Elevated Serum Leptin Concentrations in Type 2 Diabetic Patients With Microalbuminuria and Macroalbuminuria

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Leptin levels are elevated in end-stage renal disease, suggesting an impairment of renal leptin degradation. The present study aimed to determine whether leptin levels are also elevated in patients with earlier stages of renal disease, ie, microalbuminuric and macroalbuminuric nephropathy. A total of 60 subjects were assigned to two study groups. Group A contained 10 type 2 diabetics with macroalbuminuria, 10 type 2 diabetics with normoalbuminuria, and 10 healthy control subjects. Group B contained 10 type 2 diabetics with microalbuminuria, 10 type 2 diabetics with normoalbuminuria, and 10 healthy controls. The subgroups of both study groups were matched for sex and body fatness. In group A, macroalbuminuric diabetic patients had higher serum leptin levels than the normoalbuminuric diabetics (11.90 ± 2.98 *v* 4.13 ± 0.92 ng/mL, $P < .002$) and control subjects (4.78 ± 1.37 ng/mL, $P < .006$). In group B, microalbuminuric diabetics had higher serum leptin levels than the normoalbuminuric diabetics (21.16 ± 5.80 *v* 8.74 ± 1.89 ng/mL, $P < .04$) and control subjects (10.06 ± 3.00 ng/mL, $P < .06$). In both groups A and B, creatinine clearance was inversely correlated with the serum leptin level after adjusting for body fat. In conclusion, serum leptin levels are elevated in type 2 diabetic patients with microalbuminuria and macroalbuminuria, suggesting that renal leptin degradation is already impaired in the early stages of renal disease.

CIRCULATING LEPTIN is primarily eliminated by the kidney in both rodents[1-3] and humans.[4-6] Renal removal of leptin from the plasma involves tissue degradation rather than mere urinary excretion, since little or no leptin appears in the urine.[1,5,7] In patients with end-stage renal disease, leptin levels are reported to be elevated,[6,8-13] implying that renal leptin degradation is impaired in such patients. Furthermore, Sharma et al[6] have provided evidence that renal leptin uptake and degradation is also impaired in patients with mild to moderate renal insufficiency. However, it has not been investigated as to whether leptin levels are elevated in patients with early stages of renal disease, ie, microalbuminuric and macroalbuminuric nephropathy.

SUBJECTS AND METHODS

Subjects were selected from inpatients and outpatients of the Department of Internal Medicine and Endocrinology at the University of Mainz. All subjects were caucasian, and all provided informed consent. None of the subjects had any endocrine disorder other than diabetes. Group A contained 10 type 2 diabetics with macroalbuminuria (>300 mg/24 h), 10 type 2 diabetics with normoalbuminuria (<30 mg/24 h), and 10 healthy control subjects matched for sex, age, and percent body fat. Group B contained 10 type 2 diabetics with microalbuminuria (30 to 300 mg/24 h), 10 type 2 diabetics with normoalbuminuria, and 10 healthy controls matched for sex and percent body fat. The diagnosis of microalbuminuria and macroalbuminuria was established by determination of the urinary albumin excretion in two subsequent 24-hour urine collections separated by at least 4 weeks.[14] In subjects with persistent albuminuria, urinary tract infection was excluded by quantitative culture of a midstream urinary specimen. In patients receiving angiotensin-converting enzyme inhibitors, these agents were withdrawn at least 3 days before determination of urinary albumin excretion, whereas the use of other antihypertensive agents, including diuretics and calcium-channel antagonists, was continued throughout the study. Diabetic patients without insulin treatment received oral hypoglycemic agents including sulfonylurea (six subjects) and metformin (two subjects). All diabetic patients were additionally screened for diabetic retinopathy by ophthalmoscopy and for diabetic neuropathy by physical examination and cardiovascular-reflex testing (ProSciCard-System; MediSyst, Linden, Germany). All subjects reported having a stable body weight (±2 kg) for at least 2 months.

Blood samples for determination of leptin, creatinine, triglycerides, lipoproteins, and hemoglobin A_{1c} (HbA_{1c}) were obtained between 7:30 and 8:30 AM after an overnight fast (>10 hours). Blood for leptin measurements was immediately centrifuged after sampling, and the serum was stored at −24°C until analysis. The percent body fat was determined by bioelectrical impedance analysis (Body Composition Analyser, Akren-RJL BIA 101/S; DATA INPUT, Frankfurt, Germany). This estimate of body fatness was chosen for matching the subjects because it was found to be a superior estimate of body fatness compared with other estimates, eg, the body mass index.[15] Additionally, the percent body fat has been shown to correlate strongly with the serum leptin concentration.[16]

The serum leptin level was measured by a commercial radioimmunoassay (Human-Leptin-RIA LEP-R40; Mediagnost, Tübingen, Germany) with an interassay coefficient of variation (CV) less than 7.6%, intraassay CV less than 5.0%, and sensitivity less than 0.04 ng/mL. Urinary albumin excretion was measured by immunonephelometry (N Antiserum gegen Human-Albumin; Behring, Marburg, Germany) with an interassay CV less than 4.4% and an intraassay CV less than 4.3%. The HbA_{1c} level was measured by high-performance liquid chromatography (normal, <6.8%). Other parameters, ie, lipids and creatinine, were determined by routine clinical methods.

Data analyses were performed by the SPSS statistics program (Version 6.0, SPSS, Chicago, IL). An unpaired Student's *t* test was used for the comparison of variables between subgroups. Analysis of covariance (ANCOVA) was used to compare leptin levels in the subgroups, with percent body fat as a covariate. Pearson's and partial correlation coefficients and stepwise forward multiple linear regression analysis were used to detect associations between serum leptin and the other variables. Results are presented as the mean ± SE. A *P* value less than .05 was considered significant.

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RESULTS

Group A

The clinical and metabolic characteristics of group A are shown in Table 1. The serum leptin concentration correlated with percent body fat in diabetics with macroalbuminuria ($r = .77$, $P < .01$), diabetics with normoalbuminuria ($r = .79$, $P < .01$), and control subjects ($r = .55$, $P < .05$; Fig 1). Diabetic patients with macroalbuminuria had higher serum leptin levels than diabetics with normoalbuminuria (11.90 ± 2.98 *v* 4.13 ± 0.92 ng/dL, $P < .002$ adjusted for body fat by ANCOVA) and control subjects (4.78 ± 1.37 ng/dL, $P < .006$ adjusted for body fat). In macroalbuminuric patients, serum leptin was not correlated with the degree of albuminuria ($r = .07$, $P = .42$). When the data for all three subgroups were pooled, we found no significant correlation between the serum leptin level and creatinine clearance in bivariate analysis ($r = -.28$, $P = .07$; Fig 2). However, after adjusting for body fat, serum leptin was inversely correlated with creatinine clearance ($r = -.41$, $P < .02$).

Table 1. Clinical and Metabolic Characteristics of Group A

Characteristic	Controls	Diabetics	Diabetics With Macroalbuminuria
Sex ratio (male/female)	7/3	7/3	7/3
Age (yr)	60.5 ± 5.8	59.2 ± 3.1	61.3 ± 2.7
Body fat (%)	27.7 ± 1.4	27.2 ± 2.0	27.0 ± 2.1
Weight (kg)	78.1 ± 5.2	74.6 ± 3.6	79.26 ± 5.9
Height (cm)	168.8 ± 3.4	171.7 ± 2.3	171.9 ± 3.1
Urinary albumin excretion (mg/24 h)	—	<30	2,310.4 ± 552.3
Creatinine (μmol/L)	77 ± 4‡	76 ± 8‡	163 ± 15
Creatinine clearance (mL/s)	1.67 ± 0.15‡	1.61 ± 0.15‡	0.84 ± 0.08
Triglycerides (mmol/L)	2.13 ± 0.43	1.62 ± 0.14†	3.49 ± 0.98
Total cholesterol (mmol/L)	5.84 ± 0.34	6.22 ± 0.48	6.57 ± 0.73
LDL cholesterol (mmol/L)	3.86 ± 0.28	4.48 ± 1.02	4.38 ± 0.66
HDL cholesterol (mmol/L)	1.06 ± 0.10	1.32 ± 0.14*	0.94 ± 0.07
HbA_{1c} (%)	—	7.11 ± 0.49	7.48 ± 0.52
Diabetes duration (yr)	—	12.8 ± 3.3	15.6 ± 2.7
Insulin treatment (yes/no)	—	8/2	8/2
Daily insulin dose (U)	—	55 ± 9	51 ± 11
Diabetic retinopathy (yes/no)	—	3/7	10/0
Diabetic neuropathy (yes/no)	—	6/4	9/1
Hypertension (yes/no)	0/10	4/6	10/0

NOTE. Data are the mean ± SE where indicated.

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein.

*$P < .05$, †$P < .01$; ‡$P < .001$ *v* macroalbuminuric.

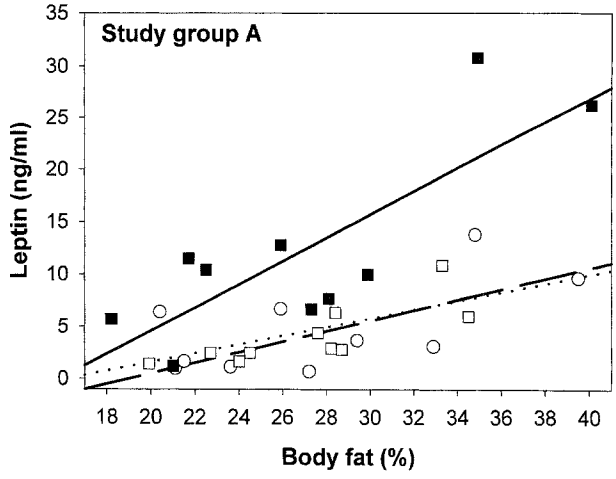

Fig 1. Correlation between the serum leptin concentration and percent body fat in 10 diabetics with macroalbuminuria (■, —, y = 1.11x − 17.64, r = .77, P < .01), 10 diabetics with normoalbuminuria (□, ---, y = 0.50x − 9.60, r = .79, P < .01), and 10 healthy control subjects (○, . . ., y = 0.42x − 6.79, r = .55, P < .05). The slope of the regression lines did not differ significantly between subgroups (P > .05 for all comparisons).

Group B

The clinical and metabolic characteristics of group B are presented in Table 2. Serum leptin correlated with percent body fat in diabetics with microalbuminuria ($r = .69$, $P < .05$), diabetics with normoalbuminuria ($r = .73$, $P < .01$), and control subjects ($r = .82$, $P < .01$; Fig 3). Diabetic patients with microalbuminuria had higher serum leptin levels than diabetics with normoalbuminuria (21.16 ± 5.80 *v* 8.74 ± 1.89 ng/dL, $P < 0.05$ adjusted for body fat by ANCOVA) and also tended to have higher levels than the control subjects (10.06 ± 3.00 ng/dL, $P < .06$ adjusted for body fat). In microalbuminuric patients, serum leptin tended to correlate inversely with the

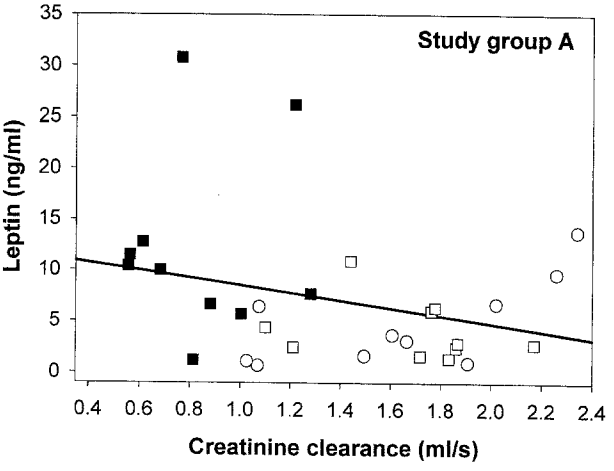

Fig 2. Correlation between the serum leptin concentration and creatinine clearance in the pooled data for group A (y = −3.67x + 12.18, r = −.28, P = .07) including 10 diabetics with macroalbuminuria (■), 10 diabetics with normoalbuminuria (□), and 10 healthy control subjects (○).

Table 2. Clinical and Metabolic Characteristics of Group B

Characteristic	Controls	Diabetics	Diabetics With Microalbuminuria
Sex ratio (male/female)	4/6	4/6	4/6
Age (yr)	64.9 ± 5.5	55.6 ± 2.7*	66.8 ± 3.36
Body fat (%)	33.3 ± 1.7	33.1 ± 1.2	33.2 ± 0.9
Weight (kg)	77.6 ± 5.8	85.8 ± 5.5	80.8 ± 4.6
Height (cm)	166.7 ± 3.1	171.3 ± 2.7	170.5 ± 4.1
Urinary albumin excretion (mg/24 h)	—	<30	170.9 ± 40.1
Creatinine (μmol/L)	72 ± 5.7†	81 ± 7.5*	111 ± 11.7
Creatinine clearance (mL/s)	1.63 ± 0.10†	1.64 ± 0.05‡	1.15 ± 0.08
Triglycerides (mmol/L)	1.59 ± 0.20*	2.09 ± 0.27	3.54 ± 1.14
Total cholesterol (mmol/L)	5.79 ± 0.48	6.0 ± 0.37	5.61 ± 0.54
LDL cholesterol (mmol/L)	3.88 ± 0.38	3.80 ± 0.35	3.68 ± 0.51
HDL cholesterol (mmol/L)	1.16 ± 0.11	1.27 ± 0.13*	0.89 ± 0.06
HbA_{1c} (%)	—	8.01 ± 0.42	8.42 ± 0.36
Diabetes duration (yr)	—	15.0 ± 3.1	19.9 ± 3.0
Insulin treatment (yes/no)	—	7/3	9/1
Daily insulin dose (U)	—	71 ± 8	64 ± 6
Diabetic retinopathy (yes/no)	—	1/9	8/2
Diabetic neuropathy (yes/no)	—	7/3	10/0
Hypertension (yes/no)	0/10	7/3	10/0

NOTE. Data are the mean ± SE.

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein.

*$P < .05$, †$P < .01$, ‡$P < .001$ *v* microalbuminuric.

degree of albuminuria ($r = -.50$, $P = .07$). When the data for the subgroups of group B were pooled, we found an inverse correlation between the serum leptin level and creatinine clearance ($r = -.42$, $P < .01$, Fig 4), which remained significant after adjusting for body fat ($r = -.45$, $P < .006$).

Pooled Data for Groups A and B

Multiple regression analysis of the pooled data for groups A and B showed that the percent body fat ($\beta = 0.61$, $P < .001$) and creatinine clearance ($\beta = -0.35$, $P < .002$), but none of the other variables, were independently related to serum leptin levels ($R^2 = .41$).

DISCUSSION

The present data indicate that serum leptin concentrations are elevated in type 2 diabetic patients with microalbuminuria and macroalbuminuria. Type 2 diabetes per se was found not to affect serum leptin levels, confirming the results of previous studies.[17-21] The potential confounding influence of sex and body fatness, the major determinants of serum leptin,[20-24] was controlled by carefully matching the subgroups for these variables. In both study groups, the duration of diabetes, daily dose of subcutaneous insulin, and glycemic control (HbA_{1c}) did

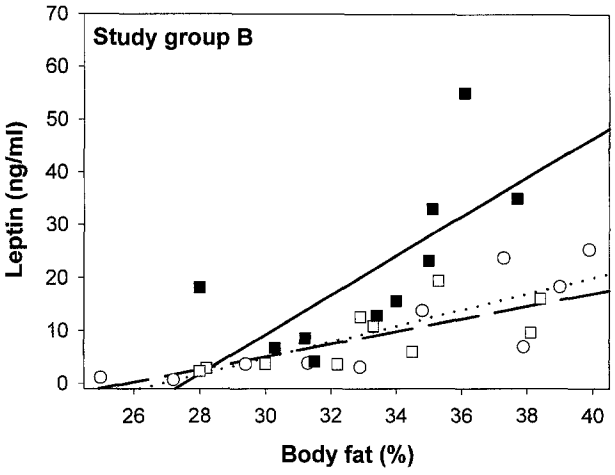

Fig 3. Correlation between the serum leptin concentration and percent body fat in 10 diabetics with microalbuminuria (■, —, 3.71x − 102.22, $r = .69$, $P < .05$), 10 diabetics with normoalbuminuria (□, ---, y = 1.19x − 30.79, $r = .73$, $P < .01$), and 10 healthy control subjects (○, . . ., y = 1.52x − 40.77, $r = .82$, $P < .01$). The slope of the regression lines did not differ significantly between subgroups ($P > .05$ for all comparisons).

not differ between diabetics with and without albuminuria. Thus, the differences found for serum leptin between groups can be most likely attributed to albuminuric nephropathy.

The human kidney plays a substantial role in leptin removal from the plasma by absorbing and degrading the peptide.[5] Leptin levels have been found to be elevated in patients with end-stage renal disease of various etiologies.[6-13] The present study extends the previous findings of elevated leptin to the earlier stages of renal disease, suggesting that renal leptin degradation is already impaired at these stages of nephropathy. Merabet et al[10] found a tendency for higher leptin levels in patients with renal failure caused by diabetes or primary hypertension versus other etiologies of renal failure, suggesting

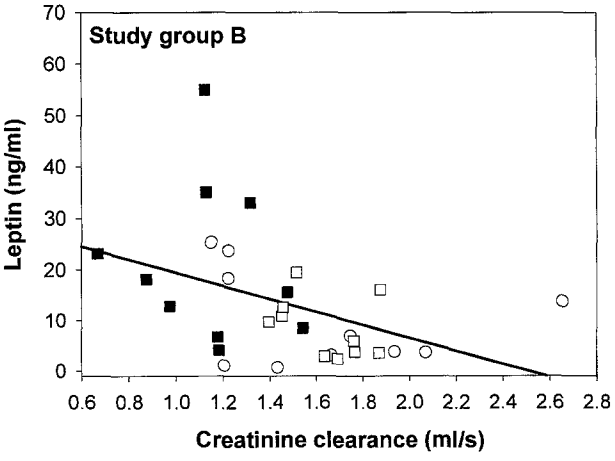

Fig 4. Correlation between the serum leptin concentration and creatinine clearance in the pooled data for group B (y = −12.87x + 32.31, $r = -.42$, $P < .01$) including 10 diabetics with microalbuminuria (■), 10 diabetics with normoalbuminuria (□), and 10 healthy control subjects (○).

that the impairment of renal leptin degradation could be especially pronounced in diabetic and hypertensive nephropathy. Although the prevalence of albuminuria in type 2 diabetics is not specific for diabetic nephropathy,[25] the present finding of elevated leptin levels even in diabetic patients with microalbuminuria may provide further support for this view.

Renal function as estimated by creatinine clearance was found to be independently related to serum leptin in the present study. This result agrees with the previous finding by Shoji et al[26] that renal function (assessed by 1/creatinine) is an independent determinant of plasma leptin levels in type 2 diabetic patients with various stages of diabetic nephropathy. Together, these findings and our results suggest that leptin levels may increase with the progression of diabetic nephropathy. However, longitudinal studies are required to confirm this hypothesis.

The physiological effects of elevated leptin in patients with renal disease can only be speculated, since leptin action in humans remains to be determined. However, considering the effect of leptin on blood pressure[27] and energy balance[28-30] in rodents, one may speculate that elevated leptin levels may contribute to the hypertension and poor nutritional status found in patients with advanced renal disease.

In summary, the present results provide evidence that serum leptin concentrations are elevated in type 2 diabetics with microalbuminuria and macroalbuminuria. This novel finding suggests that renal leptin degradation may be impaired in these early stages of renal disease.

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