$ ) among women ( $15.2 \pm 7.9 \mu\text{g/ml}$ ) than among men ( $14.0 \pm 7.4 \mu\text{g/ml}$ ). For both genders, plasma ADPN levels were inversely related to body mass index values, plasma leptin levels, insulin levels, serum triglyceride levels, and homeo-

static model assessment index values. Furthermore, plasma ADPN levels were directly related to HDL cholesterol levels and inversely related to von Willebrand factor levels. Plasma ADPN levels were lower ( $P < 0.05$ ) among patients who experienced new cardiovascular events ( $13.7 \pm 7.3 \mu\text{g/ml}$ ) than among event-free patients ( $15.8 \pm 7.8 \mu\text{g/ml}$ ). There was a 3% risk reduction for each  $1 \mu\text{g/ml}$  increase in plasma ADPN levels, and the relative risk of adverse cardiovascular events was 1.56 times (95% confidence interval, 1.12 to 1.99 times) higher among patients in the first ADPN tertile, compared with those in the third tertile. Plasma ADPN levels are an inverse predictor of cardiovascular outcomes among patients with end-stage renal disease. Furthermore, ADPN is related to several metabolic risk factors in a manner consistent with the hypothesis that this protein acts as a protective factor for the cardiovascular system.

Atherosclerotic complications are the leading cause of the high cardiovascular mortality rates among patients with end-stage renal disease (ESRD) (1). Classic risk factors only partly explain the high cardiovascular risk of these patients (2), and it is now recognized that factors peculiar to chronic uremia, such as anemia and hyperphosphatemia, and emerging (*e.g.*, inflammation and hyperhomocysteinemia) and/or unknown risk factors play relevant roles among these patients.

There is growing interest in adipose tissue as an endocrine organ. In addition to leptin, which is a signal of satiety for the central nervous system and is related to insulin and glucose metabolism (3), adipocytes secrete a variety of biologically active molecules, which may influence the function as well as the structural integrity of the cardiovascular system. Therefore, it seems likely that cytokines synthesized in fat cells, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (4) and plasminogen activator inhibitor-1 (5), have roles in insulin resistance and atherosclerotic complications in diabetes mellitus. Adiponectin (ADPN) is a recently discovered protein (6) that circulates in high concentrations (approximately 0.01% of total plasma protein) (7). This adipocyte protein seems to play a protective role in experimental models of vascular injury, perhaps because it suppresses the attachment of monocytes to endothelial cells (8), which is a fundamental step in experimental vascular damage as well as an early event in the atherosclerotic process.

Disturbances in the endocrine function of adipose tissue are

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of particular interest in ESRD because adipocyte cytokines may, in theory, be involved in the high cardiovascular risk associated with this syndrome. Levels of leptin, the most well studied adipocyte hormone, are markedly increased in chronic renal failure, and it is suspected that the accumulation of this substance contributes to alterations in glucose homeostasis and insulin sensitivity among these patients. Interestingly, like leptin levels, plasma ADPN levels are dependent on the GFR being markedly increased among patients with pronounced renal impairment (9). Because ADPN has an important protective role for the endothelium, we measured the plasma concentrations of this substance in a large cohort of patients with ESRD who were undergoing chronic dialysis, we sought its functional correlates, and we tested its predictive power for cardiovascular events.

## Materials and Methods

### Protocol

The protocol conformed to the ethical guidelines of our institutions, and informed consent was obtained from each participant. All studies were performed on nondialysis days, between 8 a.m. and 10 a.m.

### Study Cohort

Two hundred twenty-seven hemodialysis patients (125 male and 102 female patients, all Caucasian) who had been receiving regular dialysis treatment (RDT) for at least 6 mo and did not exhibit clinical evidence of heart failure (defined as dyspnea in addition to two of the following conditions: increased jugular pressure, bibasilar crackles, pulmonary venous hypertension, or interstitial edema in chest x-rays, requiring hospitalization or extra ultrafiltration) were eligible for the study. The demographic, clinical, and biochemical characteristics of these patients are detailed in Table 1. These patients were recruited between January 1997 and May 1998 and represented approximately 70% of the entire hemodialysis population of four dialysis units. The remaining 30% of the patients were excluded because of the presence of circulatory congestion or major infections (20%) or because of hospitalization for treatment of intercurrent illnesses or logistic reasons/unwillingness to participate in the study (10%). Patients were being treated thrice-weekly with standard bicarbonate hemodialysis ( $\text{Na}^+$ , 138 mM;  $\text{HCO}_3^-$ , 35 mM;  $\text{K}^+$ , 1.5 mM;  $\text{Ca}^{2+}$ , 1.25 mM;  $\text{Mg}^{2+}$ , 0.75 mM) or high-flux hemodialysis with either cellulose or semisynthetic membranes (dialysis filter surface area, 1.1 to 1.7  $\text{m}^2$ ). Dry weight was targeted in each case to achieve a normotensive, edema-free state. Eighty-five patients were habitual smokers ( $21 \pm 16$  cigarettes/d). One hundred twenty-three patients were receiving erythropoietin treatment. Eighty-two patients were receiving antihypertensive drugs (58 received single therapy with angiotensin-converting enzyme inhibitors,  $\text{AT}_1$  receptor antagonists, calcium channel blockers, or  $\alpha$ - or  $\beta$ -blockers, and 24 received double or triple therapy with various combinations of these drugs).

### Follow-Up Study

After the initial assessment, patients were monitored for  $31 \pm 13$  mo (range, 1 to 45 mo). During the follow-up period, cardiovascular events (ECG documented anginal episodes or myocardial infarctions, heart failure, ECG documented arrhythmia, transient ischemic attacks, or strokes, peripheral vascular disease, or major arterial or venous thrombotic episodes, except arteriovenous fistula thrombosis) were accurately recorded. Each death was reviewed and assigned an un-

Table 1. Demographic, clinical, and biochemical characteristics of the study population<sup>a</sup>

Somatometric data	
age (yr)	59.9 $\pm$ 15.0
gender (male/female)	125 /102
BMI ( $\text{kg}/\text{m}^2$ )	24.5 $\pm$ 4.4
duration of RDT (mo)	43 (21 to 110)
Cardiovascular risk factors	
systolic pressure (mmHg)	139.7 $\pm$ 25.0
diastolic pressure (mmHg)	76.0 $\pm$ 12.8
diabetic patients (%)	15
smokers (%)	37
serum total cholesterol level of >200 mg/dl (%)	51
LVH (%)	75
Previous cardiovascular events	
myocardial infarction (%)	11
angina pectoris (%)	33
stroke (%)	9
transient ischemic attack (%)	11
Biochemical data	
hemoglobin level (g/dl)	10.8 $\pm$ 1.9
serum total cholesterol level (mg/dl)	207.7 $\pm$ 57.8
serum HDL cholesterol level (mg/dl)	41.3 $\pm$ 11.8
serum LDL cholesterol level (mg/dl)	131.4 $\pm$ 49.3
serum triglyceride level (mg/dl)	175.4 $\pm$ 86.0
serum albumin level (g/dl)	4.2 $\pm$ 0.5
serum calcium level (mM)	2.3 $\pm$ 0.2
serum phosphate level (mM)	2.0 $\pm$ 0.4
serum CRP level (mg/L)	7.4 (3.4 to 16.0)
von Willebrand factor (%)	117.3 $\pm$ 30.5
plasma homocysteine level ( $\mu\text{M}$ )	27.0 (19.8 to 42.6)
plasma leptin level (ng/ml)	10.0 (4.8 to 30.7)
serum glucose level (mM)	5.55 $\pm$ 3.17
plasma insulin level ( $\mu\text{U}/\text{ml}$ )	19.7 (13.7 to 29.7)
HOMA-R index [ $\text{mM} \cdot (\mu\text{U}/\text{ml})$ ]	4.03 (2.71 to 6.80)
Kt/V	1.21 $\pm$ 0.27

<sup>a</sup> Data are mean  $\pm$  SD, median (interquartile range), or percent frequency, as appropriate. Kt/V denotes fractional urea clearance. BMI, body mass index; CRP, C-reactive protein; HOMA-R, homeostatic model assessment; RDT, regular dialysis treatment; LVH, left ventricular hypertrophy.

derlying cause by a panel of five physicians. As part of the review process, all available medical information regarding each death was collected. This information always included study and hospitalization records. In the case of an out-of-hospital death, family members were interviewed by telephone, for better assessment of the circumstances surrounding the death.

### Laboratory Measurements

Fasting blood samples for analysis of the levels of lipids, albumin (bromocresol green), hemoglobin, calcium, phosphate, insulin, glu-

cose, C-reactive protein (CRP), von Willebrand factor, and homocysteine were obtained from all patients on a midweek nondialysis day. Plasma homocysteine levels were determined with a HPLC method based on ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate fluorescence derivatization (10). CRP levels were measured by using a commercially available kit (Behring Scoppito, l'Aquila, Italy). Serum insulin levels were measured by using a RIA kit (Sorin Saluggia, Vercelli, Italy). Insulin sensitivity was estimated by using the homeostatic model assessment (HOMA-R) index [*i.e.*, plasma glucose level  $\times$  (plasma insulin level/22.5)], which was validated with the euglycemic-hyperinsulinemic clamp method (11). Plasma leptin measurements were made in a single assay, using a sensitive RIA developed at Linco Research Laboratories (St. Louis, MO). The assay was based on a polyclonal antibody raised in rabbits against highly purified recombinant human leptin. The recovery of leptin with the RIA ranged from 99 to 105% in the range of 0.5 to 100  $\mu\text{g/L}$ . The intra-assay coefficient of variation ranged from 3.4 to 8.3%. Plasma ADPN concentrations were measured by using a sensitive enzyme-linked immunosorbent assay (7), in the laboratory of the Department of Internal Medicine and Molecular Science of the Medical School of Osaka (Osaka, Japan).

The reference group consisted of 15 healthy, normotensive, Caucasian subjects matched to dialysis patients with respect to gender (six male and nine female patients *versus* 125 male and 102 female patients) and body mass index (BMI) ( $24.9 \pm 1.3 \text{ kg/m}^2$  *versus*  $24.5 \pm 4.3 \text{ kg/m}^2$ ).

### Echocardiography

All echocardiographic measurements were performed according to the recommendations of the American Society of Echocardiography (12). Left ventricular mass (LVM) was calculated according to the formula described by Devereux *et al.* (13) and was indexed to height<sup>2.7</sup> (LVM index) (14). The height-based indexing of LVM was specifically chosen to minimize potential distortions attributable to extracellular volume expansion (with surface area indexing being weight-sensitive). Left ventricular hypertrophy (LVH) was defined as a LVM index of  $>47 \text{ g/m}^{2.7}$  for women or  $>50 \text{ g/m}^{2.7}$  for men.

### Statistical Analyses

Data are reported as mean  $\pm$  SD or as median and interquartile range, as appropriate. Between groups, comparisons were made with the *t* test or the Mann-Whitney test. Relationships between plasma ADPN levels and other covariates were analyzed by using the least-squares method. Data that did not demonstrate a Gaussian distribution were logarithmically transformed.

Time-to-event analysis of cardiovascular outcomes was performed by using the Kaplan-Meier method. The independent prognostic power of plasma ADPN levels for death or fatal or nonfatal cardiovascular events was analyzed by using the Cox proportional-hazards model. Variables with independent effects on survival rates were identified by constructing hierarchical models, which included Framingham risk factors [age, gender, smoking (yes/no), diabetes mellitus, systolic pressure, serum cholesterol levels, LVH, and previous cardiovascular events], factors peculiar to ESRD (duration of RDT, serum albumin levels, hemoglobin levels, and calcium-phosphate product values), and emerging risk factors (serum CRP and plasma homocysteine levels). Hazard ratios (HR) and their 95% confidence intervals (CI) were calculated with the use of the estimated regression coefficients and their standard errors in the Cox regression analysis. All calculations were performed by using a standard statistical package (SPSS for Windows, version 9.0.1; SPSS, Chicago, IL).

## Results

### Plasma ADPN Levels

The main clinical and biochemical characteristics of the study population are summarized in Table 1. Plasma ADPN levels among dialysis patients ( $15.0 \pm 7.7 \mu\text{g/ml}$ ) were approximately 2.5 times higher ( $P < 0.0001$ ) than the average value for healthy subjects ( $6.3 \pm 2.0 \mu\text{g/ml}$ ) and exceeded the upper limit of the normal range (cut-off,  $>10.0 \mu\text{g/ml}$ ) for the majority of dialysis patients (170 of 227 patients, *i.e.*, 75%). Among dialysis patients, plasma ADPN levels were higher ( $P = 0.03$ ) for women ( $15.2 \pm 7.9 \mu\text{g/ml}$ ) than for men ( $14.0 \pm 7.4 \mu\text{g/ml}$ ), and this difference remained significant after data adjustment for BMI ( $P = 0.01$ ). The between-gender difference in plasma ADPN levels among dialysis patients was similar to that observed for healthy control subjects (men,  $5.2 \pm 1.8 \mu\text{g/ml}$ ; women,  $7.1 \pm 1.9 \mu\text{g/ml}$ ).

### Biochemical and Clinical Correlates of Plasma ADPN Levels among Dialysis Patients

Plasma ADPN levels were strongly and inversely related to BMI and plasma leptin levels (Figure 1), as well as to insulin levels and HOMA-R indices among both men and women (Figure 2) and serum glucose levels among men only ( $r = -0.27$ ,  $P = 0.002$ ). Plasma ADPN levels were also directly related to plasma HDL cholesterol levels and were inversely related to serum triglyceride levels; such associations were again apparent for both genders (Figure 3). Slight inverse correlations were observed between ADPN and von Willebrand factor levels (men,  $r = -0.17$ ,  $P = 0.098$ ; women,  $r = -0.19$ ,  $P = 0.04$ ). Plasma ADPN levels were slightly related to Kt/V ( $r = 0.15$ ,  $P = 0.02$ ). Plasma ADPN levels were lower ( $P < 0.05$ ) among diabetic women ( $11.9 \pm 1.7 \mu\text{g/ml}$ ) than among nondiabetic women ( $15.1 \pm 1.6 \mu\text{g/ml}$ ), whereas they were identical among diabetic ( $12.0 \pm 1.8 \mu\text{g/ml}$ ) and nondiabetic ( $12.3 \pm 1.7 \mu\text{g/ml}$ ) men. Overall, plasma ADPN levels were unrelated to age ( $P = 0.21$ ), smoking ( $P = 0.13$ ), duration of RDT ( $P = 0.15$ ), mean arterial pressure ( $P = 0.75$ ), heart rate ( $P = 0.30$ ), serum albumin levels ( $P = 0.07$ ), CRP levels ( $P = 0.17$ ), or LDL cholesterol levels ( $P = 0.48$ ).

### Plasma ADPN Levels and Drug Therapy

Plasma ADPN levels were higher ( $P = 0.006$ ) among patients who were receiving treatment with erythropoietin ( $16.3 \pm 8.2 \mu\text{g/ml}$ ) than among the remaining patients ( $13.5 \pm 6.9 \mu\text{g/ml}$ ), and this difference remained significant after data adjustment for gender and BMI ( $P = 0.04$ ). Antihypertensive treatment had no effect on plasma ADPN levels, either among women (treated,  $16.0 \pm 6.9 \mu\text{g/ml}$ ; untreated,  $16.4 \pm 8.5 \mu\text{g/ml}$ ;  $P = 0.77$ ) or among men (treated,  $13.9 \pm 7.7 \mu\text{g/ml}$ ; untreated,  $14.1 \pm 7.3 \mu\text{g/ml}$ ;  $P = 0.84$ ).

### Plasma ADPN Levels, Survival Rates, and Cardiovascular Events

Overall, 78 patients died, 50 of them (64%) as a result of cardiovascular causes (Table 2). Plasma ADPN levels failed to predict overall mortality rates, which were explained by serum

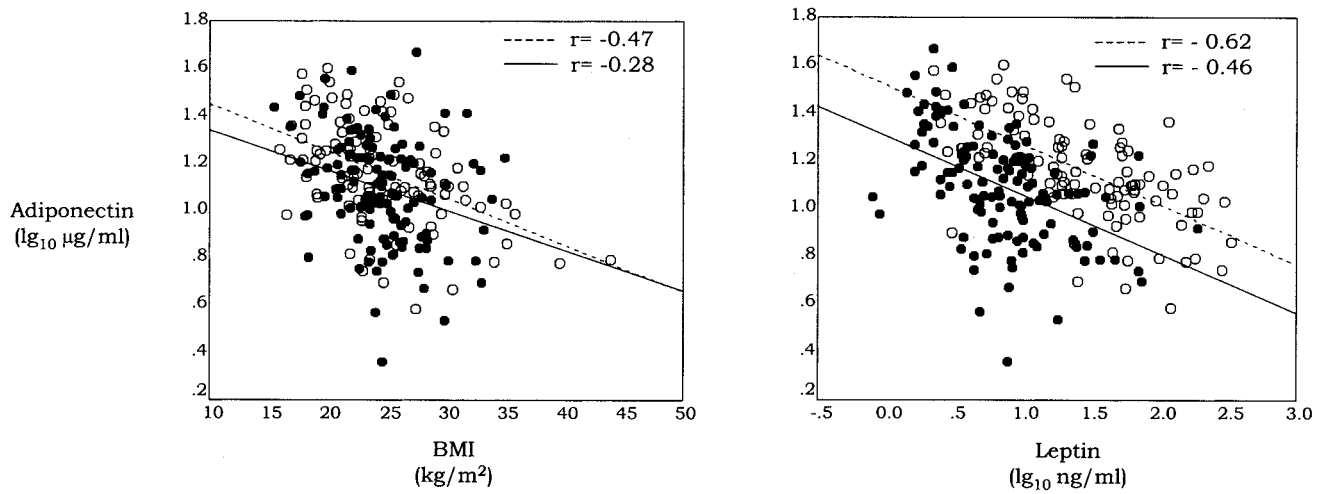


Figure 1. Relationships between plasma adiponectin (ADPN) levels and body mass index (BMI) and plasma leptin levels (●, male patients; ○, female patients). The regression lines are presented separately for male patients (—) and female patients (---).

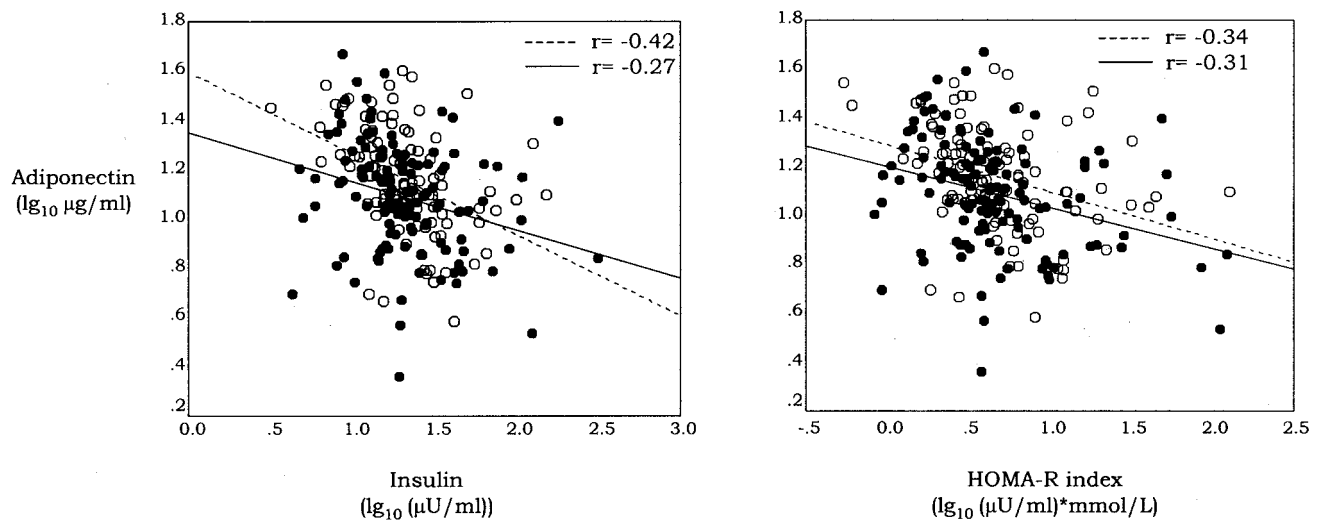


Figure 2. Relationships between plasma ADPN levels and plasma insulin levels and homeostatic model assessment (HOMA-R) indices (●, male patients; ○, female patients). The regression lines are presented separately for male patients (—) and female patients (---).

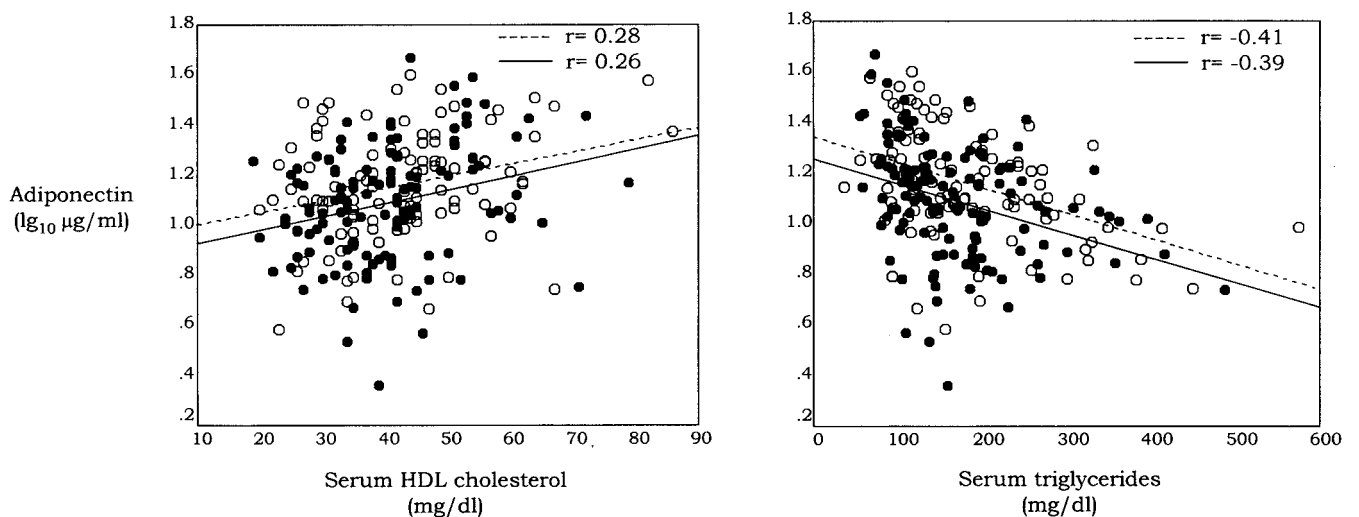


Figure 3. Relationships between plasma ADPN levels and serum HDL cholesterol and triglyceride levels (●, male patients; ○, female patients). The regression lines are presented separately for male patients (—) and female patients (---).



cholesterol levels (HR, 1.01; 95% CI, 1.00 to 1.01;  $P = 0.001$ ), male gender (HR, 2.84; 95% CI, 1.47 to 5.46;  $P = 0.002$ ), previous cardiovascular events (HR, 2.49; 95% CI, 1.41 to 4.42;  $P = 0.002$ ), age (HR, 1.04; 95% CI, 1.01 to 1.06;  $P = 0.003$ ), serum albumin levels (HR, 0.42; 95% CI, 0.24 to 0.75;  $P = 0.003$ ), serum CRP levels (HR, 1.01; 95% CI, 1.00 to 1.02;  $P = 0.01$ ), diabetes mellitus (HR, 2.28; 95% CI, 1.13 to 4.59;  $P = 0.02$ ), and LVH (HR, 2.66; 95% CI, 1.02 to 6.94;  $P = 0.04$ ).

Ninety-five cardiovascular events (fatal or nonfatal) occurred during the follow-up period (Table 2). Plasma ADPN levels were lower ( $P < 0.05$ ) among patients who experienced new cardiovascular events ( $13.7 \pm 7.3 \mu\text{g/ml}$ ), compared with event-free patients ( $15.8 \pm 7.8 \mu\text{g/ml}$ ). In the unadjusted analysis (Table 3), there was a 3% risk reduction for each 1  $\mu\text{g/ml}$  increase in plasma ADPN levels and the relative risk of

adverse cardiovascular events was 1.56 (95% CI, 1.12 to 1.99) higher for patients in the first ADPN tertile than for those in the third tertile (Figure 4). The point estimate remained almost unchanged after adjustment for Framingham risk factors (including echocardiographically detected LVH and previous cardiovascular events), factors peculiar to ESRD, and emerging risk factors such as serum CRP levels and plasma homocysteine levels (Table 3).

## Discussion

Plasma ADPN levels are markedly increased among patients with ESRD and are related to several risk factors, such as insulin levels, serum triglyceride levels, HDL cholesterol levels, and von Willebrand factor levels, in a way consistent with the hypothesis that this protein acts as a protective factor for the cardiovascular system. More importantly, ADPN seems to be a strong and independent (inverse) predictor of cardiovascular outcomes among these patients.

In recent years, evidence that adipocytes produce a variety of molecules that are metabolically active and that interfere with several organ systems has been accrued. Overproduction of TNF- $\alpha$  by adipose cells is involved in insulin resistance in obesity, whereas plasma activator inhibitor-1, which is also synthesized in adipose tissue, is a well recognized causative factor for vascular thrombosis (4,5). The gene product of the obese gene leptin is considered to be a fundamental signal of satiety to the brain. This pleiotropic protein is strictly related to body mass and gender and has a variety of actions, ranging from interference with sympathetic activity to hematopoiesis and reproductive function (3). The kidney is the principal site of elimination of circulating leptin, which in part explains why levels of this substance are increased among patients with ESRD. Among these patients, the relationships between leptin levels and BMI and fat mass are well maintained but seem steeper and upwardly displaced (15,16). Furthermore, leptin levels are directly related to plasma insulin levels, and hyperinsulinemia likely contributes to increases in plasma leptin levels among dialysis patients (17); this possibility is in line with the observed effect of insulin growth factor-1 (18). Interestingly, there seems to be a link between erythropoietin and leptin, because erythropoietin treatment induces a significant decline of leptinemia among hemodialysis patients (19), whereas erythropoietin doses and plasma leptin levels are inversely related among patients with moderate-to-severe renal failure (20).

ADPN is a novel, collagen-like, protein of the collectin family (6,21) that is exclusively synthesized in adipocytes. Although adipose tissue is the only source of ADPN, the relationship of this protein to fat and body mass is opposite to that of leptin, and ADPN levels are significantly reduced among obese subjects, in comparison with lean, healthy, control subjects (7). The link between body mass and ADPN seems to be a causal one, because weight loss induces a marked increase in plasma ADPN levels among both normal individuals and type 2 diabetic patients (22). Like plasma leptin levels (3), plasma ADPN concentrations seem to be gender-dependent, being higher among women than men (22). Interestingly,

Table 2. Cardiovascular events (fatal and nonfatal) and causes of death in the study cohort

	No. of Cases
Cardiovascular events (fatal and nonfatal)	
stroke	19
myocardial infarction	16
arrhythmia	15
heart failure	12
angina pectoris	10
transient ischemic attack	8
sudden death	4
mesenteric infarction	4
pulmonary embolism	2
major venous thrombosis	4
retinal artery thrombosis	1
total	95
Causes of death	
cardiovascular	
stroke	15
myocardial infarction	13
heart failure	8
arrhythmia	4
sudden death	4
mesenteric infarction	4
pulmonary embolism	2
other causes	
sepsis/infection	7
cachexia	7
neoplasia	5
hyperkalemia	4
gastrointestinal hemorrhage	2
diabetes mellitus, hyperosmolar coma	1
treatment withdrawal	1
respiratory failure	1
total	78

Table 3. Crude and adjusted relative risks of fatal and nonfatal cardiovascular events<sup>a</sup>

Variables (Units of Increase)	Model 1 (Unadjusted)		Model 2		Model 3		Model 4	
	Hazard Ratio	P Value	Hazard Ratio	P Value	Hazard Ratio	P Value	Hazard Ratio	P Value
ADPN levels (1 µg/ml)	0.97 (0.93 to 0.99)	0.03	0.96 (0.93 to 0.99)	0.03	0.96 (0.93 to 0.99)	0.02	0.97 (0.93 to 0.99)	0.04
Framingham risk factors								
age (1 yr)			1.02 (0.99 to 1.04)	0.14	1.02 (1.00 to 1.04)	0.06	1.03 (1.00 to 1.05)	0.02
male gender			1.19 (0.65 to 2.17)	0.57	1.35 (0.72 to 2.53)	0.35	1.31 (0.70 to 2.46)	0.40
smoking			1.22 (0.69 to 2.16)	0.49	1.15 (0.65 to 2.04)	0.63	1.10 (0.61 to 2.00)	0.75
systolic pressure (1 mmHg)			1.00 (0.99 to 1.01)	0.89	1.00 (0.99 to 1.02)	0.51	1.00 (0.99 to 1.01)	0.66
cholesterol level (1 mg/dl)			1.00 (0.99 to 1.01)	0.21	1.00 (0.99 to 1.01)	0.15	1.00 (0.99 to 1.01)	0.30
diabetes mellitus			1.11 (0.59 to 2.09)	0.75	1.48 (0.75 to 2.96)	0.26	1.72 (0.85 to 3.48)	0.13
LVH (echocardiography)			2.95 (1.28 to 6.79)	0.01	2.14 (0.88 to 5.20)	0.09	1.94 (0.79 to 4.78)	0.15
previous cardiovascular events			2.38 (1.39 to 4.05)	0.001	2.05 (1.17 to 3.58)	0.01	2.16 (1.22 to 3.80)	0.008
Factors peculiar to ESRD								
hemoglobin levels (1 g/dl)					1.03 (0.88 to 1.20)	0.71	1.01 (0.86 to 1.17)	0.92
albumin levels (1 g/dl)					0.84 (0.47 to 1.50)	0.54	0.77 (0.43 to 1.39)	0.39
calcium phosphate (1 mmol <sup>2</sup> /L <sup>2</sup> )					1.10 (0.86 to 1.40)	0.45	1.12 (0.87 to 1.44)	0.37
duration of RDT (10 mo)					1.04 (1.00 to 1.08)	0.04	1.04 (1.00 to 1.08)	0.04
Emerging risk factors								
CRP levels (1 mg/L)							0.99 (0.98 to 1.01)	0.40
homocysteine levels (10 µM)							1.10 (1.01 to 1.19)	0.03

<sup>a</sup> Data are expressed as hazard ratios, 95% confidence interval, and P values. Data adjustment for traditional risk factors (model 2), as well as for factors peculiar to end-stage renal disease (ESRD) (model 3) and emerging risk factors (model 4), did not modify the relationship between adiponectin (ADPN) levels and cardiovascular events.

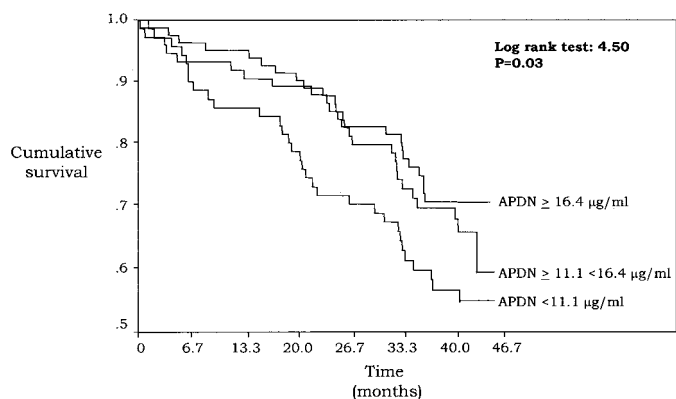


Figure 4. Kaplan-Meier survival curves for cardiovascular events (fatal and nonfatal) in the study cohort. Patients were stratified into three tertiles according to plasma ADPN concentrations (first tertile, ADPN levels of  $<11.1 \mu\text{g/ml}$ ; second tertile,  $>11.1$  to  $<16.4 \mu\text{g/ml}$ ; third tertile,  $\geq 16.4 \mu\text{g/ml}$ ).

plasma ADPN levels, independently of body mass, are subnormal among diabetic patients and seem to be inversely related to plasma glucose, insulin, and triglyceride levels (22). In addition to these metabolic relationships, ADPN is emerging as a pleiotropic cytokine linked not only to fat mass but also to other fundamental body functions, such as hematopoiesis and immunity (23).

The role of the kidney in the metabolism of ADPN has been barely investigated. Studies in our laboratory (9) and data reported here demonstrate that, independently of diabetes mellitus, ADPN plasma concentrations are markedly increased among dialysis patients and seem to be related to metabolic risk factors such as insulin levels and insulin sensitivity (HOMA-R index), triglyceride levels, and HDL cholesterol levels. Furthermore, our data demonstrate that the gender dependence of plasma ADPN levels is maintained in advanced renal failure. ADPN levels are inversely related to BMI, which is again in line with data for healthy subjects and diabetic patients (22), but, because of the increased plasma concentrations, this relationship is shifted upward in renal failure. Although ADPN and leptin levels were both increased among dialysis patients, the two hormones were inversely correlated. This phenomenon suggests that, in addition to renal failure, other factors play a role (perhaps an important one) in the regulation of the plasma concentrations of these substances in uremia. A recent longitudinal study elegantly demonstrated that leptin behaves as a negative acute-phase reactant among dialysis patients (24). The fact that ADPN levels were unrelated to serum CRP levels suggests that inflammatory processes do not play a major role in the increased plasma levels of this substance. However, the cross-sectional nature of our observations does not allow us to draw firm conclusions regarding the relationship between ADPN and inflammation. Hyperinsulinemia, which is a well known metabolic complication of chronic renal failure and a potential cause of hyperleptinemia (25), might downregulate plasma ADPN levels among dialysis patients, as suggested by the inverse correlation

between levels of this cytokine and insulin levels. Our finding that plasma ADPN levels were higher among patients who were receiving erythropoietin treatment, which again mirrors the behavior of leptin (19,20), suggests the possible involvement of this substance in hematopoiesis (23).

To date, chronic renal failure is the only disease state known to be associated with increased plasma ADPN concentrations; therefore, it represents an useful model for elucidation of the potential clinical implications of this substance. Most of the interest in ADPN depends on its potential protective role for the cardiovascular system. Physiologic concentrations of this substance exhibit inhibitory effects on TNF- $\alpha$ -induced monocyte adhesion and adhesion molecule expression, and ADPN seems to act as an endogenous regulator of endothelial cells in response to inflammatory stimuli (8). In line with these *in vitro* data, it was recently reported that ADPN levels were reduced among patients with coronary artery disease (8), as well as patients with type 2 diabetes mellitus (22). Atherosclerosis is currently regarded as an inflammatory disease (26), and there is evidence that atherosclerosis is strongly associated with inflammation among uremic patients (27). If the inflammatory response detected in atherosclerotic lesions is effectively counteracted by ADPN *in vivo*, then this protein may have the potential for prevention and/or retardation of atherogenesis in various diseases, including chronic renal failure. The directions of the relationships between ADPN and several metabolic risk factors, such as insulin, triglycerides, and HDL cholesterol, are all in agreement with the hypothesis that ADPN may have a protective role for the cardiovascular system among dialysis patients. Although it is slight, the relationship with von Willebrand factor (a biochemical marker of endothelial dysfunction) supports such a hypothesis. Undoubtedly the most stimulating finding of our study is the independent association between ADPN levels and cardiovascular events in a comprehensive analysis including both traditional and nontraditional risk factors. Relatively higher ADPN levels were associated with better cardiovascular outcomes among dialysis patients. However, it must be noted that, on average, ADPN levels were increased, in comparison with healthy subjects, not only among patients who experienced relatively few cardiovascular events (third ADPN tertile in Figure 4) but also (albeit to a much lesser extent) among those who developed cardiovascular complications in large proportions (first ADPN tertile). It can be hypothesized that in ESRD the biologic phenomena underlying the cardiovascular protective role of ADPN must be downregulated, perhaps at the receptor level, thus resetting at a higher plasma concentration the relationship between this protein and cardiovascular damage and clinical complications. This hypothesis must be fully tested in *in vitro* and *in vivo* experiments, to better elucidate the role of this most interesting cytokine in human diseases.

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