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ORIGINAL

Low circulating adiponectin levels are associated with insulin resistance in non-obese peritoneal dialysis patients

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Abstract. In patients with end-stage renal disease (ESRD), circulating adipokine levels are increased due to decreased renal clearance, irrespective of obesity. However, whether adipokines play a role in the development of insulin resistance (IR) in non-obese ESRD patients is unknown. We conducted a cross-sectional study to identify factors associated with IR in 62 non-obese patients on peritoneal dialysis (PD). Non-obesity was defined as body mass index (BMI) <25 kg/m^2. IR was determined using homeostatic model assessment–IR (HOMA-IR). We also measured serum concentrations of adiponectin, leptin, resistin, high-sensitivity C-reactive protein (hsCRP), and IL-6. The average BMI of the study patients was 21.6 kg/m^2. When patients were divided into two groups according to the median value of HOMA-IR, serum adiponectin levels were significantly lower in patients with HOMA-IR >1.85 than in those with HOMA-IR ≤1.85, whereas serum concentrations of leptin and resistin did not differ between the two groups. In addition, log-transformed HOMA-IR was negatively correlated with adiponectin (γ = -0.464, P < 0.001) and log-transformed high-density lipoprotein cholesterol (γ = -0.250, P = 0.050) and positively correlated with age (γ = 0.334, P = 0.008) and triglyceride (γ = 0.392, P = 0.002). However, resistin, log-transformed leptin and log-transformed hsCRP were not associated with HOMA-IR. In a multiple linear regression model, adiponectin was independently associated with HOMA-IR (β = -0.023, P = 0.015). In conclusion, this study suggests that low circulating adiponectin levels might be involved in IR even in non-obese patients undergoing PD.

Key words: Insulin resistance, Non-obese, Peritoneal dialysis, Adipokine, Chronic inflammation

CARDIOVASCULAR DISEASE is the leading cause of death in patients with end-stage renal disease (ESRD) [1]. In addition to well-known traditional risk factors such as hypertension, diabetes, smoking, and dyslipidemia, several non-traditional risk factors can contribute to increased cardiovascular morbidity and mortality in these patients [2]. Among these factors, insulin resistance (IR) is strongly related to hypertension and dyslipidemia [3] and is involved in the development of cardiovascular disease.

The linkage between obesity and IR has been most widely studied in investigations of the pathogenesis of IR, and adipose tissue is reported to be a key contributor to IR. Adipose tissue, as an endocrine organ, produces several adipokines such as leptin and adiponectin, which are involved in modulation of insulin action in metabolic tissues such as skeletal muscle and the liver [4]. In addition, macrophages, which infiltrate adipose tissue, serve as a major source of locally produced pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), ultimately desensitizing insulin action [5]. Therefore, obesity can cause IR by altering adipokine profiles and causing an excess production of pro-inflammatory cytokines.

However, a substantial number of ESRD patients are not obese [6]. Interestingly, even in non-obese ESRD patients, IR is prevalent and an independent predictor of cardiovascular mortality [7]. There are a few possible explanations for this phenomenon. It is speculated that uremia-associated factors such as chronic inflammation are involved in the pathogenesis of IR [8]. Furthermore, circulating concentrations of adipok-

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ines are markedly elevated in ESRD patients because of their decreased renal clearance [9], and these altered adipokine profiles may worsen IR even in non-obese individuals. However, whether adipokines or inflammation play a role in the development of IR in these non-obese patients has not yet been explored. Therefore, in this study, we investigated the roles of adipokine and inflammation in IR in non-obese patients.

Subjects and Methods

Patients

This cross-sectional single-center study involved 135 ESRD patients receiving peritoneal dialysis (PD) at Yonsei University Severance Hospital, Seoul, Korea. In this study, PD patients were our particular concern. These individuals are at high risk of IR because a substantial amount of glucose is absorbed *via* the peritoneal capillary vessels, which elevates serum insulin level [10]. Patients were considered eligible if they aged 20 to 75 years and had maintained PD > 3 months. Any of the following criteria were excluded from participation; BMI >25 kg/m^2, history of overt infections during the 3 months prior to the study, history of malignancy or other chronic inflammatory disease (e.g., rheumatoid arthritis or systemic lupus erythematosus), evidence of severe malnutrition with subjective global assessment (SGA) score ≤5 or serum albumin level <3.5 g/dL, prior history of kidney transplantation, current use of glucocorticoids or peroxisome proliferator-activated receptor agonists that may affect adiponectin level. After excluding patients who met one of these criteria, a total of 62 patients were included (Fig. 1).

To assess obesity, we measured body mass index (BMI) and waist circumference (WC). However, in this study, we primarily used BMI as the indicator of obesity because WC may not reliably reflect abdominal visceral fat content in ESRD patients undergoing PD [11]. In addition, according to definition of obesity proposed by Korean Society for the Study of Obesity [12], patients with BMI <25 kg/m^2 were considered non-obese because epidemiological studies of Asian populations have shown that the threshold values of anthropometric indices for association with cardiovascular risk factors are considerably lower than those conventionally used in Caucasians [13, 14], and because strong evidence now shows that Asian people have more body fat than their Caucasian counterparts at the same BMI [15, 16]. Height, body weight, and WC were measured with an empty peritoneal cavity after completion of dialysate drainage. All participants gave their written informed consent prior to study inclusion.

Data Collection

Demographic and clinical data were recorded at study entry. After an overnight fast, venous samples were drawn for assessment of the following laboratory data: hemoglobin, blood urea nitrogen (BUN), creatinine, albumin, calcium, phosphorus, intact parathyroid hormone (PTH), total cholesterol, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, and triglyceride. The preceding overnight dwell was regulated to 1.5% dextrose dialysate to standardize the glucose load. Fasting concentrations of glucose, total cholesterol, HDL- and LDL-cholesterol, and triglyceride were measured with an auto-analyzer using an enzymatic colorimetric method (Hitachi 7150, Hitachi Ltd., Tokyo, Japan). Cystatin C was measured by using a particle-enhanced nephelometric immunoassay (Dade Behring Ltd, Marburg, Germany). Nutritional status was determined using SGA score

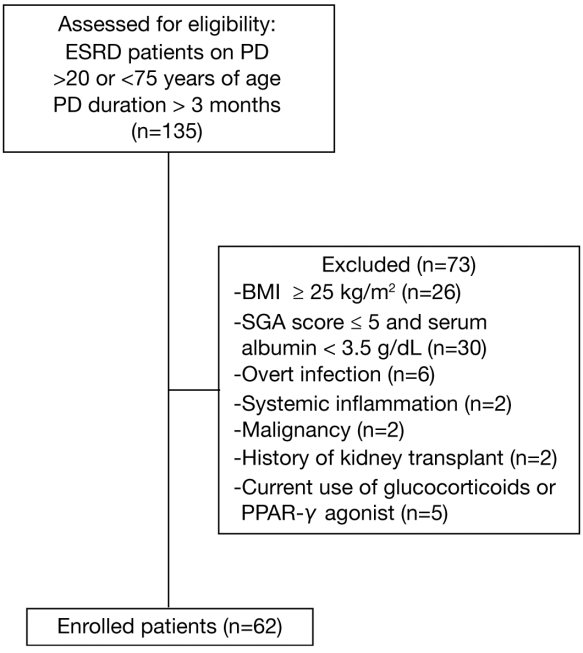

Fig. 1 Flow diagram indicating patient recruitment and exclusion ESRD, end-stage renal disease; PD, peritoneal dialysis; SGA, subjective global assessment; PPAR-γ, peroxisome proliferator activated receptor gamma; BMI, body mass index

[17]. Definition of metabolic syndrome essentially followed that of the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) definition [18]. However, WC cutoff point for central obesity was defined to be 90 cm for men and 85 cm for women according to Korean Society for the Study of Obesity [12].

Because the use of non-glucose based solution may lead to less glucose absorption *via* peritoneal cavity, we also evaluated icodextrin solution users, who were defined as one bag exchange of this solution per day for > 1 month before the study.

Measurement of Homeostasis Model Assessment of Insulin Resistance

From the serum fasting levels of glucose and insulin, the homeostatic model assessment –insulin resistance (HOMA-IR) was calculated according to the following formula [19]: glucose (mg/dL) X insulin (μIU/ml)/405. Insulin levels were determined by chemiluminescent assay system (Immulite 2000, Los Angeles, CA, USA).

Measurement of Serum Inflammatory Markers and Adipokines

High-sensitivity C-reactive protein (hsCRP) concentration was measured using a latex-enhanced immunonephelometric method on a BN II analyzer (Dade Behring, Newark, DE, USA). The concentrations of IL-6, adiponectin (R&D Systems Europe, Abingdon, Oxon, UK), TNF-α, leptin (R&D Systems Inc., Minneapolis, MN, USA), and resistin (LINCO Research, St Charles, MS, USA) were measured using enzyme-linked immunosorbent assay (ELISA) kits. The ELISA system had an intra-assay coefficients of variability (CV) of 3.48% and an inter-assay CV of 4.36%.

Assessment of Dialysis Adequacy, Residual Renal Function, and Glucose Absorption

Urea kinetic studies were conducted using a 24-hour collection of dialysate and urine at the time of study enrollment. Dialysis adequacy was determined by measuring total weekly urea clearance (Kt/V_{urea}) using the Watson equation [20], and residual renal function (RRF) was determined as the average of urea and creatinine clearance from a 24-hour urine collection. In addition, the amount of glucose absorption *via* peritoneal cavity was determined using the equation based on peritoneal equilibration test (PET) D/D_0 glucose at 4 hour as previously suggested [21].

Statistical Analyses

Statistical analysis was performed using SPSS for Windows, version 13.0 (SPSS Inc., Chicago, IL, USA). All data are expressed as mean ± standard deviation (SD). The Kolmogorov-Smirnov test was used to analyze the normality of the distribution of the parameters measured. Data with a skewed distribution, such as HOMA-IR and hsCRP, were expressed as log transformations. Geometric means for all log-normally distributed continuous variables were calculated and reported with geometric SD. Data for RRF are presented as median values and interquartile ranges. For normally distributed data, variables were compared between two groups using Student's *t*-test and the Chi-square test. In addition, Pearson's correlation tests were used to evaluate the relationships between HOMA-IR and clinical and laboratory parameters. For skewed data, the Mann-Whitney U-test was conducted to compare variables between two groups, and Spearman's correlation coefficient was determined to identify the relationship between co-variates. To define independent factors associated with log-transformed HOMA-IR (log HOMA-IR), multiple linear regression analyses were conducted, using variables such as age, gender and other variables that were significantly associated with log HOMA-IR in correlation analyses. *P*-value <0.05 was considered statistically significant.

Results

Baseline Demographic, Clinical and Laboratory Findings

The baseline characteristics of the study patients are shown in Table 1. The mean age was 48.6 years, 53.2% were male, and the mean PD duration was 73.2 months. Diabetic patients accounted for 6.5% of all patients. The average BMI was 21.6 kg/m^2. Metabolic syndrome was present in 14 patients (22.6%) The mean systolic and diastolic blood pressures were 132.5 ± 18.2 and 79.9 ± 9.3 mmHg, respectively, and study patients were taking an average of 2.4 antihypertensive pills daily. The geometric mean values of HOMA-IR, leptin, and hsCRP were 2.07, 14.7 ng/mL, and 0.76 mg/L respectively, while the average adiponectin concentration was 17.7 μg/ml. We obtained data for HOMA-IR in healthy non-obese subjects (n = 30), who were recruited for an earlier study [22]. Compared with

Table 1 Baseline characteristics of all patients

	Total (n=62)	HOMA-IR≤1.85	HOMA-IR>1.85	*P*-value
Age (years)	48.6 ± 11.8	47.0 ± 12.2	50.2 ± 11.3	0.286
Male gender	33 (53.2%)	16 (51.6%)	17 (54.8%)	0.799
Height (cm)	161.2 ± 8.1	162.0 ± 8.2	160.4 ± 8.0	0.425
Weight (kg)	56.2 ± 8.4	56.9 ± 7.6	55.5 ± 9.2	0.526
BMI (kg/m^2)	21.6 ± 2.1	21.6 ± 2.0	21.5 ± 2.3	0.788
Diabetes	4 (6.5%)	1 (3.2%)	3 (9.7%)	0.612
SBP (mmHg)	132.5 ± 18.2	136.8 ± 18.3	128.3 ± 17.2	0.062
SGA	6.5 ± 0.4	6.4 ± 0.5	6.5 ± 0.4	0.852
Dialysis duration (months)	73.2 ± 52.5	87.2 ± 48.6	69.3 ± 53.2	0.254
Metabolic syndrome	14 (22.6%)	5 (16.1%)	9 (29.0%)	0.224
Number of factors	1.8 ± 0.9	1.6 ± 0.8	1.9 ± 1.0	0.102
Icodextrin users, n (%)	19 (30.6%)	11 (35.5%)	8 (25.8%)	0.277
Medication				
RAS blockades	46 (74.2%)	22 (71.0%)	24 (77.4%)	0.562
Active vitamin D	13 (21.0%)	7 (22.6%)	6 (19.4%)	0.755
Statin	9 (14.5%)	3 (9.7%)	6 (19.4%)	0.473
Laboratory values				
Hemoglobin (g/dL)	10.8 ± 1.7	10.8 ± 1.4	11.4 ± 1.8	0.235
Calcium (mg/dL)	8.9 ± 0.7	8.8 ± 0.6	9.1 ± 0.8	0.199
Phosphorus (mg/dL)	5.1 ± 1.3	4.8 ± 1.3	5.3 ±1.2	0.122
PTH (pg/ml)*	135.2 ± 4.1	168.2 ± 3.8	108.7 ± 4.3	0.223
Albumin (g/dL)	3.9 ± 0.3	3.9 ± 0.3	3.9 ± 0.3	0.872
Total cholesterol (mg/dL)	178.6 ± 34.7	179.4 ± 35.3	177.9 ± 34.8	0.868
Triglyceride (mg/dL)	123.4 ± 62.2	103.4 ± 52.2	143.5 ± 65.7	0.010
LDL cholesterol (mg/dL)	105.5 ± 31.7	109.7 ± 33.3	101.2 ± 29.8	0.295
HDL cholesterol (mg/dL)*	49.3 ± 1.3	52.5 ± 1.4	46.2 ± 1.3	0.076
Fasting glucose (mg/dL)	89.0 ± 13.2	86.6 ± 8.5	91.4 ± 16.5	0.157
Insulin (μU/mL)	9.4 ± 6.4	6.7 ± 1.3	13.2 ± 7.1	<0.001
HOMA-IR*	2.07 ± 1.65	1.43 ± 1.21	2.99 ± 1.55	<0.001
HbA1c (%)	5.05 ± 0.74	4.95 ± 0.54	5.15 ± 0.85	0.275
hsCRP (mg/L)*	0.76 ± 3.17	0.54 ± 2.97	1.09 ± 3.09	0.017
Leptin (ng/mL)*	14.7 ± 4.7	13.2 ± 4.7	16.5 ± 4.8	0.570
Adiponectin (μg/mL)	17.7 ± 9.0	21.1 ± 8.0	14.4 ± 8.8	0.003
IL-6 (pg/mL)	6.07 ± 4.24	6.04 ± 3.25	6.13 ± 4.22	0.537
TNF-α (pg/mL)	5.51 ± 3.73	5.35 ± 3.28	5.68 ± 4.19	0.242
Resistin (ng/mL)	27.4 ± 14.2	26.1 ± 15.6	29.6 ± 11.7	0.460
Cystatin C (mg/L)	4.80 ± 2.52	4.76 ± 2.21	4.85 ± 3.02	0.478
Kt/V_{urea}	2.1 ±0.4	2.0 ± 0.3	2.2 ± 0.5	0.087
RRF (ml/min/1.73m^2)	0.35 (0-3.47)	0.25 (0-2.35)	0.43 (0-3.47)	0.175
D/P_{Cr} at 4 hr	0.67 ± 0.10	0.67 ± 0.10	0.66 ± 0.10	0.710
D/D_0 glucose at 4 hr	0.36 ± 0.08	0.36 ± 0.08	0.36 ± 0.07	0.993
Glucose infused (g/day)	119.2 ± 24.3	113.2 ± 23.0	125.3 ± 24.5	0.050
Glucose absorbed (g/day)	76.1 ± 15.7	72.3 ± 16.9	80.0 ± 19.8	0.104

Data are expressed as mean ± SD or geometric mean ± geometric SD*. RRF was expressed as median and interquartile ranges. HOMA-IR, homeostasis model assessment insulin resistance; BMI, body mass index; SBP, systolic blood pressure; SGA, subjective global assessment; RAS, renin angiotensin system; PTH, parathyroid hormone; LDL, low-density lipoprotein; HDL, high-density lipoprotein; IL-6, interleukin-6; RRF, residual renal function.

healthy controls, HOMA-IR was significantly higher in non-obese PD patients, suggesting that our study subjects had greater insulin-resistance than these healthy non-obese patients (2.07 ± 1.65 *vs*. 1.21 ± 0.85, P = 0.013, data not shown).

Comparisons of Demographic, Clinical and Laboratory Parameters According to HOMA-IR

Study patients were assigned to two groups according to median HOMA-IR. Demographic, clinical, and laboratory parameters were compared between the two groups (Table 1). Compared with patients with HOMA-IR <1.85 (L group), patients with HOMA-IR >1.85 (H group) had lower serum adiponectin levels (14.4 ± 8.8 *vs*. 21.1 ± 8.0 μg/mL, P = 0.003) and higher hsCRP levels (1.09 ± 3.09 *vs*. 0.54 ± 2.97 mg/L, P = 0.017), whereas serum concentrations of leptin, resistin, cystatin C did not differ between the two groups. Serum TNF-α levels were slightly elevated in the H group than in the L group, but it was not statistically significant. In addition, patients in the H group had significantly higher triglyceride levels (143.5 ± 65.7 *vs*. 103.4 ± 52.2 mg/dL, P = 0.010) than those in the L group. However, the proportion of icodextrin users, RRF, and the prevalence of metabolic syndrome were not different between the two groups. The amount of peritoneal glucose exposure and glucose absorption was slightly increased in the H group than in the L group, but it did not reach statistical significance.

Factors Associated with HOMA-IR

Bivariate correlation analyses were conducted to elucidate the relationships between HOMA-IR and other variables (Table 2 and Fig. 2). Log HOMA-IR

Table 2 Correlations of HOMA-IR with other variables

		Age	BMI	Log HOMA-IR	Log Leptin	APDN	Log IL-6	Resistin	Log hsCRP	T-chol	TG	LDL	Log HDL	Glucose absorbed	Kt/V_{urea}
Log HOMA-IR	γ	0.334	-0.067	1	0.069	-0.464	0.094	0.045	0.175	0.075	0.392	-0.088	-0.250	0.274	0.195
	P- value	0.008	0.603		0.596	<0.001	0.467	0.782	0.174	0.564	0.002	0.497	0.050	0.031	0.136
Log Leptin	γ	-0.105	0.184	-	1	0.021	0.114	0.235	-0.192	0.254	0.383	0.250	0.064	0.236	0.257
	P- value	0.416	0.152	-		0.870	0.485	0.145	0.135	0.046	0.002	0.050	0.620	0.118	0.047
APDN	γ	-0.140	-0.261	-	-	1	0.037	0.140	-0.070	0.125	-0.302	0.254	0.278	-0.188	0.053
	P- value	0.277	0.040	-	-		0.822	0.388	0.587	0.333	0.017	0.046	0.029	0.178	0.686
Log IL-6	γ	0.237	0.205	-	-	-	1	0.017	0.280	-0.174	-0.097	-0.158	-0.122	0.046	-0.275
	P- value	0.141	0.204	-	-	-		0.917	0.080	0.282	0.553	0.329	0.454	0.777	0.090
Resistin	γ	-0.196	-0.048	-	-	-	-	1	-0.124	-0.130	0.036	-0.085	-0.068	0.265	0.312
	P- value	0.226	0.771	-	-	-	-		0.447	0.426	0.824	0.603	0.677	0.098	0.053
Log hsCRP	γ	0.189	0.131	-	-	-	-	-	1	-0.144	-0.061	-0.059	-0.264	0.123	-0.196
	P- value	0.142	0.311	-	-	-	-	-		0.264	0.637	0.651	0.038	0.341	0.133
T-chol	γ	0.207	-0.039	-	-	-	-	-	-	1	0.489	0.855	0.082	-0.095	0.291
	P- value	0.106	0.762	-	-	-	-	-	-		<0.001	<0.001	0.527	0.463	0.024
TG	γ	0.135	-0.078	-	-	-	-	-	-	-	1	0.251	-0.424	0.249	0.317
	P- value	0.295	0.546	-	-	-	-	-	-	-		0.049	0.001	0.105	0.014
LDL	γ	0.110	-0.067	-	-	-	-	-	-	-	-	1	-0.100	-0.049	0.297
	P- value	0.396	0.604	-	-	-	-	-	-	-	-		0.440	0.705	0.021
Log HDL	γ	-0.152	-0.061	-	-	-	-	-	-	-	-	-	1	-0.024	-0.068
	P- value	0.238	0.636	-	-	-	-	-	-	-	-	-		0.851	0.606
Glucose absorbed	γ	-0.133	0.149	-	-	-	-	-	-	-	-	-	-	1	-0.221
	P- value	0.314	0.249	-	-	-	-	-	-	-	-	-	-		0.090

HOMA-IR, homeostasis model assessment insulin resistance; BMI, body mass index; APDN, adiponectin; ; IL-6, interleukin-6; hsCRP, high sensitive C-reactive protein; T-chol, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein

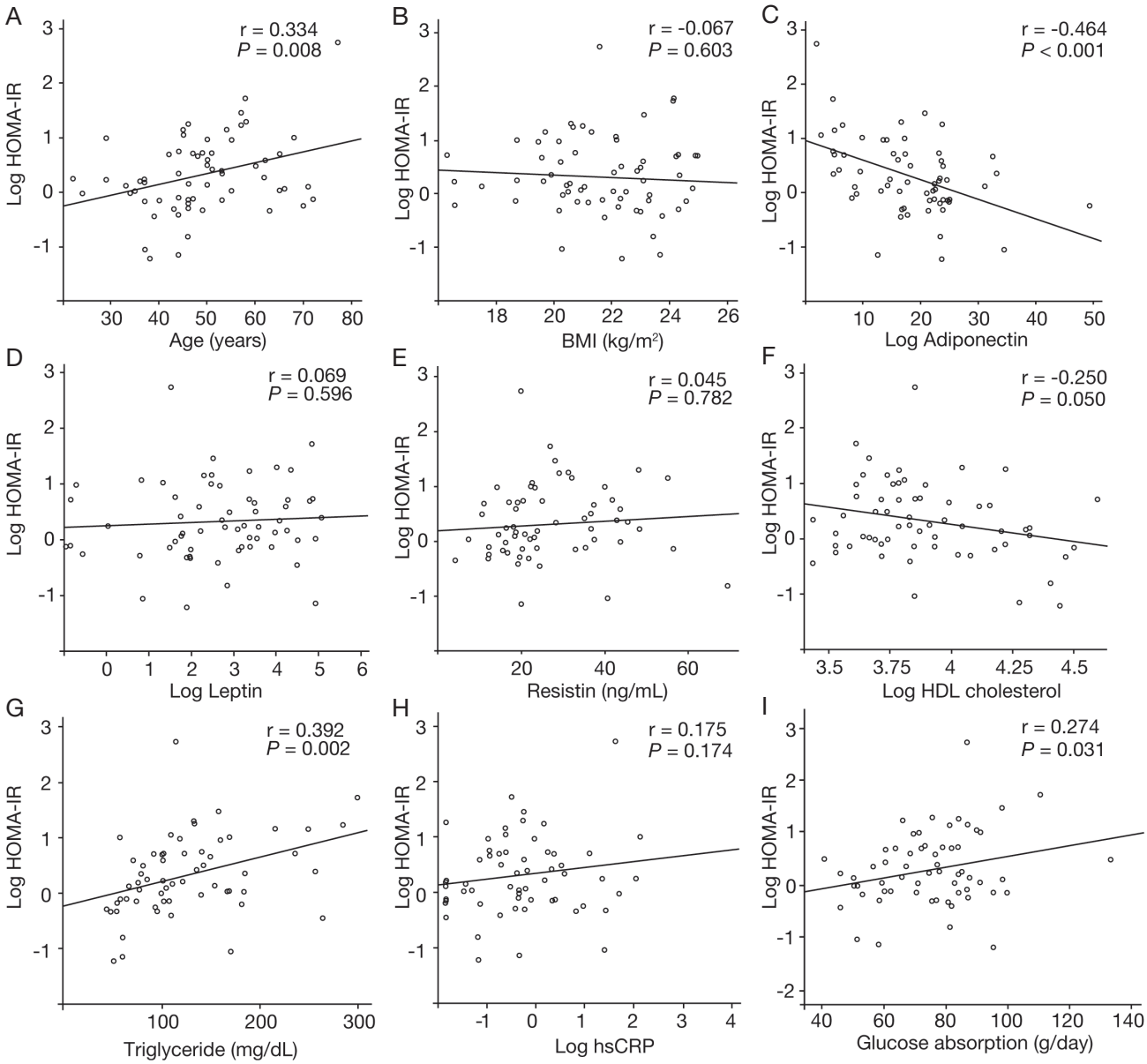

Fig. 2 Correlations of HOMA-IR with other variables.
Log HOMA-IR was inversely correlated with adiponectin (C) and log HDL cholesterol (F) but was positively related to age (A), triglyceride (G), and peritoneal glucose absorption (I). However, it was not associated with BMI (B), log leptin (D), resistin (E), and log hsCRP (H). HOMA-IR, homeostasis model assessment insulin resistance; BMI, body mass index; HDL, high-density lipoprotein; hsCRP, high sensitive C-reactive protein; IL, interleukin

was inversely correlated with adiponectin (γ = -0.464, $P < 0.001$) and log HDL cholesterol (γ = -0.250, P = 0.050) but was positively related to age (γ = 0.334, P = 0.008), triglyceride (γ = 0.392, P = 0.002), and peritoneal glucose absorption (γ = 0.274, P = 0.031). However, log leptin and log hsCRP were not associated with log HOMA-IR.

In contrast, serum adiponectin level was negatively correlated with BMI (γ = -0.261, P = 0.040) and triglyceride (γ = -0.302, P = 0.017), while being positively correlated with LDL cholesterol (γ = 0.254, P = 0.046) and log HDL (γ = 0.278, P = 0.029).

To further identify factors that were independently associated with HOMA-IR, multiple linear regression analysis was conducted. Age, gender, and variables that were significantly correlated with HOMA-IR in the

Table 3 Multiple linear regression analysis for HOMA-IR in non-obese patients with BMI ≤25 kg/m^2

Variables	β ± SE	*P*-value
Age	0.007 ± 0.007	0.114
Male gender	0.110 ± 0.173	0.529
DM	0.664 ± 0.326	0.047
Adiponectin	-0.023 ± 0.009	0.015
Triglyceride	0.003 ± 0.002	0.026
Log HDL	0.185 ± 0.309	0.552
Log hsCRP	0.094 ± 0.068	0.176
Glucose absorption	0.006 ± 0.004	0.146

DM, diabetes; HDL, high-density lipoprotein; hsCRP, high sensitive C-reactive protein

Table 4 Multiple linear regression analysis for HOMA-IR in non-obese patients with waist circumference < 90 cm for men and < 85 cm for women (n=66)

Variables	β ± SE	*P*-value
Age	0.012 ± 0.006	0.080
Male gender	0.022 ± 0.159	0.892
DM	0.662 ± 0.252	0.011
Adiponectin	-0.024 ± 0.008	0.007
Triglyceride	0.003 ± 0.002	0.037
Log HDL	0.030 ± 0.272	0.897
Log hsCRP	0.087 ± 0.052	0.160
Glucose absorption	0.008 ± 0.012	0.529

DM, diabetes; HDL, high-density lipoprotein; hsCRP, high sensitive C-reactive protein

bivariate correlation analyses were used to construct a multiple linear regression model. To address potential multicollinearity, variance inflation factors (VIF) were analyzed. We found no variables with VIF >10, suggesting that multicollinearity was unlikely. In our model, adiponectin (β ± SE, -0.023 ± 0.009; $P = 0.015$), DM (β ± SE, 0.664 ± 0.326; $P = 0.047$), and triglyceride (β ± SE, 0.003 ± 0.002; $P = 0.026$) were identified as independent factors associated with HOMA-IR (Table 3). This finding was corroborated by a separate analysis using the definition of obesity based on WC (Table 4), suggesting that low circulating adiponectin levels were significantly associated with HOMA-IR.

Discussion

In this cross-sectional study of 62 'non-obese' PD patients, we showed that HOMA-IR was independently and negatively correlated with circulating adiponectin level. However, we failed to find any significant association between HOMA-IR and other adipokines or inflammatory cytokines. These findings suggest that, among these parameters, low circulating adiponectin level might play a role in the pathogenesis of IR in non-obese ESRD patients.

Although IR is multifactorial in origin, obesity has long been linked with IR. In obesity, white adipose tissue serves as a major source of locally produced pro-inflammatory cytokines such as Il-6 and TNF-α [5], which can alter insulin sensitivity by triggering key steps in the insulin signaling pathway. In addition, several adipokines derived from adipocytes are known to be involved in IR pathogenesis. Leptin increases in obesity because of leptin resistance [23]. Leptin can suppress insulin secretion from the pancreas [24], and hyperleptinemia is observed in chronic kidney disease (CKD) patients with hyperinsulinemia [25]. In contrast, adiponectin, which has anti-inflammatory and insulin-sensitizing effects, decreases in obesity [26]. Taken together, inflammatory cytokines and altered adipokines from adipose tissue act together to hinder insulin action, resulting in an insulin resistant state. Interestingly, because adipokines are normally excreted by the kidney, their levels are elevated in ESRD patients, even if they are not obese [9, 27]. In addition, chronic inflammation is a key feature of CKD [28]. However, whether the effects of adipokines and vinflammation on IR exist in 'non-obese' ESRD patients is currently unknown. Therefore, investigating this issue is interesting because circulating levels in these patients are altered irrespective of obesity.

A major finding of our study is that adiponectin showed an independent and negative association with IR even in non-obese PD patients. This finding is in agreement with previous studies of ESRD patients that included both obese and non-obese patients [29, 30]. In addition, circulating adiponectin level was inversely associated with adverse metabolic risk factors [29, 30], and its decreased level independently predicted cardiovascular outcome [29, 31]. This indicated that, even if adiponectin is elevated due to decreased renal clearance, low adiponectin level appears to be unfavorable in ESRD patients. Interestingly, an association between hypoadiponectinemia and IR was previously suggested in 'non-obese' patients without CKD [32-34]. In these studies, circulating adiponectin level was decreased and independently associated with IR parameters in non-obese but otherwise insu-

lin-resistant states. These findings suggest that hypoadiponectinemia might be involved in IR even in non-obese patients even though adiposity, which is a strong suppressor of adiponectin, is not evident. However, whether adiponectin is protective in non-obese uremic conditions requires further investigation.

Recently, high-molecular-weight (HMW) adiponectin is considered biologically active and it is reported that HMW-to-total adiponectin ratio (HMWR) was more predictive of insulin resistance and metabolic syndrome [35]. However, this finding was not replicated in a subsequent study by Bluher et al showing that HMWR was not superior to total adiponectin in assessing insulin sensitivity [36]. To explore this issue, we measured HMW adiponectin using ELSIA method and found that there was a highly significant correlation between HMW and total adiponectin ($\gamma = 0.660$, $P < 0.001$). When HMWR was entered instead of total adiponectin in a multivariate regression analysis, it was independently correlated with HOMA-IR ($\beta \pm SE$, -0.025 ± 0.007; $P = 0.022$, data not shown). In addition, the area under receiver operating characteristic curve of total adiponectin and HMWR for the prediction of HOMA-IR >1.85 was 0.66 and 0.68, respectively, suggesting that both forms of adiponectin was comparable in predicting insulin resistance.

In this study, leptin was not associated with IR. This finding contradicted the results of a previous study by Zoccali *et al*. showing that leptin positively correlated with HOMA-IR in ESRD patients undergoing hemodialysis [37]. This discrepancy could be explained by differences in the characteristics of study subjects and dialysis modalities. Our study included only non-overweight and non-obese PD patients in whom the amount of adipose tissue was substantially decreased. Therefore, it can be surmised that less leptin was produced in the 'non-obese' condition. In fact, our study patients had relatively lower leptin levels than did ESRD patients in previous studies [9,38,39]. In addition, serum resistin level was not associated with IR in this study. Resistin is also secreted by adipocytes and its circulating level is increased in CKD patients [40]. Although a previous study suggested a potential link between obesity and IR [41], subsequent studies did not find a significant association between resistin and IR in uremic patients [40, 42]. In line with these findings, serum resistin level did not correlate with HOMA-IR in our study. Taken together, it can be speculated that leptin and resistin do not contribute to IR in non-obese ESRD patients despite the elevated levels due to decreased renal clearance.

In addition to obesity, uremia-associated factors such as chronic inflammation or oxidative stress might contribute to IR in ESRD patients [8]. However, in the present study, we did not find that chronic inflammation was significantly associated with IR, although we observed a trend of positive correlation between hsCRP and HOMA-IR. This might stem from the fact that our study subjects had strongly attenuated inflammation. The mean concentration of hsCRP was 0.76 mg/L. This value is considerably lower than earlier reported [43], given the fact that these non-obese patients of our study were undergoing PD. Interestingly, adiposity is reported to amplify inflammation and oxidative stress in CKD patients [43]. In addition, a Japanese study of 1,624 non-diabetic subjects found that the association between CRP and HOMA-IR was decreased after adjustment for BMI [44]. Therefore, in non-obese patients, inflammation might be lower, resulting in a weakened association with IR.

A unique feature of PD is a large amount of glucose absorption *via* peritoneal cavity. This may aggravate insulin resistance in PD patients. In this study, the amount of peritoneal glucose absorption was slightly elevated in patients with HOMA-IR > 1.85 than those with HOMA-IR < 1.85 (72.3 ± 16.9 *vs*. 80.0 ± 19.8 g/day, $P=0.104$), and it was significantly correlated with log HOMA-IR ($\gamma = 0.274$, $P=0.031$). However, peritoneal glucose absorption was not independently associated with HOMA-IR in the multivariate analysis. In addition, it was not significantly correlated with serum adiponectin levels. These findings suggest that insulin resistance is not entirely determined by peritoneal glucose absorption.

Several shortcomings of this study should be discussed. First, as a cross-sectional study at a single center, it was subject to biases inherent to this study design. In addition, a cross-sectional design does not allow for cause-and-effect relationships to be drawn between adiponectin and IR. In fact, despite the previously reported insulin-sensitizing effect of adiponectin [26], circulating adiponectin level was reduced during hyperinsulinemic euglycemic clamp studies, suggesting a possible suppressive effect of insulin on adiponectin [45, 46]. Second, we used HOMA-IR as the IR indicator rather than the hyperinsulinemic euglycemic clamp test, which is regarded as the gold standard for measuring IR. However, this method is cumber-

some and inconvenient. HOMA-IR is reported to be well correlated with the hyperinsulinemic euglycemic test and has been validated in CKD patients [47]. Third, in this study, we primarily used BMI as an indicator of obesity. Recently, WC has been suggested as a better parameter of central obesity than BMI in the general population [48]. However, a concern in ESRD patients undergoing PD is that WC might not reliably reflect abdominal visceral fat content because of the presence of a Tenckhoff catheter in situ, lax skin after repeated distention of the abdomen by PD fluid, and potential residual peritoneal dialysate within the abdominal cavity. This renders measurement of WC inaccurate in this group. Nevertheless, to clarify this concern, we conducted a separate analysis using the definition of obesity based on WC. We found that an independent association between low adiponectin level and HOMA-IR still remained (Table 4). Finally, patients with hemodialysis were not included in this study. However, patients with PD were of our particular interest because they are more likely insulin resistant than those with HD due to large amount of glucose absorption *via* peritoneal cavity as aforementioned. Therefore, it needs to be verified whether our findings can be generalized to patients with HD.

In conclusion, this study showed that low circulating adiponectin level was independently associated with HOMA-IR in non-obese ESRD patients undergoing PD. Our findings also suggested that leptin and resistin from adipose tissue do not appear to contribute to IR in the non-obese condition even though their circulating concentrations were elevated due to decreased renal clearance, irrespective of obesity.

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References

1. Collins AJ, Li S, Ma JZ, Herzog C (2001) Cardiovascular disease in end-stage renal disease patients. *Am J Kidney Dis* 38:S26-29.
2. Zoccali C (2000) Cardiovascular risk in uraemic patients-is it fully explained by classical risk factors? *Nephrol Dial Transplant* 15:454-457.
3. Reaven GM (1988) Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 37:1595-1607.
4. Sethi JK, Vidal-Puig AJ (2007) Thematic review series: adipocyte biology. Adipose tissue function and plasticity orchestrate nutritional adaptation. *J Lipid Res* 48:1253-1262.
5. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112:1796-1808.
6. Guarnieri G, Faccini L, Lipartiti T, Ranieri F, Spangaro F, Giuntini D, Toigo G, Dardi F, Berquier-Vidali F, Raimondi A (1980) Simple methods for nutritional assessment in hemodialyzed patients. *Am J Clin Nutr* 33:1598-1607.
7. Shinohara K, Shoji T, Emoto M, Tahara H, Koyama H, Ishimura E, Miki T, Tabata T, Nishizawa Y (2002) Insulin resistance as an independent predictor of cardiovascular mortality in patients with end-stage renal disease. *J Am Soc Nephrol* 13:1894-1900.
8. Guarnieri G, Zanetti M, Vinci P, Cattin MR, Barazzoni R (2009) Insulin resistance in chronic uremia. *J Ren Nutr* 19:20-24.
9. Taskapan MC, Taskapan H, Sahin I, Keskin L, Atmaca H, Ozyalin F (2007) Serum leptin, resistin, and lipid levels in patients with end stage renal failure with regard to dialysis modality. *Ren Fail* 29:147-154.
10. Lin SH, Lin YF, Kuo SW, Hsu YJ, Hung YJ (2003) Rosiglitazone improves glucose metabolism in non-diabetic uremic patients on CAPD. *Am J Kidney Dis* 42:774-780.
11. Nurmi J, Korkeila M, Honkanen E, Lindgren L, Castren M (2010) Effect of peritoneal dialysis on abdominal circumference. *Perit Dial Int* 30:215-217.
12. Lee SY, Park HS, Kim DJ, Han JH, Kim SM, Cho GJ, Kim DY, Kwon HS, Kim SR, Lee CB, Oh SJ, Park CY, Yoo HJ (2007) Appropriate waist circumference cutoff points for central obesity in Korean adults. *Diabetes Res Clin Pract* 75:72-80.
13. Deurenberg-Yap M, Chew SK, Deurenberg P (2002) Elevated body fat percentage and cardiovascular risks at low body mass index levels among Singaporean Chinese, Malays and Indians. *Obes Rev* 3:209-215.
14. Ko GT, Chan JC, Cockram CS, Woo J (1999) Prediction of hypertension, diabetes, dyslipidaemia or albuminu-

ria using simple anthropometric indexes in Hong Kong Chinese. *Int J Obes Relat Metab Disord* 23:1136-1142.
15. Ko GT, Tang J, Chan JC, Sung R, Wu MM, Wai HP, Chen R (2001) Lower BMI cut-off value to define obesity in Hong Kong Chinese: an analysis based on body fat assessment by bioelectrical impedance. *Br J Nutr* 85:239-242.
16. Yajnik CS, Yudkin JS (2004) The Y-Y paradox. *Lancet* 363:163.
17. Detsky AS, McLaughlin JR, Baker JP, Johnston N, Whittaker S, Mendelson RA, Jeejeebhoy KN (1987) What is subjective global assessment of nutritional status? *JPEN J Parenter Enteral Nutr* 11:8-13.
18. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Jr., Spertus JA, Costa F (2005) Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 112:2735-2752.
19. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412-419.
20. Watson PE, Watson ID, Batt RD (1980) Total body water volumes for adult males and females estimated from simple anthropometric measurements. *Am J Clin Nutr* 33:27-39.
21. Bodnar DM, Busch S, Fuchs J, Piedmonte M, Schreiber M (1993) Estimating glucose absorption in peritoneal dialysis using peritoneal equilibration tests. *Adv Perit Dial* 9:114-118.
22. Han SH, Choi SH, Cho BJ, Lee Y, Lim S, Park YJ, Moon MK, Lee HK, Kang SW, Han DS, Kim YB, Jang HC, Park KS (2010) Serum fibroblast growth factor-21 concentration is associated with residual renal function and insulin resistance in end-stage renal disease patients receiving long-term peritoneal dialysis. *Metabolism* 59:1656-1662.
23. Enriori PJ, Evans AE, Sinnayah P, Cowley MA (2006) Leptin resistance and obesity. *Obesity (Silver Spring)* 14 Suppl 5:254S-258S.
24. Kieffer TJ, Heller RS, Leech CA, Holz GG, Habener JF (1997) Leptin suppression of insulin secretion by the activation of ATP-sensitive K+ channels in pancreatic beta-cells. *Diabetes* 46:1087-1093.
25. Stenvinkel P, Heimburger O, Lonnqvist F (1997) Serum leptin concentrations correlate to plasma insulin concentrations independent of body fat content in chronic renal failure. *Nephrol Dial Transplant* 12:1321-1325.
26. Kadowaki T, Yamauchi T (2005) Adiponectin and adiponectin receptors. *Endocr Rev* 26:439-451.
27. Kataoka H, Sharma K (2006) Renal handling of adipokines. *Contrib Nephrol* 151:91-105.
28. Oberg BP, McMenamin E, Lucas FL, McMonagle E, Morrow J, Ikizler TA, Himmelfarb J (2004) Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. *Kidney Int* 65:1009-1016.
29. Zoccali C, Mallamaci F, Tripepi G, Benedetto FA, Cutrupi S, Parlongo S, Malatino LS, Bonanno G, Seminara G, Rapisarda F, Fatuzzo P, Buemi M, Nicocia G, Tanaka S, Ouchi N, Kihara S, Funahashi T, Matsuzawa Y (2002) Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. *J Am Soc Nephrol* 13:134-141.
30. Huang JW, Yen CJ, Chiang HW, Hung KY, Tsai TJ, Wu KD (2004) Adiponectin in peritoneal dialysis patients: a comparison with hemodialysis patients and subjects with normal renal function. *Am J Kidney Dis* 43:1047-1055.
31. Rao M, Li L, Tighiouart H, Jaber BL, Pereira BJ, Balakrishnan VS (2008) Plasma adiponectin levels and clinical outcomes among haemodialysis patients. *Nephrol Dial Transplant* 23:2619-2628.
32. Pellme F, Smith U, Funahashi T, Matsuzawa Y, Brekke H, Wiklund O, Taskinen MR, Jansson PA (2003) Circulating adiponectin levels are reduced in nonobese but insulin-resistant first-degree relatives of type 2 diabetic patients. *Diabetes* 52:1182-1186.
33. Tai ES, Tan ML, Stevens RD, Low YL, Muehlbauer MJ, Goh DL, Ilkayeva OR, Wenner BR, Bain JR, Lee JJ, Lim SC, Khoo CM, Shah SH, Newgard CB (2010) Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. *Diabetologia* 53:757-767.
34. Taniguchi A, Fukushima M, Ohya M, Nakai Y, Yoshii S, Nagasaka S, Matsumoto K, Taki Y, Kuroe A, Nishimura F, Seino Y (2006) Interleukin 6, adiponectin, leptin, and insulin resistance in nonobese Japanese type 2 diabetic patients. *Metabolism* 55:258-262.
35. Hara K, Horikoshi M, Yamauchi T, Yago H, Miyazaki O, Ebinuma H, Imai Y, Nagai R, Kadowaki T (2006) Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. *Diabetes Care* 29:1357-1362.
36. Bluher M, Brennan AM, Kelesidis T, Kratzsch J, Fasshauer M, Kralisch S, Williams CJ, Mantzoros CS (2007) Total and high-molecular weight adiponectin in relation to metabolic variables at baseline and in response to an exercise treatment program: comparative evaluation of three assays. *Diabetes Care* 30:280-285.
37. Zoccali C, Tripepi G, Cambareri F, Catalano F, Finocchiaro P, Cutrupi S, Pizzini P, Testa A, Spoto B, Panuccio V, Enia G, Mallamaci F (2005) Adipose tissue cytokines, insulin sensitivity, inflammation, and cardiovascular outcomes in end-stage renal disease patients. *J Ren Nutr* 15:125-130.

38. Merabet E, Dagogo-Jack S, Coyne DW, Klein S, Santiago JV, Hmiel SP, Landt M (1997) Increased plasma leptin concentration in end-stage renal disease. *J Clin Endocrinol Metab* 82:847-850.
39. Diez JJ, Iglesias P, Fernandez-Reyes MJ, Aguilera A, Bajo MA, Alvarez-Fidalgo P, Codoceo R, Selgas R (2005) Serum concentrations of leptin, adiponectin and resistin, and their relationship with cardiovascular disease in patients with end-stage renal disease. *Clin Endocrinol (Oxf)* 62:242-249.
40. Axelsson J, Bergsten A, Qureshi AR, Heimburger O, Barany P, Lonnqvist F, Lindholm B, Nordfors L, Alvestrand A, Stenvinkel P (2006) Elevated resistin levels in chronic kidney disease are associated with decreased glomerular filtration rate and inflammation, but not with insulin resistance. *Kidney Int* 69:596-604.
41. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA (2001) The hormone resistin links obesity to diabetes. *Nature* 409:307-312.
42. Kielstein JT, Becker B, Graf S, Brabant G, Haller H, Fliser D (2003) Increased resistin blood levels are not associated with insulin resistance in patients with renal disease. *Am J Kidney Dis* 42:62-66.
43. Ramos LF, Shintani A, Ikizler TA, Himmelfarb J (2008) Oxidative stress and inflammation are associated with adiposity in moderate to severe CKD. *J Am Soc Nephrol* 19:593-599.
44. Nakanishi N, Shiraishi T, Wada M (2005) Association between C-reactive protein and insulin resistance in a Japanese population: the Minoh Study. *Intern Med* 44:542-547.
45. Brame LA, Considine RV, Yamauchi M, Baron AD, Mather KJ (2005) Insulin and endothelin in the acute regulation of adiponectin in vivo in humans. *Obes Res* 13:582-588.
46. Mohlig M, Wegewitz U, Osterhoff M, Isken F, Ristow M, Pfeiffer AF, Spranger J (2002) Insulin decreases human adiponectin plasma levels. *Horm Metab Res* 34:655-658.
47. Shoji T, Emoto M, Nishizawa Y (2001) HOMA index to assess insulin resistance in renal failure patients. *Nephron* 89:348-349.
48. Pouliot MC, Despres JP, Lemieux S, Moorjani S, Bouchard C, Tremblay A, Nadeau A, Lupien PJ (1994) Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol* 73:460-468.