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ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 1414 – 1421
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Adipokines influencing metabolic and cardiovascular disease are differentially regulated in maintenance hemodialysis

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Received 6 November 2007; accepted 19 May 2008

Abstract

Adipokines including leptin, adiponectin, visfatin, resistin, and interleukin (IL)-6 significantly influence energy metabolism, insulin sensitivity, and cardiovascular health. In the current study, we investigated serum levels of these adipokines in diabetic and nondiabetic patients on maintenance hemodialysis (MD) as compared with controls with a glomerular filtration rate greater than 50 mL/min. Serum leptin, adiponectin, high–molecular-weight (HMW) adiponectin, visfatin, resistin, and IL-6 were determined by enzyme-linked immunosorbent assay in control (n = 60) and MD (n = 60) patients and correlated to clinical and biochemical measures of renal function, glucose, and lipid metabolism, as well as inflammation. Adiponectin, visfatin, resistin, and IL-6 were significantly elevated in MD patients as compared with controls. In multivariate analyses, sex and body mass index were independently correlated with serum leptin levels in both controls and MD patients. Furthermore, insulin resistance was independently and negatively associated with adiponectin and HMW adiponectin in both groups. Moreover, circulating resistin levels were independently correlated with serum visfatin concentrations in control and MD patients. However, various independent associations were only found in either controls or patients on MD. Thus, serum IL-6 levels were strongly and independently associated with C reactive protein and resistin in MD patients but not control subjects. We show that levels of various adipokines are significantly increased in MD patients. Furthermore, regulation of adipokines in vivo strongly depends on renal function. Regulation of HMW adiponectin is similar as compared with total adiponectin in the patients studied.

1. Introduction

The incidence of obesity is rapidly increasing in industrialized countries. When weight is gained, both hyperplasia and hypertrophy of adipocytes are found. Adipose tissue secretes various proteins, so-called adipokines. Within the last years, it has become obvious that obesity and various components of the metabolic syndrome such as insulin resistance and hypertension are strongly linked because of the differential secretory function of adipose tissue. Furthermore, there is an increasing body of evidence that adipokines significantly contribute to the increased risk of cardiovascular disease found in obesity [1,2].

Various adipokines have been better characterized in recent years. Among those, adiponectin is an insulin-sensitizing and vasoprotective fat-secreted factor [3]. Here, activation of adenosine monophosphate kinase, which in turn inhibits acetyl coenzyme A carboxylase, appears as a primary mechanism for the beneficial effects of this adipokine [3]. Moreover, adiponectin forms several complexes; and the high–molecular-weight (HMW) complex has been postulated as the active form of the protein [4]. Recently, Fukuhara et al [5] isolated visfatin as a novel adipokine that improves glucose tolerance by binding to the insulin receptor and might play a role in the development of obesity-associated insulin resistance and type 2 diabetes mellitus (T2DM). However, the article was subsequently retracted because several aspects of the original work including insulin receptor binding and activation by visfatin could not be repeated in all experiments [6]. Resistin and interleukin (IL)-6 have been characterized as insulin

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doi:10.1016/j.metabol.2008.05.011

resistance–inducing adipokines that potentially increase the risk of cardiovascular disease. Thus, data obtained in rodents convincingly show that resistin inhibits insulin signaling in insulin-sensitive tissues, leading to glucose intolerance [7,8]. However, the importance of resistin in human obesity and insulin resistance is less clear. Human adipocytes do not secrete substantial amounts of resistin, and its serum levels do not correlate to insulin resistance and obesity [9]. For IL-6, on the other hand, the data in humans are more convincing. Thus, IL-6 plasma concentrations independently predict the future risk of T2DM and cardiovascular disease in large epidemiologic studies [1]. Interleukin-6 impairs intracellular insulin signaling in fat cells and hepatocytes with decreased activation of insulin receptor substrates that is potentially mediated via up-regulation of suppressor of cytokine signaling protein synthesis [10]. Leptin is an adipokine that primarily influences appetite. Thus, leptin decreases orexigenic and increases anorexigenic peptide synthesis in the hypothalamus; and an inactivating mutation of the leptin gene or its receptor leads to massive obesity in both rodents and humans [11]. Furthermore, convincing evidence has been presented that circulating leptin is also a potent proatherogenic adipokine [12].

Whereas the connection between leptin, adiponectin, visfatin, resistin, and IL-6 on one hand and components of the metabolic syndrome on the other hand has been studied in detail, little is known about the regulation of these adipokines in renal dysfunction. Therefore, we determined serum levels of leptin, adiponectin, visfatin, resistin, and IL-6 in 60 patients on maintenance hemodialysis (MD) (32 diabetic and 28 nondiabetic subjects) and 60 controls (30 diabetic and 30 nondiabetic subjects) with a glomerular filtration rate (GFR) greater than 50 mL/min and correlated concentrations of these adipokines to clinical and biochemical measures of renal function, glucose, and lipid metabolism, as well as inflammation, in both groups. Furthermore, we measured HMW adiponectin with a specific novel enzyme-linked immunosorbent assay (ELISA) system to elucidate whether regulation of this type of adiponectin might be different as compared with total adiponectin.

2. Subjects and methods

2.1. Subjects

One hundred twenty white men (n = 62) and women (n = 58) were recruited, with 60 patients having a GFR greater than 50 mL/min (controls) as assessed by Cockroft-Gault formula and 60 patients being on MD. Body mass index (BMI) was calculated as weight divided by squared height. Waist to hip ratio was calculated after waist and hip circumferences were determined. The age of the study population ranged from 32 to 85 years, and BMI was from 18.7 to 46.1 kg/m^2. *Type 2 diabetes mellitus* in control and MD patients was defined as fasting blood glucose of at least 126 mg/dL or use of insulin or oral hypoglycemic medications. When these criteria were applied to the study population, 30 of 60 controls and 32 of 60 MD patients presented with T2DM. In the 30 controls who did not meet these criteria for T2DM, the disease was further excluded by performing 75-g oral glucose tolerance tests. Here, these patients showed 2-hour glucose levels of less than 200 mg/dL. In contrast, T2DM was not excluded by 75-g oral glucose tolerance tests in the 28 MD patients who did not meet the above-mentioned criteria for T2DM because of the necessary fluid restriction. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as previously described [13]. Patients with severe conditions including generalized inflammation or end-stage malignant diseases were excluded from the study. The study was approved by the local ethics committee, and all subjects gave written informed consent before taking part in the study.

2.2. Assays

Blood samples were taken after an overnight fast. In MD patients, blood was drawn just before the hemodialysis started. Serum insulin was determined with a 2-site chemiluminescent enzyme immunometric assay for the Immulite automated analyzer (Diagnostic Products, Los Angeles, CA). Adiponectin (Mediagnost, Reutlingen, Germany), HMW adiponectin (Fujirebio, Tokyo, Japan), resistin (Mediagnost), and visfatin (Adipogen, Seoul, South Korea) serum levels were measured with commercially available ELISAs according to the manu-

Table 1
Baseline characteristics of the study population

	Control	MD
n	60	60
Age (y)	62 ± 10	64 ± 12
Sex (male/female)	27/33	35/25
Diabetic/nondiabetic	30/30	32/28
BMI (kg/m^2)	30.2 ± 5.5	27.2 ± 5.1*
SBP (mm Hg)	127 ± 14	123 ± 22
DBP (mm Hg)	74 ± 10	70 ± 11
Creatinine (μmol/L)	76 ± 16	761 ± 270*
GFR (mL/min)	100 ± 36	10 ± 5*
PTH (pmol/L)	4.1 ± 1.6	22.1 ± 20.2*
FG (mmol/L)	6.5 ± 2.3	5.5 ± 2.2*
FI (pmol/L)	59.9 ± 70.2	73.7 ± 108.4
HOMA-IR	2.5 ± 2.4	3.3 ± 7.3
FFA (mmol/L)	0.6 ± 0.3	0.6 ± 0.4
Cholesterol (mmol/L)	5.2 ± 1.1	4.4 ± 1.0*
TG (mmol/L)	1.5 ± 0.8	2.1 ± 1.3*
CRP (mg/L)	3.6 ± 2.9	16.1 ± 25.2*
Leptin (μg/L)	23.0 ± 23.2	50.2 ± 71.3
Adiponectin (mg/L)	6.7 ± 3.8	16.3 ± 11.1*
HMW (mg/L)	8.2 ± 5.1	16.1 ± 9.2*
Visfatin (μg/L)	2.5 ± 3.9	4.5 ± 3.8*
Resistin (μg/L)	5.8 ± 1.7	11.4 ± 4.6*
IL-6 (ng/L)	2.7 ± 1.5	13.2 ± 12.9*

Values for mean ± SD are shown. DBP indicates diastolic blood pressure; FG, fasting glucose; FI, fasting insulin; SBP, systolic blood pressure; TG, triglycerides.

* $P < .05$ as compared with control as assessed by Mann-Whitney U test.

facturers' instructions. Leptin levels were determined using an in-house assay as described previously [14]. Serum creatinine, parathyroid hormone (PTH), free fatty acids (FFA), cholesterol, triglycerides, IL-6, and C-reactive protein (CRP) were measured by standard laboratory methods in a certified laboratory.

2.3. Statistical analysis

For statistical analysis, SPSS software version 11.5 (SPSS, Chicago, IL) was used. Differences between controls and MD patients were assessed as indicated in the figure legends. Correlations were performed using the Spearman rank correlation method. To adjust the effects of covariates and identify independent relationships, multivariate linear regression analyses were performed. Here, distribution was tested for normality using Shapiro-Wilk W test; and non-normally distributed parameters were logarithmically transformed before performing multivariate analyses. A P value less than .05 was considered as statistically significant in all analyses.

3. Results

3.1. Serum levels of adiponectin, HMW adiponectin, visfatin, resistin, and IL-6 are increased in MD patients as compared with controls

Clinical characteristics of the subjects studied (control and MD) are summarized in Table 1. Furthermore, the characteristics of the subgroups further divided into nondiabetic and diabetic subjects are presented in Table 2. Mean serum adiponectin (16.3 ± 11.1 vs 6.7 ± 3.8 mg/L), HMW adiponectin (16.1 ± 9.2 vs 8.2 ± 5.1 mg/L), visfatin (4.5 ± 3.8 vs 2.5 ± 3.9 μg/L), resistin (11.4 ± 4.6 vs 5.8 ± 1.7 μg/L), and IL-6 (13.2 ± 12.9 vs 2.7 ± 1.5 ng/L) were significantly increased in MD patients as compared with controls (Table 1). Mean leptin concentrations were about 2-fold elevated in MD patients (50.2 ± 71.3 μg/L) as compared with control subjects (23.0 ± 23.3 μg/L); however, this difference did not reach statistical significance (Table 1). Serum levels of the adipokines studied were not significantly different between diabetic as compared with nondiabetic subjects (data not shown). Mean serum leptin levels were significantly higher in women (51.9 ± 66.3 μg/L) as compared with men (22.3 ± 35.7 μg/L), whereas resistin concentrations were significantly decreased in female as compared with male subjects (7.7 ± 3.9 vs 9.4 ± 4.8 μg/L). In contrast, there was no sex difference in adiponectin, HMW adiponectin, visfatin, and IL-6 levels (data not shown). Because mean concentrations of adiponectin, HMW adiponectin, visfatin, resistin, and IL-6 were significantly increased in MD patients as compared with controls, all subsequent analyses were performed in the 2 subgroups separately.

3.2. Adipokine concentrations in relation to diabetes mellitus and sex in controls and MD patients

In controls, leptin concentrations were significantly increased in women (33.8 ± 25.9 μg/L) as compared with men (9.8 ± 8.4 μg/L). In all other cases, there was no significant

Table 2
Baseline characteristics of the study population further divided into controls without diabetes (control/T2DM−) or with diabetes (control/T2DM+) and MD patients without diabetes (MD/T2DM−) or with diabetes (MD/T2DM+)

	Control/T2DM−	Control/T2DM+	MD/T2DM−	MD/T2DM+
n	30	30	28	32
Age (y)	61 ± 11	63 ± 10	60 ± 14	67 ± 10
Sex (male/female)	11/19	16/14	15/13	20/12
BMI (kg/m^2)	29.8 ± 5.8	30.6 ± 5.2	26.1 ± 5.1*,†	28.2 ± 5.0
SBP (mm Hg)	126 ± 16	129 ± 12	123 ± 24	123 ± 21
DBP (mm Hg)	75 ± 11	72 ± 9	72 ± 12	69 ± 9
Creatinine (μmol/L)	77 ± 15	75 ± 18	780 ± 272*,†	743 ± 271*,†
GFR (mL/min)	94 ± 32	107 ± 39	10 ± 5*,†	10 ± 4*,†
PTH (pmol/L)	4.4 ± 1.7	3.8 ± 1.3	24.1 ± 25.9*,†	20.4 ± 13.6*,†
FG (mmol/L)	5.2 ± 0.8	7.8 ± 2.6*	4.7 ± 0.9†	6.2 ± 2.7†,‡
FI (pmol/L)	45.8 ± 26.2	74.0 ± 94.5	56.4 ± 81.7	88.9 ± 126.8
HOMA-IR	1.6 ± 1.0	3.4 ± 3.0	1.9 ± 3.1†	4.5 ± 9.4
FFA (mmol/L)	0.5 ± 0.2	0.6 ± 0.3	0.6 ± 0.3	0.7 ± 0.4
Cholesterol (mmol/L)	5.5 ± 0.8	4.9 ± 1.2	4.5 ± 1.0*	4.3 ± 1.1*
TG (mmol/L)	1.2 ± 0.5	1.7 ± 1.0	1.7 ± 0.5*	2.4 ± 1.7*
CRP (mg/L)	3.9 ± 3.3	3.3 ± 2.5	14.0 ± 25.9	17.9 ± 24.8*,†
Leptin (μg/L)	23.9 ± 21.3	22.1 ± 25.4	32.0 ± 53.8	66.1 ± 81.2†,‡
Adiponectin (mg/L)	7.6 ± 4.1	5.8 ± 3.4	17.3 ± 10.8*,†	15.5 ± 11.4*,†
HMW (mg/L)	8.7 ± 4.7	7.6 ± 5.4	18.5 ± 8.5*,†	14.0 ± 9.4†
Visfatin (μg/L)	3.4 ± 4.7	1.7 ± 2.8	4.3 ± 2.6*,†	4.6 ± 4.6†
Resistin (μg/L)	6.3 ± 1.6	5.2 ± 1.5	12.8 ± 5.6*,†	10.1 ± 2.9*,†
IL-6 (ng/L)	2.7 ± 1.3	2.7 ± 1.7	13.1 ± 13.6*,†	13.3 ± 12.4*,†

Means ± SD are shown. Parameters were analyzed by analysis of variance followed by Bonferroni post hoc analysis.

* $P < .05$ as compared with control/T2DM−.

† As compared with control/T2DM+.

‡ As compared with MD/T2DM−.

difference in adipokine levels depending on diabetes mellitus and sex in the controls (Table 2 and data not shown).

In MD patients, adiponectin was significantly decreased in men (14.2 ± 10.6 mg/L) as compared with women (19.2 ± 11.3 mg/L). Furthermore, leptin concentrations were significantly higher in diabetic as compared with nondiabetic patients (66.1 ± 81.2 vs 32.0 ± 53.8 μg/L) (Table 2). In all other cases, a significant difference in adipokine concentrations could not be elucidated depending on diabetes mellitus and sex (Table 2 and data not shown).

3.3. Univariate correlations

The results of univariate analyses for the adipokines studied are shown in Table 3 for controls and in Table 4 for MD patients.

In controls, leptin serum levels positively correlated with BMI, fasting insulin, HOMA-IR, FFA, triglycerides, and CRP (Table 3). For adiponectin, a positive association was observed with FFA and cholesterol, whereas a negative correlation existed with HOMA-IR (Table 3). Similar to adiponectin, HMW adiponectin on one hand and FFA and cholesterol on the other hand were positively correlated (Table 3). There was a strong correlation between adiponectin and HMW adiponectin (Table 3). For visfatin, a significant positive correlation existed with resistin (Table 3). Besides the positive correlation with visfatin, resistin was positively associated with creatinine and negatively correlated with GFR, fasting glucose, and triglycerides (Table 3). Interleukin-6 significantly and positively correlated with CRP in controls (Table 3).

Table 3
Univariate correlations in control patients

	Leptin	Adiponectin	HMW	Visfatin	Resistin	IL-6
Age	$r = -0.158$	$r = 0.085$	$r = 0.141$	$r = 0.125$	$r = 0.103$	$r = -0.140$
	$P = .228$	$P = .519$	$P = .284$	$P = .343$	$P = .434$	$P = .287$
BMI	$r = 0.604$	$r = -0.161$	$r = -0.184$	$r = -0.086$	$r = -0.114$	$r = 0.156$
	$P = .000$*	$P = .218$	$P = .160$	$P = .514$	$P = .387$	$P = .234$
SBP	$r = 0.003$	$r = 0.024$	$r = 0.074$	$r = -0.045$	$r = -0.062$	$r = -0.055$
	$P = .984$	$P = .857$	$P = .573$	$P = .732$	$P = .637$	$P = .679$
DBP	$r = 0.086$	$r = 0.113$	$r = 0.100$	$r = -0.091$	$r = 0.006$	$r = 0.049$
	$P = .514$	$P = .390$	$P = .449$	$P = .488$	$P = .966$	$P = .708$
Creatinine	$r = -0.170$	$r = -0.094$	$r = -0.069$	$r = 0.145$	$r = 0.364$	$r = 0.039$
	$P = .195$	$P = .473$	$P = .603$	$P = .268$	$P = .004$*	$P = .770$
GFR	$r = 0.092$	$r = -0.156$	$r = -0.150$	$r = -0.228$	$r = -0.430$	$r = 0.008$
	$P = .483$	$P = .234$	$P = .253$	$P = .080$	$P = .001$*	$P = .949$
PTH	$r = 0.181$	$r = -0.036$	$r = -0.010$	$r = 0.033$	$r = 0.197$	$r = 0.176$
	$P = .167$	$P = .786$	$P = .942$	$P = .803$	$P = .132$	$P = .179$
FG	$r = -0.082$	$r = -0.126$	$r = -0.074$	$r = -0.092$	$r = -0.270$	$r = -0.156$
	$P = .533$	$P = .336$	$P = .573$	$P = .485$	$P = .037$*	$P = .235$
FI	$r = 0.424$	$r = -0.205$	$r = -0.173$	$r = -0.067$	$r = -0.049$	$r = 0.119$
	$P = .001$*	$P = .115$	$P = .186$	$P = .612$	$P = .709$	$P = .367$
HOMA-IR	$r = 0.292$	$r = -0.284$	$r = -0.233$	$r = -0.066$	$r = -0.144$	$r = 0.032$
	$P = .024$*	$P = .028$*	$P = .073$	$P = .616$	$P = .272$	$P = .805$
FFA	$r = 0.309$	$r = 0.283$	$r = 0.317$	$r = -0.035$	$r = -0.074$	$r = 0.081$
	$P = .016$*	$P = .028$*	$P = .014$*	$P = .791$	$P = .577$	$P = .540$
Cholesterol	$r = 0.250$	$r = 0.359$	$r = 0.337$	$r = -0.166$	$r = -0.237$	$r = 0.168$
	$P = .054$	$P = .005$*	$P = .009$*	$P = .204$	$P = .068$	$P = .200$
TG	$r = 0.328$	$r = -0.147$	$r = -0.168$	$r = -0.080$	$r = -0.275$	$r = 0.068$
	$P = .011$*	$P = .262$	$P = .198$	$P = .542$	$P = .033$*	$P = .604$
CRP	$r = 0.292$	$r = -0.006$	$r = -0.079$	$r = 0.089$	$r = 0.067$	$r = 0.293$
	$P = .023$*	$P = .952$	$P = .547$	$P = .500$	$P = .612$	$P = .023$*
Leptin	–	$r = 0.164$	$r = 0.110$	$r = -0.006$	$r = -0.170$	$r = 0.202$
		$P = .211$	$P = .402$	$P = .962$	$P = .193$	$P = .122$
Adiponectin	$r = 0.164$	–	$r = 0.950$	$r = -0.237$	$r = -0.131$	$r = 0.096$
	$P = .211$		$P = .000$*	$P = .068$	$P = .318$	$P = .466$
HMW	$r = 0.110$	$r = 0.950$	–	$r = -0.197$	$r = -0.156$	$r = -0.022$
	$P = .402$	$P = .000$*		$P = .131$	$P = .233$	$P = .869$
Visfatin	$r = -0.006$	$r = -0.237$	$r = -0.197$	–	$r = 0.418$	$r = -0.018$
	$P = .962$	$P = .068$	$P = .131$		$P = .001$*	$P = .891$
Resistin	$r = -0.170$	$r = -0.131$	$r = -0.156$	$r = 0.418$	–	$r = 0.121$
	$P = .193$	$P = .318$	$P = .233$	$P = .001$*		$P = .358$
IL-6	$r = 0.202$	$r = 0.096$	$r = -0.022$	$r = -0.018$	$r = 0.121$	–
	$P = .122$	$P = .466$	$P = .869$	$P = .891$	$P = .358$	

The r and P values are given.

* Significant correlation as assessed by Spearman correlation method.

Table 4
Univariate correlations in MD patients

	Leptin	Adiponectin	HMW	Visfatin	Resistin	IL-6
Age	$r = 0.015$	$r = -0.104$	$r = -0.116$	$r = 0.151$	$r = 0.025$	$r = 0.203$
	$P = .907$	$P = .431$	$P = .379$	$P = .249$	$P = .849$	$P = .119$
BMI	$r = 0.601$	$r = -0.117$	$r = -0.202$	$r = -0.226$	$r = -0.065$	$r = 0.172$
	$P = .000$*	$P = .373$	$P = .123$	$P = .082$	$P = .620$	$P = .188$
SBP	$r = 0.048$	$r = 0.043$	$r = -0.041$	$r = -0.149$	$r = 0.194$	$r = 0.226$
	$P = .718$	$P = .745$	$P = .758$	$P = .255$	$P = .138$	$P = .083$
DBP	$r = 0.173$	$r = 0.008$	$r = -0.037$	$r = -0.182$	$r = 0.079$	$r = -0.120$
	$P = .186$	$P = .949$	$P = .780$	$P = .163$	$P = .549$	$P = .363$
Creatinine	$r = -0.126$	$r = -0.209$	$r = -0.160$	$r = -0.069$	$r = 0.004$	$r = -0.091$
	$P = .336$	$P = .110$	$P = .222$	$P = .601$	$P = .977$	$P = .491$
GFR	$r = 0.257$	$r = 0.083$	$r = 0.029$	$r = -0.091$	$r = 0.103$	$r = 0.119$
	$P = .048$*	$P = .530$	$P = .829$	$P = .489$	$P = .433$	$P = .365$
PTH	$r = 0.065$	$r = 0.002$	$r = -0.050$	$r = -0.117$	$r = -0.077$	$r = 0.001$
	$P = .623$	$P = .990$	$P = .703$	$P = .372$	$P = .558$	$P = .992$
FG	$r = 0.151$	$r = -0.278$	$r = -0.307$	$r = -0.119$	$r = -0.030$	$r = 0.070$
	$P = .251$	$P = .032$*	$P = .017$*	$P = .364$	$P = .822$	$P = .596$
FI	$r = 0.481$	$r = -0.557$	$r = -0.589$	$r = -0.249$	$r = -0.057$	$r = -0.054$
	$P = .000$*	$P = .000$*	$P = .000$*	$P = .055$	$P = .667$	$P = .684$
HOMA-IR	$r = 0.460$	$r = -0.522$	$r = -0.572$	$r = -0.231$	$r = -0.022$	$r = -0.021$
	$P = .000$*	$P = .000$*	$P = .000$*	$P = .075$	$P = .868$	$P = .876$
FFA	$r = 0.021$	$r = 0.348$	$r = 0.271$	$r = 0.242$	$r = 0.123$	$r = 0.270$
	$P = .872$	$P = .006$*	$P = .036$*	$P = .062$	$P = .350$	$P = .037$*
Cholesterol	$r = 0.266$	$r = -0.015$	$r = -0.108$	$r = 0.014$	$r = -0.315$	$r = -0.230$
	$P = .040$*	$P = .908$	$P = .410$	$P = .917$	$P = .014$*	$P = .077$
TG	$r = 0.318$	$r = -0.384$	$r = -0.426$	$r = -0.113$	$r = -0.259$	$r = -0.098$
	$P = .013$*	$P = .002$*	$P = .001$*	$P = .391$	$P = .045$*	$P = .458$
CRP	$r = 0.081$	$r = 0.103$	$r = 0.126$	$r = 0.003$	$r = 0.350$	$r = 0.650$
	$P = .539$	$P = .436$	$P = .338$	$P = .981$	$P = .006$*	$P = .000$*
Leptin	–	$r = -0.213$	$r = -0.354$	$r = -0.264$	$r = -0.205$	$r = -0.054$
		$P = .102$	$P = .005$*	$P = .041$*	$P = .116$	$P = .679$
Adiponectin	$r = -0.213$	–	$r = 0.904$	$r = 0.215$	$r = -0.030$	$r = 0.167$
	$P = .102$		$P = .000$*	$P = .099$	$P = .822$	$P = .203$
HMW	$r = -0.354$	$r = 0.904$	–	$r = 0.252$	$r = 0.018$	$r = 0.135$
	$P = .005$*	$P = .000$*		$P = .052$	$P = .890$	$P = .304$
Visfatin	$r = -0.264$	$r = 0.215$	$r = 0.252$	–	$r = 0.278$	$r = 0.163$
	$P = .041$*	$P = .099$	$P = .052$		$P = .032$*	$P = .213$
Resistin	$r = -0.205$	$r = -0.030$	$r = 0.018$	$r = 0.278$	–	$r = 0.522$
	$P = .116$	$P = .822$	$P = .890$	$P = .032$*		$P = .000$*
IL-6	$r = -0.054$	$r = 0.167$	$r = 0.135$	$r = 0.163$	$r = 0.522$	–
	$P = .679$	$P = .203$	$P = .304$	$P = .213$	$P = .000$*	

The r and P values are given.
* Significant correlation as assessed by Spearman correlation method.

In MD patients, a positive association was found between leptin on one hand and BMI, GFR, fasting insulin, HOMA-IR, cholesterol, and triglycerides on the other hand in univariate analyses (Table 4). Furthermore, leptin was negatively correlated with HMW adiponectin and visfatin (Table 4). Adiponectin correlated positively with FFA and negatively with fasting glucose, fasting insulin, HOMA-IR, and triglycerides (Table 4). The same associations were found for HMW adiponectin (Table 4). In addition, a negative correlation existed between HMW adiponectin and leptin (Table 4). Again, adiponectin and HMW adiponectin were strongly and positively correlated (Table 4). Visfatin serum levels positively correlated with resistin and negatively correlated with leptin (Table 4). Serum concentrations of resistin positively correlated with visfatin, CRP, and IL-6 and negatively correlated with triglycerides and cholesterol (Table 4). Interleukin-6 positively correlated with FFA, CRP, and resistin in the MD patients in univariate analyses (Table 4).

3.4. Multivariate correlations

Parameters showing significant correlations with adipokine levels in univariate analyses were tested in multivariate analyses for independent associations. Furthermore, age and sex were included in each model. The results are shown in Table 5 (controls) and Table 6 (MD patients).

In controls, leptin serum levels were independently correlated with sex, BMI, FFA, and triglycerides (Table 5). The homeostasis model assessment of insulin resistance, FFA, and cholesterol were independently correlated with both circulating adiponectin and HMW adiponectin (Table 5). In addition, sex was independently associated with total

Table 5
Multivariate linear regression analyses in control patients with leptin, adiponectin, HMW adiponectin, visfatin, resistin, or IL-6 as dependent variables

Dependent variable	Independent variable	β	P
Leptin	Age	−0.021	.778
	Sex	0.561	.000*
	BMI	0.432	.000*
	FI	0.006	.940
	FFA	0.180	.014*
	TG	0.163	.039*
	CRP	0.009	.902
Adiponectin	Age	0.148	.162
	Sex	0.266	.020*
	HOMA-IR	−0.359	.002*
	FFA	0.281	.011*
	Cholesterol	0.337	.003*
HMW	Age	0.188	.081
	Sex	0.201	.079
	HOMA-IR	−0.336	.004*
	FFA	0.340	.003*
	Cholesterol	0.305	.007*
Visfatin	Age	0.090	.471
	Sex	0.021	.863
	Resistin	0.377	.003*
Resistin	Age	−0.170	.323
	Sex	−0.089	.447
	GFR	−0.379	.037*
	FG	−0.122	.303
	TG	−0.196	.114
	Visfatin	0.310	.009*
IL-6	Age	−0.031	.815
	Sex	0.184	.170
	CRP	0.110	.410

Independent variables tested include age and sex, as well as parameters showing a significant correlation with the respective adipokine in univariate analysis (Table 3). The β coefficients and P values are given.
* Significant correlation.

adiponectin (Table 5). Resistin remained independently correlated with serum visfatin concentrations (Table 5). Glomerular filtration rate and visfatin were independently associated with resistin serum levels (Table 5). The significant correlation between IL-6 and CRP was no longer present in controls after adjusting for age and sex (Table 5).

In MD patients, sex, BMI, and GFR were independently correlated with leptin concentrations (Table 6). For adiponectin and HMW adiponectin, an independent association was found with HOMA-IR and triglycerides (Table 6). Visfatin remained significantly associated with resistin after adjustment for age, sex, and leptin (Table 6). Serum resistin concentrations were independently related to circulating IL-6 and visfatin (Table 6). Both CRP and resistin were significantly and independently correlated with serum IL-6 levels in MD patients (Table 6).

4. Discussion

We demonstrate that adiponectin, HMW adiponectin, visfatin, resistin, and IL-6 are significantly increased in patients undergoing MD as compared with controls with a GFR greater than 50 mL/min. Our results point to the fact that renal function should be taken into consideration when interpreting adipokine levels. In the current study, blood has been drawn in MD patients just before the hemodialysis started. If elevated levels of several adipokines in MD patients are due to impaired renal metabolism/clearance or if they are significantly affected by hemodialysis, time from the last hemodialysis might have a significant effect on adipokine concentrations. This hypothesis should be tested in future studies by obtaining blood samples in MD patients in a serial fashion, that is, between one hemodialysis and the next.

In our hands, sex and BMI are independently correlated with leptin serum concentrations in multivariate analyses in

Table 6
Multivariate linear regression analyses in MD patients with leptin, adiponectin, HMW adiponectin, visfatin, resistin, or IL-6 as dependent variables

Dependent variable	Independent variable	β	P
Leptin	Age	−0.015	.882
	Sex	0.358	.003*
	BMI	0.325	.007*
	GFR	0.282	.021*
	FI	0.228	.095
	Cholesterol	0.071	.526
	TG	−0.015	.904
	HMW	−0.264	.055
	Visfatin	−0.005	.960
Adiponectin	Age	−0.106	.315
	Sex	0.180	.095
	HOMA-IR	−0.327	.017*
	FFA	0.157	.179
	TG	−0.301	.015*
HMW	Age	−0.093	.366
	Sex	0.171	.127
	HOMA-IR	−0.302	.041*
	FFA	0.110	.342
	TG	−0.314	.010*
	Leptin	−0.161	.201
Visfatin	Age	−0.011	.934
	Sex	0.179	.177
	Leptin	−0.148	.263
	Resistin	0.306	.023*
Resistin	Age	−0.169	.121
	Sex	−0.163	.145
	Cholesterol	−0.187	.168
	TG	−0.128	.288
	CRP	−0.022	.890
	Visfatin	0.234	.041*
	IL-6	0.465	.003*
IL-6	Age	0.143	.129
	Sex	−0.010	.911
	FFA	0.065	.499
	CRP	0.510	.000*
	Resistin	0.345	.001*

Independent variables tested include age and sex, as well as parameters showing a significant correlation with the respective adipokine in univariate analysis (Table 4). The β and P values are given.
* Significant correlation.

both control and MD patients. These results confirm previous studies indicating that leptin levels are increased when body weight is gained and are sex-dependent with increased levels found in women [1,15].

In the current study, circulating adiponectin is significantly increased in MD as compared with controls in accordance with other studies [16,17]. An independent negative correlation is found between adiponectin and HOMA-IR in both control and MD patients. These findings indicate that, although mean adiponectin levels are significantly increased in MD patients, the strong relationship between insulin sensitivity and concentrations of this adipokine seen in non-MD patients in various studies [1] persists when renal function deteriorates. Convincing evidence has been presented in recent years that adiponectin has potent insulin-sensitizing and antiatherogenic effects [3]. Here, it needs to be determined in future studies whether increased levels of adiponectin in MD might have some protective effects against glucose intolerance and cardiovascular disease. Similar to adiponectin, HMW adiponectin, which has been postulated as the active form of the protein [4], is increased in MD patients. This finding is consistent with a recent report [18] where HMW adiponectin quantification was not ELISA-based but was performed by fast protein liquid chromatography. Furthermore, we show for the first time that an independent relationship with HOMA-IR exists not only for adiponectin but also for HMW adiponectin in both control and MD patients. Moreover, there is a strong positive correlation between adiponectin and HMW adiponectin in univariate analysis in both groups, with an r value greater than 0.9. Taking these results into consideration, it appears questionable whether determination of HMW adiponectin has an additional value as compared with quantifying total adiponectin in the population studied.

The finding that visfatin is increased in MD patients is in accordance with a recent study [19]. We show for the first time that resistin levels are independently correlated with serum visfatin concentrations in both control and MD patients. It is interesting to note in this context that visfatin has proinflammatory functions besides its potential insulin-mimetic effects. Thus, visfatin activates human leukocytes and induces the production of proinflammatory cytokines including IL-1β, tumor necrosis factor–α, and IL-6 [20]. In agreement with this role, visfatin is up-regulated in a variety of pathophysiologic conditions of the immune system including psoriasis [21] and rheumatoid arthritis [22]. Because resistin has recently been characterized as a proinflammatory cytokine with insulin resistance–inducing effects [23,24], it will be interesting to determine in future studies whether visfatin might directly regulate resistin expression and secretion in humans. Along the same line, the independent association between IL-6 levels and circulating resistin in MD patients might be due to direct stimulation of resistin by IL-6. In accordance with this hypothesis, up-regulation of resistin messenger RNA is found in adipose tissue of control and diabetic patients after IL-6 infusion in vivo [25].

Various independent associations between adipokines and measures of renal function, glucose, and lipid metabolism, as well as inflammation, are only found in either controls or MD patients in the present study. These results emphasize that regulation of various adipokines in end-stage renal disease is different as compared with patients with a GFR greater than 50 mL/min.

Taken together, we show that levels of adiponectin, HMW adiponectin, visfatin, resistin, and IL-6 are significantly increased in MD patients. Furthermore, regulation of adipokines in vivo depends on renal function. Moreover, regulation of HMW adiponectin is similar to adiponectin in the patients studied.

Acknowledgment

This study was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG), KFO 152: “Atherobesity”, project FA476/4-1 (TP 4) to MF, project BL833/1-1 (TP3) to MB, the IZKF Leipzig to MF (Project B25), and the Deutsche Diabetes-Stiftung to MF.

References

[1] Fasshauer M, Paschke R. Regulation of adipocytokines and insulin resistance. Diabetologia 2003;46:1594-603.
[2] Trujillo ME, Scherer PE. Adipose tissue–derived factors: impact on health and disease. Endocr Rev 2006;27:762-78.
[3] Fasshauer M, Paschke R, Stumvoll M. Adiponectin, obesity, and cardiovascular disease. Biochimie 2004;86:779-84.
[4] Pajvani UB, Hawkins M, Combs TP, et al. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J Biol Chem 2004;279:12152-62.
[5] Fukuhara A, Matsuda M, Nishizawa M, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 2005;307:426-30.
[6] Fukuhara A, Matsuda M, Nishizawa M, et al. Retraction: visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 2007;318:565.
[7] Satoh H, Nguyen MT, Miles PD, et al. Adenovirus-mediated chronic “hyper-resistinemia” leads to in vivo insulin resistance in normal rats. J Clin Invest 2004;114:224-31.
[8] Steppan CM, Bailey ST, Bhat S, et al. The hormone resistin links obesity to diabetes. Nature 2001;409:307-12.
[9] Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM. Resistin gene expression in human adipocytes is not related to insulin resistance. Obes Res 2002;10:1-5.
[10] Fasshauer M, Kralisch S, Klier M, et al. Insulin resistance–inducing cytokines differentially regulate SOCS mRNA expression via growth factor– and Jak/Stat signaling pathways in 3T3-L1 adipocytes. J Endocrinol 2004;181:129-38.
[11] Ahima RS, Flier JS. Leptin. Annu Rev Physiol 2000;62:413-37.
[12] Schafer K, Halle M, Goeschen C, et al. Leptin promotes vascular remodeling and neointimal growth in mice. Arterioscler Thromb Vasc Biol 2004;24:112-7.
[13] Ziegelmeier M, Bachmann A, Seeger J, et al. Serum levels of the adipokine RBP-4 in relation to renal function. Diabetes Care 2007;30:2588-92.

[14] Kratzsch J, Berthold A, Lammert A, et al. A rapid, quantitative immunofunctional assay for measuring human leptin. Horm Res 2002; 57:127-32.

[15] Horn R, Geldszus R, Potter E, von zur MA, Brabant G. Radioimmunoassay for the detection of leptin in human serum. Exp Clin Endocrinol Diabetes 1996;104:454-8.

[16] Zoccali C, Mallamaci F, Tripepi G, et al. Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. J am Soc Nephrol 2002;13:134-41.

[17] Huang JW, Yen CJ, Chiang HW, et al. Adiponectin in peritoneal dialysis patients: a comparison with hemodialysis patients and subjects with normal renal function. Am J Kidney Dis 2004;43: 1047-55.

[18] Shen YY, Charlesworth JA, Kelly JJ, Loi KW, Peake PW. Up-regulation of adiponectin, its isoforms and receptors in end-stage kidney disease. Nephrol Dial Transplant 2007;22:171-8.

[19] Axelsson J, Witasp A, Carrero JJ, et al. Circulating levels of visfatin/pre–B-cell colony–enhancing factor 1 in relation to genotype, GFR, body composition, and survival in patients with CKD. Am J Kidney Dis 2007;49:237-44.

[20] Moschen AR, Kaser A, Enrich B, et al. Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol 2007; 178:1748-58.

[21] Koczan D, Guthke R, Thiesen HJ, et al. Gene expression profiling of peripheral blood mononuclear leukocytes from psoriasis patients identifies new immune regulatory molecules. Eur J Dermatol 2005;15: 251-7.

[22] Nowell MA, Richards PJ, Fielding CA, et al. Regulation of pre–B cell colony–enhancing factor by STAT-3–dependent interleukin-6 trans-signaling: implications in the pathogenesis of rheumatoid arthritis. Arthritis Rheum 2006;54:2084-95.

[23] Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. J Immunol 2005; 174:5789-95.

[24] Nagaev I, Bokarewa M, Tarkowski A, Smith U. Human resistin is a systemic immune-derived proinflammatory cytokine targeting both leukocytes and adipocytes. PLoS ONE 2006;1:e31.

[25] Carey AL, Petersen EW, Bruce CR, et al. Discordant gene expression in skeletal muscle and adipose tissue of patients with type 2 diabetes: effect of interleukin-6 infusion. Diabetologia 2006;49:1000-7.