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Low serum adiponectin is associated with high circulating oxidized low-density lipoprotein in patients with type 2 diabetes mellitus and coronary artery disease

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Abstract

Decrease in adiponectin level, a common feature in patients with type 2 diabetes mellitus, is considered to predict cardiovascular events. Elevated oxidized low-density lipoprotein (oxLDL), formed within the arterial wall, is commonly seen as part of the atherogenic profile. We investigated the association of adiponectin and oxLDL in 58 patients with type 2 diabetes mellitus and ischemic coronary artery disease. In addition to adiponectin, the serum lipid profile (including oxLDL), plasminogen activator inhibitor 1, high-sensitivity C-reactive protein, and whole-body glucose uptake determined by euglycemic-hyperinsulinemic clamp were evaluated. The average adiponectin level was 7.1 ± 3.5 μg/mL and was higher in female than in male patients (P = .011). Adiponectin level correlated with whole-body glucose uptake (P = .037) and high-density lipoprotein (HDL) cholesterol concentration (P = .007) and was inversely associated with oxLDL (P = .005), triglycerides (P = .010), and plasminogen activator inhibitor 1 (P = .004). No association was found between adiponectin and high-sensitivity C-reactive protein or LDL cholesterol levels. In multiple linear regression analysis, adiponectin contributed to oxLDL concentration, whereas total cholesterol, LDL and HDL cholesterol, and triglycerides did not. In conclusion, our results suggest that low adiponectin concentration indicates increased oxidative state in the arterial wall, which further supports previous data on the role of adipose tissue in atherogenesis.

1. Introduction

Patients with type 2 diabetes mellitus (T2DM) are at high risk of cardiovascular events. The exact mechanism responsible for the development of atherosclerotic disease in these patients is still unknown. Oxidized low-density lipoprotein (oxLDL) is a key player in macrophage foam cell formation, which forms the basis of atherosclerotic disease, and circulating oxLDL is strongly associated with coronary artery disease (CAD) [1].

Quite recently, adipose tissue has been recognized as a metabolically active organ; in particular, accumulation of visceral adipose tissue is an important risk factor for cardiovascular events [2]. Adiponectin is an adipose tissue–derived hormone with beneficial effects on fat metabolism and insulin sensitivity. Previously, low adiponectin levels have been linked to insulin-resistant conditions such as T2DM and obesity [3]. In addition, hypoadiponectinemia is independently correlated with CAD [4] and low adiponectin levels predict the severity of coronary disease [5].

Concentration of serum adiponectin is directly associated with high-density lipoprotein (HDL) cholesterol

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Table 1
Characteristics of the study subjects

Sex (male/female)	42/16
Age (y)	63.8 ± 7.4
Body weight (kg)	88.1 ± 16.3
Mean duration of diabetes (y)	7.0 ± 6.4
BMI (kg/m^2)	29.8 ± 4.2
Waist-hip ratio	0.99 ± 0.07
Blood pressure (mm Hg)	146/78 ± 22/8
Fasting plasma glucose (mmol/L)	7.5 ± 1.8
HbA_{1c} (%)	7.2 ± 0.9
Fasting C-peptide (nmol/L)	0.86 ± 0.32
Fasting insulin (pmol/L)	52.5 ± 29.9
Fasting free fatty acids (mmol/L)	0.78 ± 0.26
Whole-body glucose uptake (μmol/kg per minute)	11.7 ± 5.1
Medications (n = 58), n (%)	
β-Adrenoceptor antagonists	44 (76)
Statins	28 (48)
Angiotensin-converting enzyme inhibitors	25 (43)
Calcium antagonists	13 (22)
Acetylsalicylic acid	51 (88)
Long-acting nitrates	14 (24)

Values are presented as mean ± SD or number (%).

concentration [6] and inversely with triglyceride level [7]. Because elevated oxLDL and low adiponectin concentrations are both significant predictors of complications in patients with T2DM and because their mutual association is presently unknown, we evaluated their interrelationship in patients with T2DM and ischemic CAD, simultaneously controlling for several potentially confounding factors.

2. Materials and methods

2.1. Subjects

A total of 58 patients with T2DM and stable CAD were enrolled in the study. Patients were participants of an intervention study with rosiglitazone [8]. Inclusion criteria were ischemic coronary disease previously confirmed by single-photon emission computed tomography perfusion imaging, T2DM treated with diet only or with metformin and/or sulfonylurea, and good or moderate glycemic control (glycated hemoglobin [HbA_{1c}] <8.5%). Criteria for exclusion were unstable angina pectoris, symptomatic tachy- or bradyarrhythmias, a history of percutaneous transluminal coronary angioplasty during the preceding 6 months, long-term use of insulin, use of peroxisome proliferator–activated receptor γ agonists, or clinical signs of heart failure. None of the patients had been previously treated with rosiglitazone. All patients had past or present angina pectoris symptoms under stress but no unstable angina pectoris, and they were on stable medical therapy. Ten patients had a history of previous myocardial infarction, and 6 patients had Q waves on electrocardiogram. Coronary angiography revealed 1-vessel disease in 57% (33 of 58), 2-vessel disease in 31% (18 of 58), and 3-vessel disease in 12% (7 of 58) of the patients. The median degree of the main coronary artery stenosis was 62% (range, 9%-100%). The characteristics and medications of the study subjects are shown in Table 1. All patients gave written informed consent before participating in the study. The study was conducted according to the principles of the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland.

2.2. Study design

Patients fasted and refrained from taking caffeine-containing drinks, from smoking, and from taking all their medications with the exception of short-acting nitrates for 12 hours before the study. For the biochemical measurements, blood was drawn after an overnight fast and thereafter a euglycemic-hyperinsulinemic clamp (1 mU/kg per minute) was performed for 180 minutes. Whole-body glucose uptake, that is, whole-body insulin-sensitivity status, was determined from 80 to 140 minutes of the clamp as described earlier [9].

Coronary angiography was performed via the femoral artery with the Judkins technique after an intravenous injection of 3750 IU of heparin and 0.5 mg of sublingual nitroglycerin. Angiography was performed with 5F catheters (Cordis, Johnson & Johnson, Miami Lakes, FL). A single operator analyzed coronary artery diameters with QCA software (Quantcor stenosis evaluation software, Siemens, Munich, Germany).

2.3. Biochemical analysis

All laboratory samples, with the exception of the samples for the analysis of inflammatory markers, oxidized LDL, and adiponectin, were sent by courier to a central laboratory (Quest Diagnostics, Heston, Middlesex, UK). LDL cholesterol concentration was calculated with the Friedewald formula. Concentrations of oxLDL, plasminogen activator inhibitor 1 (PAI-1), and high-sensitivity C-reactive protein (hsCRP) were determined as described earlier [10-12]. Serum adiponectin level was determined by radioimmunoassay (Human Adiponectin RIA Kit, Linco Research, St Charles, MO). The PAI-1 assay is based on a sandwich-type

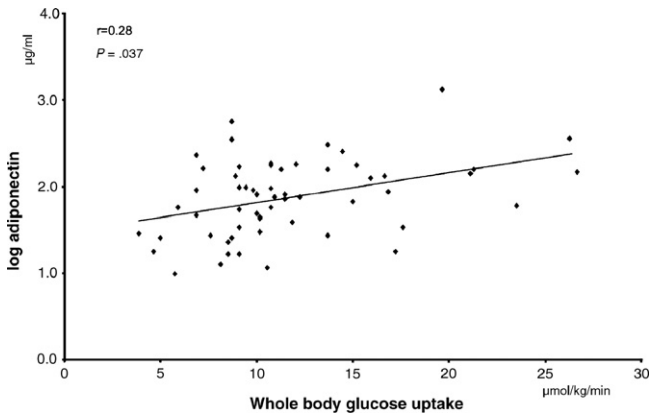

Fig. 1. Adiponectin level is significantly associated with whole-body glucose uptake.

immunoassay that uses a monoclonal antibody in an enzyme-linked immunosorbent assay plate as a capture antibody for human PAI-1 antigens. The assay is also able to detect all PAI-1 forms of plasma (free, total, active, or complex PAI in the plasma). The oxLDL and PAI-1 antigen assays are currently used only for research purposes; therefore, clinically relevant reference values are not available. For hsCRP, the reference values in apparent infections are greater than 10 mg/L. In noninfectious situations, values of less than 1, 1 to 3, and greater than 3 mg/L have been defined by the American Heart Association to represent low, average, and high risk for coronary disease, respectively [13].

2.4. Statistical methods

The results are given as mean ± SD. Because of the skewed distribution, adiponectin values were log transformed before analyses. The relationships between adiponectin and waist-hip ratio, age, and body mass index (BMI) were calculated by linear regression analysis and the sex difference in adiponectin was analyzed with 1-way analysis of variance and Tukey test. Partial Pearson correlation coefficient or partial Spearman correlation coefficient (in case of nonnormally distributed variables) was calculated to study the association of adiponectin concentration and lipoproteins or inflammatory parameters after adjustment for sex and age. Multiple linear regression analysis was performed to control for confounding variables when studying the relationship between adiponectin and oxLDL. A P value less than .05 was considered statistically significant. All statistical analyses were performed with SAS statistical analysis system, version 8.2 (Cary, NC).

3. Results

3.1. Adiponectin and clinical characteristics of the study subjects

The average serum adiponectin concentration was 7.1 ± 3.5 μg/mL (range, 2.7-22.5 μg/mL). It was significantly higher in female than in male patients (9.4+4.7 vs 6.3 ± 2.5 μg/mL, respectively, P = .011). In the whole study population, adiponectin was inversely associated with waist-hip ratio (r = −0.30, P = .025) but not with age (P = .16) or BMI (P = .18). Adiponectin level was not associated

Table 2
Association between adiponectin and serum lipids, PAI-1, and hsCRP

	Mean concentration	Correlation coefficient with adiponectin[a]	P
Total cholesterol (mmol/L)	4.40 ± 0.74	0.11	.44
LDL (mmol/L)	2.48 ± 0.67	0.04	.75
Oxidized LDL (mU/L)	37.2 ± 10.6	−0.37	.005
HDL (mmol/L)	1.12 ± 0.28	0.37	.007
Triglycerides (mmol/L)	1.78 ± 0.89	−0.35	.010
PAI-1 (AU/mL)	11.8 ± 10.2	−0.39	.004
hsCRP (mg/L)	1.7 ± 1.6	−0.06	.67

[a] Adjusted for sex and age.

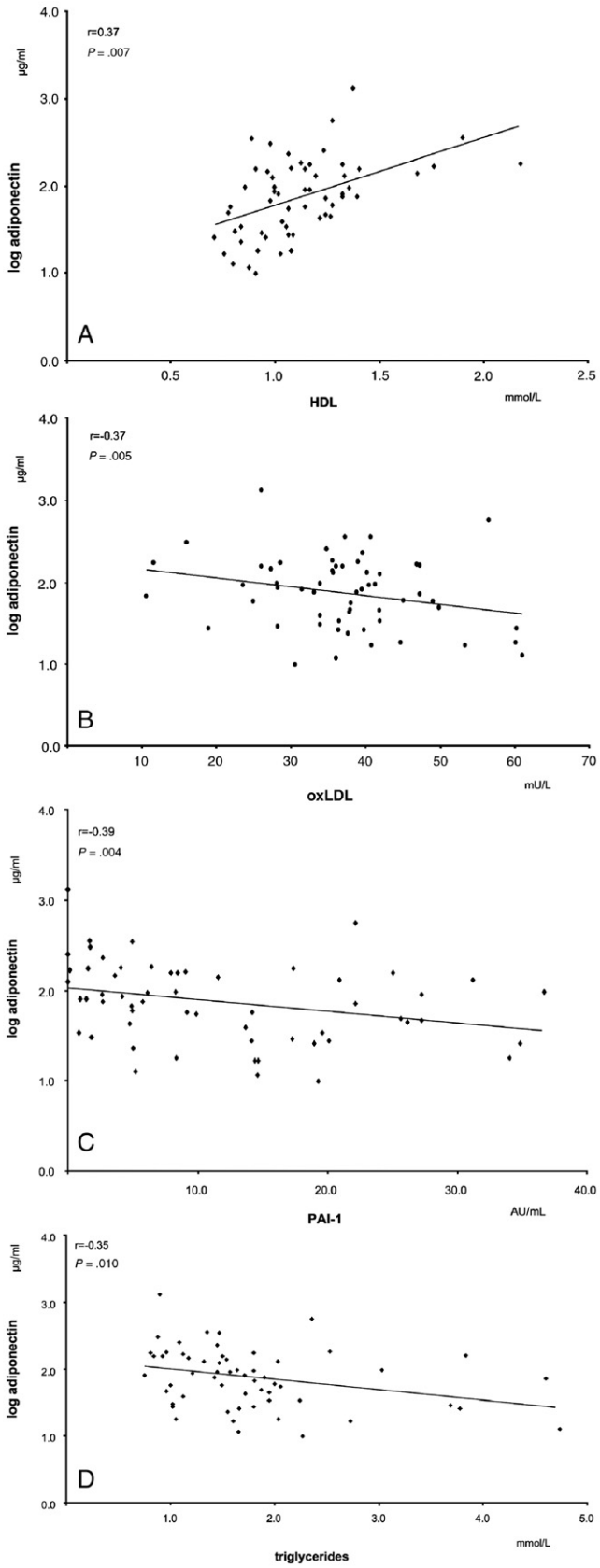

Fig. 2. Adiponectin level is directly correlated with HDL concentration (A) and inversely associated with oxLDL (B), PAI-1 (C), and triglyceride (D) concentrations.

Table 3
Multiple linear regression analysis results

Independent variable	*P*	Parameter estimate	95% Confidence limits
Model A[a]			
Sex	.018	11.3	2.04, 20.53
Waist-hip ratio	.49	19.6	−37.08, 76.25
Log adiponectin (μg/mL)	.016	−9.44	−17.05, −1.84
HDL cholesterol (mmol/L)	.27	6.08	−4.86, 17.01
Triglycerides (mmol/L)	.10	2.64	−0.54, 5.81
C-peptide (nmol/L)	.22	6.31	−3.82, 16.43
PAI-1 (AU/mL)	.23	−0.18	−0.48, 0.12
Whole-body glucose uptake (μmol/kg per minute)	.83	−0.07	−0.75, 0.60
Model B[b]			
Log adiponectin (μg/mL)	.014	−6.87	−12.31, −1.43
Total cholesterol (mmol/L)	.60	−26.98	−130.97, 77.02
LDL cholesterol (mmol/L)	.48	36.87	−67.22, 140.95
HDL cholesterol (mmol/L)	.53	32.64	−70.26, 135.54
Triglycerides (mmol/L)	.48	16.85	−30.37, 64.06

Dependent variable, oxLDL (mU/L).

[a] Multiple linear regression analysis shows that sex and adiponectin concentration are the major determinants of the circulating oxLDL concentration. $R^2 = 0.36$.

[b] Multiple linear regression analysis shows that when adiponectin and serum lipid parameters are used as predictors of oxLDL, only adiponectin is a significant contributor. $R^2 = 0.49$.

with fasting plasma glucose, insulin, or HbA_{1c}, but was inversely correlated with circulating C-peptide level ($r = -0.40$, $P = .002$). There was a significant association between adiponectin and whole-body glucose uptake ($r = 0.28$, $P = .037$; Fig. 1). The degree of coronary stenosis did not correlate with the adiponectin level.

3.2. Adiponectin, lipids, and inflammation

Serum adiponectin was significantly and directly associated with HDL cholesterol level and inversely associated with triglycerides, PAI-1, and oxLDL (Table 2; Fig. 2). During euglycemic clamp, adiponectin level was inversely associated with circulating free fatty acid concentration ($r = -0.37$, $P = .005$), whereas such a correlation did not exist in the fasting state. There was no association between oxLDL and LDL cholesterol.

In the multiple linear regression analysis (Table 3), sex and adiponectin were the only factors associated in a statistically significant manner with oxLDL. In addition, in a model including all lipoproteins, adiponectin was the only factor significantly associated with oxLDL.

4. Discussion

The current study shows that adiponectin is inversely associated with oxLDL, extending our knowledge on the associations between adiponectin and lipids in patients with insulin resistance. The results suggest a link between adipose tissue dysfunction and the atheromatous vascular damage in patients with T2DM and CAD. Furthermore, using multiple linear regression analysis, we show that adiponectin is a stronger contributor of oxLDL level than any other lipid parameter included in the model.

There is a growing body of evidence that low levels of adiponectin are associated with diabetes and obesity as well as with CAD. It has been suggested that adiponectin would predict future cardiovascular events [14]. In agreement with previous studies [6], we found a sex difference in adiponectin concentrations, with female subjects having higher levels than did male subjects. In addition, adiponectin was inversely associated with abdominal obesity as measured with waist-hip ratio, whereas no association existed with BMI. This suggests that adipose tissue distribution rather than its total mass is an important determinant of adiponectin concentration. This is in agreement with a previous study in which visceral fat depots were identified as a significant predictor of adiponectin level [15].

Circulating oxLDL originates from the oxidation taking place in the arterial wall by cell-associated lipoxygenase and/or myeloperoxidase enzymes [16]. Oxidized LDL is significantly higher in T2DM patients with CAD than in T2DM patients without CAD and is also independent of glycemic control and the duration of diabetes [17]. High oxLDL level decreases the stability of a vulnerable plague [18]. Previous in vitro studies have presented some evidence of a link between adiponectin and oxidized LDL by showing that recombinant globular adiponectin may inhibit the cellular proliferation and generation of reactive oxygen species induced by oxLDL [19]. In addition, low adiponectin levels are associated with the complexity of the coronary lesions, suggesting a relationship between adiponectin level and coronary plaque vulnerability [20]. Therefore, the results of the current study suggest that the causal relationship between adiponectin and oxLDL-induced effects seen in vitro may indeed exist also in vivo. This suggests that adiponectin may have antioxidative effects on LDL particles in the arterial wall and, thus, decrease the production of oxLDL. This relationship may be beneficial for the patients with diseased coronary arteries by promoting the plaque stability.

In the current study, we evaluated the relationship between adiponectin and the markers of high-risk profile for acute coronary event in T2DM patients with existing ischemic coronary disease. In previous studies, adiponectin decreased the expression of several adhesion molecules on the endothelial surface [21]. In addition, PAI-1, a secretory product of the inflammatory process in the vascular wall, has a strong inverse relationship with adiponectin [6]. PAI-1 is a significant player in hypercoagulable states such as obesity and myocardial infarction [22] and is primarily secreted from platelets and endothelium, although in obesity-related conditions there is some evidence of adipocyte-derived PAI-1 as well [23]. In agreement with previous studies, PAI-1 was significantly inversely associated with adiponectin, further strengthening the view that adipose tissue–derived factors may have a strong effect on local vascular wall

inflammation. Surprisingly, in the current study, hsCRP, a marker of low-grade general inflammation, was not associated with adiponectin. This may be due to the multiple sites of CRP synthesis; that is, IL-6 mediates its production in liver, in atherosclerotic plaques, and even in adipose tissue, and in various patient groups, the importance of the different sites of production may vary [24-26].

The model used in the regression analysis was designed to analyze the significance of the different variables related to oxLDL in the preliminary analysis of a simple regression line. Although we show a significant dependency of oxidized LDL and adiponectin over other confounding cardiovascular risk factors, we may not completely exclude the possibility of an unknown variable of a stronger causal relationship with the increased production of oxLDL. Furthermore, the sample size was limited and no healthy subjects were included in the study; thus, further studies in larger populations and between different patient groups need to be conducted to confirm the finding. In addition, because of the nature of the current patient population the cholesterol-lowering medication may affect the absolute values of lipid parameters. In addition, recently it has been published that statin therapy may also affect the adiponectin level [27]. However, it is noteworthy that despite the small sample size the representativeness of the sample is good with respect to the patient population (T2DM + CAD with multiple medications); furthermore, the main finding of a direct relationship between adiponectin and oxLDL, which remains significant regardless of potential confounders, allows one to speculate that this association, more than individual factors, may promote plaque vulnerability in this high-risk patient group.

In conclusion, elevated adiponectin concentration is an important marker of a decreased cardiovascular risk. The current study is the first to show that in patients with T2DM and CAD, adiponectin is inversely associated with circulating oxLDL. This further confirms a link between adipose tissue metabolism and vascular wall atheromatous processes in these patients.

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