Decreased Plasma Lipoprotein Lipase in Hypoadiponectinemia

An association independent of systemic inflammation and insulin resistance

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OBJECTIVE — Adiponectin is a plasma protein expressed in adipose tissue. Hypoadiponectinemia is associated with low HDL cholesterol and high plasma triglycerides, which also characterize lipoprotein lipase (LPL) deficiency syndromes. Recently, dramatically increased LPL activity was reported in mice overexpressing adiponectin. We therefore speculated that adiponectin may directly affect LPL in humans.

RESEARCH DESIGN AND METHODS — We measured plasma adiponectin and postheparin LPL in 206 nondiabetic men and in a second group of 110 patients with type 2 diabetes. Parameters were correlated with markers of systemic inflammation (C-reactive protein [CRP]) and insulin resistance (homeostatis model assessment of insulin resistance [HOMA-IR]).

RESULTS — Nondiabetic subjects with decreased plasma adiponectin had lower LPL activity ($r = 0.42$, $P < 0.0001$). This association of plasma adiponectin with LPL activity was confirmed in the second group of patients with type 2 diabetes ($r = 0.37$, $P < 0.0001$). Multivariate analysis revealed that adiponectin was the strongest factor influencing LPL activity, accounting for 23% of the variation in LPL activity in nondiabetic subjects and for 26% of the variation in LPL activity in type 2 diabetic patients. These associations were independent of plasma CRP and HOMA-IR.

CONCLUSIONS — These results demonstrate an association of decreased postheparin LPL activity with low plasma adiponectin that is independent of systemic inflammation and insulin resistance. Therefore, LPL may represent a link between low adiponectin levels and dyslipidemia in both nondiabetic individuals and patients with type 2 diabetes.

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Lipoprotein lipase (LPL) is a pivotal enzyme in lipid metabolism (1). Low LPL activity may be observed in the diabetic dyslipidemia of low HDL cholesterol and high plasma triglycerides (2). Plasma LPL activity has been shown to be decreased in insulin-resistant subjects without diabetes (3), and recently, overall plasma LPL activity was inversely associated with insulin resistance in patients with type 2 diabetes (4). LPL expression is also modulated by systemic inflammation. Tumor necrosis factor (TNF)-α and interleukin-6 reduce the expression of LPL at the transcriptional level and decrease LPL activity in plasma (5,6).

Adiponectin, the gene product of the apM1 (adipose most abundant gene transcript 1) gene (7), is a member of bioactive substances known as adipocytokines. Adiponectin plasma levels are negatively correlated with BMI (8), insulin resistance (9), and type 2 diabetes (9). Furthermore, adiponectin is associated with lower TNF-α expression (10) and has been shown to suppress TNF-α–induced activation of nuclear transcription factor-κB (11), thereby demonstrating anti-inflammatory potential. Recently published data suggest that low levels of adiponectin are associated with a lipid profile of low HDL cholesterol and increased plasma triglycerides (12) that is also characteristic for the hypertriglyceridemia in LPL deficiency. LPL is mainly expressed in muscle and adipose tissue (1), the unique source of adiponectin. These findings suggest a direct or indirect relationship between LPL and adiponectin by an unknown mechanism. We therefore investigated, in two different groups of patients, whether adiponectin influences LPL plasma activity and concentration and whether this relationship was affected by systemic inflammatory activity, insulin resistance, or overt diabetes.

RESEARCH DESIGN AND METHODS

— We investigated two different cohorts of patients. *1*) A total of 206 nondiabetic men were recruited from the University Hospital Heidelberg, Department of Medicine. All individuals in this group had diagnosed or suspected coronary artery disease and underwent elective coronary angiography. The overt diagnosis of diabetes, according to the American Diabetes Association criteria

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Abbreviations: CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; HOMA, homeostatis model assessment; HOMA-IR, HOMA of insulin resistance; hs-CRP, high-sensitivity CRP; LPL, lipoprotein lipase; TNF, tumor necrosis factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical characteristics of the study population*

	No incident diabetes	Incident diabetes
n	206	110
Age (years)	60.9 ± 10.4	55.9 ± 9.6
BMI (kg/m^2)	27.3 ± 3.2	28.6 ± 4.1
Serum creatinine (mg/dl)	0.99 ± 0.25	0.97 ± 0.33
Serum total cholesterol (mmol/l)	5.3 ± 1.1	5.7 ± 1.4
LDL cholesterol (mmol/l)	3.6 ± 1.0	3.8 ± 1.1
HDL cholesterol (mmol/l)	1.1 ± 0.3	1.0 ± 0.4
VLDL cholesterol (mmol/l)*	0.6 ± 0.4	1.0 ± 1.4
Triglycerides (mmol/l)*	1.5 ± 0.8	2.3 ± 2.2
Atherogenic index*	5.3 ± 1.7	6.2 ± 3.4
HbA_{1c}		7.5 ± 0.9
Glucose (mg/dl)	96.9 ± 13.0	159.4 ± 51.0
Insulin (μU/ml)†	21.2 ± 8.0	36.8 ± 26.0
HOMA-IR†	4.9 ± 1.6	13.1 ± 7.9
LPL activity (nmol · ml^{-1} · min^{-1})	175.9 ± 64.7	240.2 ± 139.9
LPL concentration (ng/ml)	298.6 ± 120.7	373.3 ± 177.8
hs-CRP (mg/l)*	2.3 ± 2.3	2.3 ± 2.4
Adiponectin (μg/ml)*	5.9 ± 4.4	5.7 ± 4.4

Data are mean ± SE or *median ± SE. Diabetes was defined by history or by a fasting plasma glucose >126 mg/dl. †Subjects were not treated with insulin.

(fasting plasma glucose ≥126 mg/dl) (13), was an exclusion criterion. 2) A total of 110 patients (82 male and 28 female subjects) with known type 2 diabetes were recruited from the Diabetes Outpatient Clinic of the University Hospital Heidelberg.

Contraindications for heparin, treatment with subcutaneous or intravenous heparin in the previous 72 h, severe kidney or liver disease, treatment with drugs known to affect LPL activity (such as fibrates) or adiponectin plasma levels (such as peroxisome proliferator–activated receptor γ agonists), and a fasting triglyceride level >11.4 mmol/l (1,000 mg/dl), suggesting secondary lipid disorders, were exclusion criteria in both nondiabetic and type 2 diabetic subjects. The study was approved by the Internal Ethics Committee of Heidelberg University, and each patient gave informed consent.

Analysis of lipids/lipoproteins

Total cholesterol, HDL cholesterol, and triglyceride concentrations were determined enzymatically with a Synchron LX-20 (Beckman Coulter, Munich, Germany). LDL and VLDL were separated by ultracentrifugation in a Beckman LM-8 ultracentrifuge in 100-μl volumes with a VT-51.2 rotor (Beckman Coulter). The atherogenic index was calculated by the following formula: (total cholesterol − HDL cholesterol)/HDL cholesterol.

LPL

After an overnight fast, venous blood samples were drawn into EDTA tubes before and 10 min after intravenous injection of 60 IU heparin (Braun Melsungen, Melsungen, Germany) per kilogram of body weight. The samples were immediately chilled to 4°C, centrifuged, and stored at −80°C until assayed. Postheparin LPL activity was determined with a triolein/phosphatidylcholine emulsion as described previously (14). Regular serum was used as the source for the LPL activator apolipoprotein C-II. Selective measurement of LPL was based on the inactivation of LPL by 1.0 mol/l NaCl. The samples were quantitated in duplicate, and postheparin plasma from pooled normal control subjects was used to correct for interassay variation. The intra-assay coefficient was 7.8% and the interassay coefficient 11.4%. LPL concentration in postheparin plasma was quantitated by enzyme-linked immunosorbent assay (ELISA) (Immundiagnostik, Bensheim, Germany) using monoclonal antibodies against the COOH-terminal domain of LPL for capture and quantitation.

Blood variables

In the morning after an overnight fast, venous blood was sampled for the measurement of plasma concentrations of adiponectin, glucose, insulin, and C-reactive protein (CRP). Adiponectin samples were quantitated in duplicate by ELISA (B-Bridge International, San Jose, CA), and plasma from four normal control subjects was used for interassay variation. Both the intra- and interassay coefficients of variation were <5.0%. Plasma glucose was measured by a glucose-oxidase method. Serum insulin immunoreactivity was determined from frozen serum by ELISA (CIS bio international, Gif-Sur-Yvette, France). Plasma concentrations of CRP in a highly sensitive assay (high-sensitivity CRP [hs-CRP]) were determined by ELISA (Dade Behring, Cupertino, CA).

Table 2—*Spearman correlation coefficients with plasma adiponectin*

	Plasma adiponectin			
	Nondiabetic		Diabetic	
Factor	*r*	*P*	*r*	*P*
Age	0.114	0.1106	−0.119	0.1533
BMI	**−0.246**	**<0.001**	**−0.225**	**<0.01**
Serum creatinine	0.116	0.1045	0.131	0.2612
Serum total cholesterol	0.084	0.2384	0.122	0.2031
LDL cholesterol	0.004	0.9582	0.095	0.3442
HDL cholesterol	**0.396**	**<0.0001**	**0.437**	**<0.0001**
Triglycerides	**−0.173**	**<0.01**	**−0.296**	**<0.01**
HOMA-IR	**−0.388**	**<0.0001**	0.035	0.8062
LPL activity	**0.417**	**<0.0001**	**0.372**	**<0.0001**
LPL concentration	**0.308**	**<0.0001**	**0.421**	**<0.0001**
hs-CRP	**−0.244**	**<0.001**	**−0.315**	**<0.01**

Correlation coefficients in bold indicate statistical significance ($P < 0.05$).

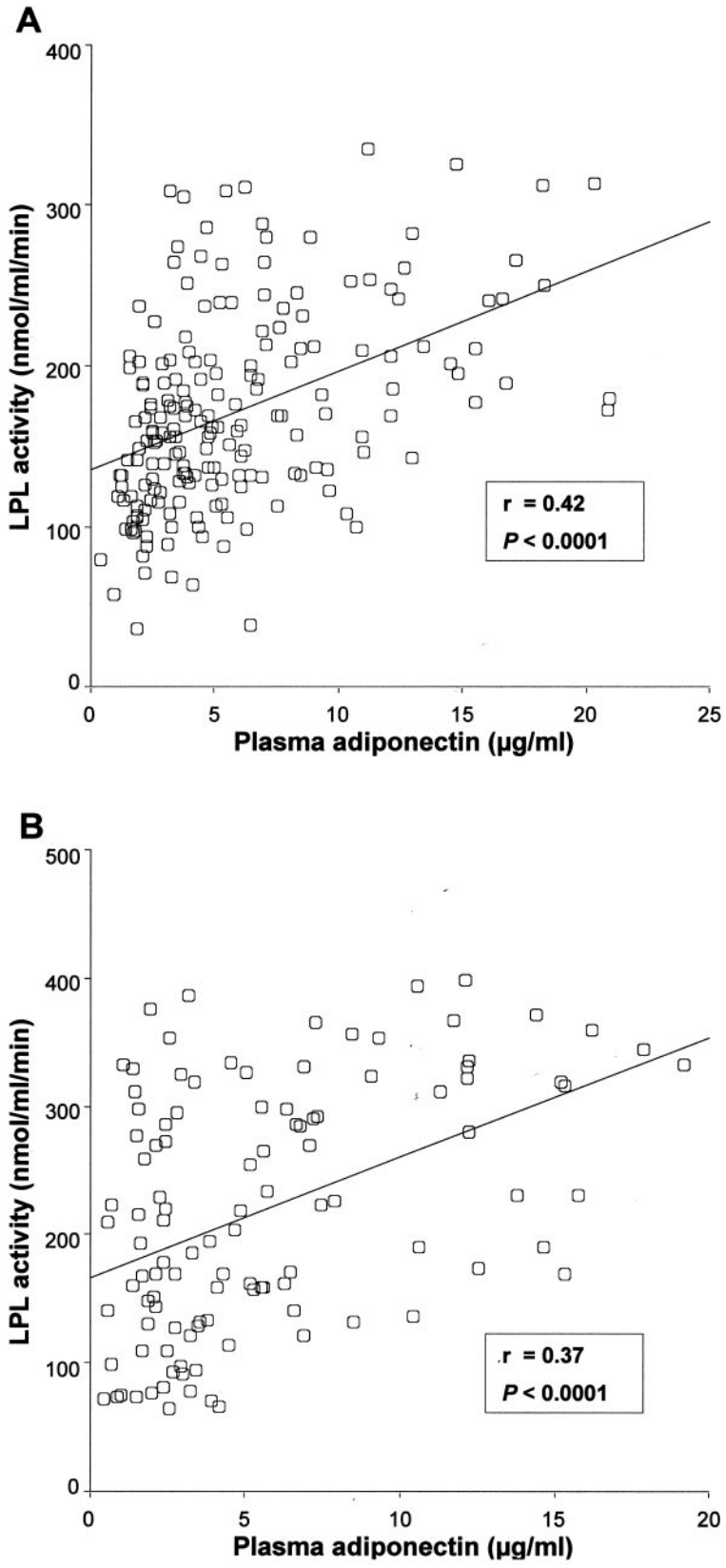

Figure 1—*Correlation between LPL activity and plasma adiponectin levels in 206 nondiabetic subjects* (A) *and 110 patients with type 2 diabetes* (B). *Spearman correlation coefficients are shown.*

Assessment of insulin resistance

The degree of insulin resistance was estimated by homeostatis model assessment (HOMA) according to the method described by Matthews et al. (15). In particular, an insulin resistance score (HOMA of insulin resistance [HOMA-IR]) was computed with the following formula: fasting serum glucose (mmol/l) × fasting serum insulin (μU/ml)/22.5.

Statistical analyses

Statistical analyses were performed with SPSS for Windows (version 11.0; SPSS, Chicago, IL). Spearman correlation coefficients were used to describe the association between adiponectin and other continuous variables of interest. Linear regression models were used to control for potentially confounding variables. The models, fitted for LPL activity as a dependent variable, included age, BMI, plasma triglycerides, HDL cholesterol, HOMA-IR, hs-CRP, and adiponectin plasma concentration, as well as independent variables, to demonstrate the relative contribution of each of these variables to the outcome variable. Variables that were not normally distributed, such as plasma adiponectin, triglycerides, and hs-CRP, were log transformed to better approximate normal distributions. Results are expressed as means ± SE. A P value <0.05 was considered statistically significant.

RESULTS — The clinical characteristics of the subjects studied are summarized in Table 1. Table 2 shows the association between adiponectin and selected variables for both nondiabetic and diabetic subjects. LPL activity and concentration were highly correlated with each other ($P < 0.001$) in both cohorts, as expected (16). Therefore, we mainly report the findings with LPL activity. In both nondiabetic and type 2 diabetic subjects, adiponectin levels were strongly correlated with postheparin plasma LPL activity (Fig. 1 and Table 2). As shown in Table 2, there was a significant negative correlation between plasma adiponectin levels and the concentration of plasma hs-CRP in both groups. HOMA-IR was negatively associated with plasma adiponectin in the nondiabetic group. Spearman correlation coefficients of plasma adiponectin with BMI and lipid parameters (HDL cholesterol and plasma triglycerides) were similar in the diabetic and nondiabetic subjects. We also found a

Table 3—*Multiple regression models predicting LPL activity*

	Nondiabetic subjects							Diabetic subjects						
	Model 1		Model 2		Model 3			Model 1		Model 2		Model 3		
Variable	β	*t*	β	*t*	β	*t*	*P*	β	*t*	β	*t*	β	*t*	*P*
Adiponectin*	0.521	3.813	0.489	3.240	0.476	3.009	**0.005**	0.357	2.181	0.430	2.229	0.443	2.184	**0.039**
HOMA-IR	−0.266	−2.004	−0.255	−1.858	−0.276	−1.801	0.080	−0.320	−1.941	−0.320	−1.821	−0.379	−2.024	0.055
hs-CRP*	−0.244	−1.846	−0.265	−1.919	−0.261	−1.859	0.071	−0.230	−1.380	−0.282	−1.690	−0.313	−1.807	0.084
Triglycerides*			0.070	0.503	0.077	0.543	0.590			−0.229	−1.352	−0.238	−1.342	0.193
HDL cholesterol			0.101	0.663	0.096	0.615	0.542			−0.265	−1.322	−0.323	−1.526	0.141
Age					0.046	0.319	0.752					−0.009	−0.049	0.961
BMI					0.121	0.863	0.312					−0.190	−1.063	0.232
r		0.664		0.670			0.671		0.564		0.628			0.652
r^2		0.441		0.448			0.450		0.318		0.395			0.425

*Log-transformed variables. *P* values in bold indicate statistical significance ($P < 0.05$).

significant relationship between postheparin LPL activity and HOMA-IR in both cohorts (data not shown). Patients with significant renal insufficiency have been shown to have elevated adiponectin plasma levels (17). In our patient cohort, however, very few subjects had increased plasma creatinine values. Adiponectin was not correlated with creatinine in either of our cohorts.

In multivariate analyses including markers of systemic inflammation (hs-CRP) and insulin resistance (HOMA-IR), the strongest predictive variable for LPL activity in the nondiabetic and diabetic groups was adiponectin in $\log_{10}$ ($r = 0.521$ and $r = 0.357$, respectively) (Table 3, model 1). When HDL cholesterol and plasma triglycerides (Table 3, model 2) and finally age and BMI (Table 3, model 3) were included in the model, adiponectin remained the strongest predictive factor for LPL activity in both groups. Regression analyses revealed that plasma adiponectin levels account for 23% of the variation in LPL activity in nondiabetic subjects and 26% of the variation in LPL activity in type 2 diabetic patients.

CONCLUSIONS — Our data in two independent cohorts of patients demonstrate a significant relationship between the plasma levels of adiponectin and postheparin plasma LPL activity. This is of special importance because investigations on the regulation of plasma LPL activity in larger patient collectives are limited because of the complex procedure of the quantification of postheparin plasma LPL activity.

Similar findings of dramatically raised LPL activity associated with increased plasma adiponectin levels have recently been shown in an animal model (18). Our data also confirm a significant negative association between the plasma adiponectin level and the concentration of hs-CRP that has been previously reported (19). Thus, a proinflammatory state with a decreased plasma concentration of adiponectin may result in reduced LPL expression.

In vitro, insulin is a strong determinant of LPL activity (1). In clinical studies, however, conflicting data about the association of plasma insulin levels with LPL activity have been published (20,21). Therefore, it has been suggested that the HOMA index, integrating plasma insulin levels with glycemia, may be the more reliable parameter (4). Our results show a negative correlation between postheparin LPL activity and HOMA-IR in both cohorts studied, which is consistent with previous reports (4). Plasma adiponectin has been shown to be inversely correlated with insulin resistance (9). This capability of adiponectin to increase insulin sensitivity may therefore point to an indirect effect of adiponectin on plasma LPL activity. Our findings, however, show that the relationship between adiponectin and postheparin LPL activity and concentration is independent of both adiponectin effects on whole-body insulin sensitivity and systemic inflammation.

Multivariate regression analyses, including HOMA-IR as a marker of insulin resistance and hs-CRP as a marker of systemic inflammation, revealed that plasma adiponectin was the strongest independent factor influencing plasma LPL activity in both groups studied and accounted for 23 and 26% of the observed variances in posthaparin LPL activity, respectively.

In a previous study, adiponectin was associated with an antiatherogenic lipid profile (12), but the mechanism by which adiponectin influences lipid metabolism has not been elucidated. We now show for the first time a significant relationship between plasma adiponectin and lipid metabolism that is independent of inflammation and insulin resistance. We speculate that adiponectin, in addition to its anti-inflammatory properties, may directly stimulate the expression of LPL. Thus, increased LPL activity stimulated by increased adiponectin levels might result in elevated plasma HDL cholesterol and antiatherogenic changes in lipid profile because a positive association between LPL activity and HDL levels is known (22).

In summary, we demonstrate for the first time that plasma levels of adiponectin are significantly correlated with LPL activity in two independent cohorts, nondiabetic individuals and patients with type 2 diabetes. This relationship is independent of systemic inflammatory activity and unaffected by the link of adiponectin with insulin resistance.

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