

## CLINICAL STUDY

## Adiponectin may play a part in the pathogenesis of diabetic retinopathy

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### Abstract

**Objectives:** To measure plasma adiponectin concentrations in patients with type 2 diabetes and to investigate any association with the severity of diabetic retinopathy, because adiponectin seems to be an important modulator for metabolic and vascular diseases.

**Methods:** Seventy-four patients (mean age  $46.8 \pm 5.1$  years; body mass index (BMI),  $26.8 \pm 2.10$  kg/m<sup>2</sup>) and 54 healthy volunteers (mean age  $46.8 \pm 5.4$  years; BMI  $26.47 \pm 2.33$  kg/m<sup>2</sup>) were included.

**Results:** Adiponectin concentrations in the patients were significantly lower than those in controls ( $4.71 \pm 2.11$  µg/ml for patients,  $n = 74$ ;  $15.95 \pm 3.72$  µg/ml for controls,  $n = 54$ ;  $P < 0.001$ ). In the patients group there was a significant negative correlation between adiponectin and homeostasis model assessment index ( $r = -0.318$ ,  $P = 0.006$  respectively). Plasma adiponectin concentrations in patients with proliferative diabetic retinopathy ( $n = 20$ ;  $3.16 \pm 1.83$  µg/ml) or non-proliferative diabetic retinopathy ( $n = 24$ ;  $3.97 \pm 1.47$  µg/ml,  $P = 0.014$ ) were significantly lower than those in patients without diabetic retinopathy ( $n = 30$ ;  $6.30 \pm 1.57$  µg/ml,  $P = 0.001$ ). When the presence of diabetes was defined as the final variable in the conditional logistic regression model with the adiponectin concentration as the continuous variable, adiponectin was significantly involved in the model.

**Conclusion:** The results show that adiponectin concentrations are lower in patients with type 2 diabetes and that the concentrations are associated with the severity of diabetic retinopathy. Our findings suggest that adiponectin may take part in the pathogenesis of diabetic retinopathy.

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### Introduction

Diabetic retinopathy is the leading cause of blindness in the industrialized world (1). Factors such as poor blood glucose control, hypertension, blood lipid concentrations and renal disease are all known to have an impact on the condition and may be involved in its progression (2, 3). Although time is the most important risk factor for the development of diabetic retinopathy (4), not all patients with poor control of diabetes over long periods of time develop retinopathy, particularly the advanced proliferative form. Conversely, strict glycaemic control cannot always prevent the development of diabetic retinopathy. Some studies of familial clustering suggest that additional components are also involved in susceptibility to the condition (5).

Adiponectin is a novel protein product of the adipose most abundant gene transcript 1 (*apM1*) gene (6). According to current data, plasma adiponectin

concentrations are decreased in obesity, insulin resistance, type 2 diabetes, coronary disease and hypertension (7–9). Several studies have indicated that adiponectin possesses anti-inflammatory properties and thus may negatively modulate the process of atherogenesis (10–12). Interestingly, the diabetes-susceptibility locus has been mapped to human chromosome 3q27, where the adiponectin gene is located (13). Formerly, we reported a direct association between the severity of diabetic retinopathy and leptin, another adipocytokine related to angiogenesis and neovascularization (14). As adiponectin is another adipocytokine associated with the same vascular complications as leptin, a similar association between adiponectin and diabetic retinopathy was worth investigation.

In the present study we measured plasma adiponectin concentrations in patients with type 2 diabetes and investigated any association between plasma adiponectin concentrations and the degree of diabetic retinopathy in these patients.

## Participants and methods

Seventy-four patients with type 2 diabetes (mean age  $46.8 \pm 5.1$  years, body mass index (BMI)  $26.8 \pm 2.10$  kg/m<sup>2</sup>, male/female ratio 41/33) were enrolled in the study. The patients were all undergoing follow-up in the Retina Division of the Ophthalmology outpatient clinics and had the inclusion criteria described below. None of them had hypertension, hyperlipidaemia, nephropathy, coronary heart disease, heart failure or renal failure. The time since diagnosis of diabetes was  $44.14 \pm 8.4$  months. All patients were being treated with sulphonylureas and were receiving no other medications. They were evaluated by standard physical examination, chest X-ray, baseline electrocardiogram, exercise electrocardiogram, two-dimensional echocardiography and routine clinical laboratory tests, including liver and kidney function tests and 24 h urinary protein measurements.

Each patient underwent ophthalmoscopic examination and fluoroangiography. The stage of diabetic retinopathy was graded in a masked fashion, in that only the code numbers were made available to the graders. The patients were then stratified into subgroups according to the stage of retinopathy. The subgroup without any retinopathy included 30 patients (18 men and 12 women, ages  $47.36 \pm 5.1$  years, BMI  $26.61 \pm 2.16$  kg/m<sup>2</sup>). The subgroup with non-proliferative diabetic retinopathy (NPDR) included 24 patients (male/female ratio 12/12, age  $46.29 \pm 5.3$  years, BMI  $27.07 \pm 2.02$  kg/m<sup>2</sup>). NPDR was diagnosed on the basis of one or more of the following findings: hard or soft exudates, intraretinal microvascular abnormalities, haemorrhage, microaneurysms, and venous beading in at least one eye (15). The subgroup with proliferative diabetic retinopathy (PDR) included 20 patients (male/female ratio 11/9, ages  $48.25 \pm 3.8$  years, BMI  $26.93 \pm 2.17$  kg/m<sup>2</sup>). The diagnosis of PDR required the presence of one or more of the following abnormalities: new vessels and fibrous tissue on disc, fibrous proliferations, and preretinal or vitreous haemorrhages, or both, in at least one eye (15).

Fifty-four healthy volunteers (mean age  $46.84 \pm 5.4$  years, BMI  $26.47 \pm 2.33$  kg/m<sup>2</sup>, male/female ratio 25/29) served as the control group. They were matched with the diabetic patients according to blood pressure, age, BMI and sex. They underwent routine physical and laboratory evaluations to ensure that none had diabetes, hypertension, hyperlipidaemia or other metabolic, hepatic or renal diseases. In addition, none of the healthy volunteers had a family history of hypertension or diabetes. All individuals gave informed consent to participate in the study, which was approved by the local ethics committee of Gülhane School of Medicine. Arterial blood pressures were measured in the right arm by mercury sphygmomanometer three times in a resting condition in the morning, and mean values were calculated for systolic and diastolic pressures.

## Biochemical assays

Serum concentrations of glucose (glucose oxidase peroxidase calorimetric method) and creatinine (Jaffé method) were determined using a Tecnicon Dax-48 system analyser (Miles, Tarrytown, NY, USA). Glycated haemoglobin (HbA<sub>1c</sub>) was measured by inhibition of latex agglutination, using a DCA 2000 analyser (Bayer, Elkhart, IN, USA). Microalbuminuria was detected by an immunoturbidimetric method with an Urinapak Micro Albumin immunokit (Miles, Tarrytown, NY, USA). Total cholesterol, high-density lipoprotein cholesterol and triglycerides were determined by an enzymatic colorimetric method with an Olympus AU 600 autoanalyser, using reagents from Olympus Diagnostics GmbH (Hamburg, Germany). Low-density lipoprotein (LDL) cholesterol was calculated by Friedewald's formula.

The basal serum concentration of insulin was determined by the coated-tube method (Diagnostic Products Corporation, Los Angeles, CA, USA). In particular, a homeostasis model of assessment insulin resistance score (HOMA-IR) was computed with the formula: (HOMA-IR) = fasting plasma glucose (mg/dl) × immunoreactive insulin (μU/ml)/405 (16).

## Radioimmunoassay

After an overnight fast, venous blood samples were drawn and promptly centrifuged, and the plasma was stored at  $-20^{\circ}\text{C}$  until adiponectin assay was performed. All samples were run in the same assay. Plasma adiponectin concentrations were measured in duplicate by RIA (Human Adiponectin RIA Kit, Linco Research, Inc., St Charles, MO, USA).

## Statistical analysis

Results are reported as means  $\pm$  s.d. Levene's test was used to evaluate the distribution characteristics of the variables. Differences between diabetic and control groups were tested for significance by *t*-test, Mann-Whitney *U* test and  $\chi^2$ -test. The relationship between variables was analysed by Pearson's correlation. The results were also analysed by one-way analysis of variance and Bonferroni-adjusted Mann-Whitney *U* and *t*-tests for comparison of subgroups. We used the odds ratio (OR) as a measure of association between outcome and exposure, and estimated the adjusted odds ratios in conditional logistic regression analysis.

Receiver operating characteristics (ROC) curve analysis was performed to determine a threshold concentration of adiponectin for development of diabetic retinopathy. Differences and correlations were considered significant at  $P < 0.05$ .

**Table 1** Clinical and laboratory features of the patient and control groups. Values are means ( $\pm$ s.d. or number (%)).

	Patients ( <i>n</i> = 74)	Controls ( <i>n</i> = 54)	<i>P</i>
Age (years)	46.8 $\pm$ 5.1	46.8 $\pm$ 5.4	0.451†
Sex (M/F)	41/33 (55/45)	25/29 (46/54)	0.371‡
Systolic BP (mmHg)	130.28 $\pm$ 13.84	126.33 $\pm$ 10.24	0.079§
Diastolic BP (mmHg)	83.63 $\pm$ 3.69	84.33 $\pm$ 4.39	0.683§
Total cholesterol (mmol/l)	11.08 $\pm$ 1.69	10.61 $\pm$ 1.33	0.093§
Triglycerides (mmol/l)	6.7 $\pm$ 1.15	6.63 $\pm$ 0.85	0.067†
LDL cholesterol (mmol/l)	6.23 $\pm$ 4.6	6.09 $\pm$ 1.04	0.395†
HDL cholesterol (mmol/l)	2.4 $\pm$ 0.34	2.52 $\pm$ 0.34	0.055†
BMI (kg/m <sup>2</sup> )	26.84 $\pm$ 2.10	26.47 $\pm$ 2.33	0.353†
Insulin ( $\mu$ U/ml)	12.72 $\pm$ 6.65	6.06 $\pm$ 1.29	<0.001†
HOMA-IR	3.11 $\pm$ 0.84	1.33 $\pm$ 0.34	<0.001†
HbA <sub>1c</sub> (%)	8.83 $\pm$ 1.34	4.30 $\pm$ 1.01	<0.001†
FPG (mmol/l)	7.65 $\pm$ 1.28	4.66 $\pm$ 0.63	<0.001†
Adiponectin ( $\mu$ g/ml)	4.71 $\pm$ 2.11	15.95 $\pm$ 3.72	<0.001†

BP, blood pressure; FPG, fasting plasma glucose. *P* values are matched for in analyses: † *t*-test; ‡  $\chi^2$ -test; § Mann–Whitney *U* test.

## Results

Clinical and laboratory data of the patients and controls are shown in Table 1. No significant differences in age, BMI or systolic and diastolic blood pressures were observed between the patient and control groups. The insulin, fasting blood glucose and HOMA-IR indexes of the patients were significantly greater, whereas the adiponectin concentrations were lower than those of the controls ( $P < 0.001$  for all).

There was a significant negative correlation between plasma adiponectin and insulin or HOMA-IR index in the group of patients ( $r = -0.524$ ,  $P < 0.001$  and  $r = -0.318$ ,  $P = 0.006$  respectively). There was no correlation between adiponectin concentration and insulin or HOMA-IR indexes in the control group, and there was no correlation between adiponectin concentration and glucose, BMI and the lipid parameters in the diabetic and control groups. The significant differences between the adiponectin concentrations of the subgroups were not affected by the sex distribution of the subgroups.

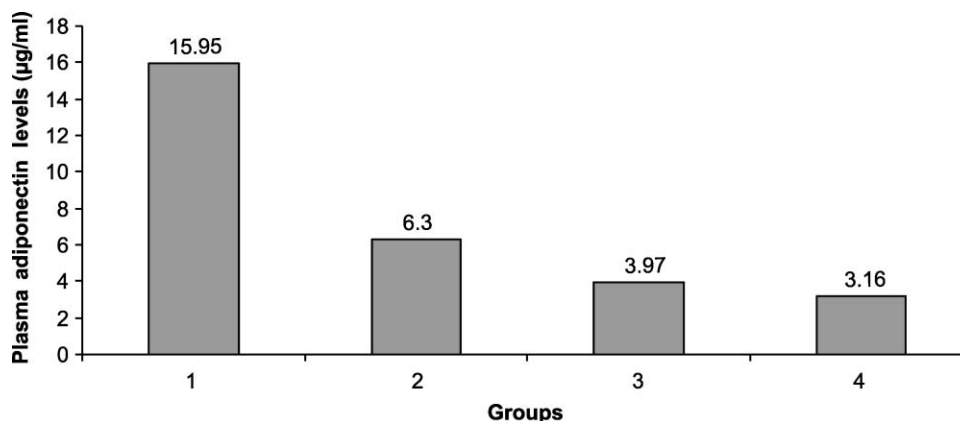
After the patients were subdivided according to severity of retinopathy, the age, sex, mean duration of diabetes, concentrations of fasting blood glucose, HbA<sub>1c</sub> and insulin, and HOMA-IR indexes were similar for each subgroup (Table 2). Plasma adiponectin concentrations in patients with PDR ( $n = 20$ ;  $3.16 \pm 1.83 \mu\text{g/ml}$ ) or NPDR ( $n = 24$ ;  $3.97 \pm 1.47 \mu\text{g/ml}$ ) were significantly lower than those in patients without retinopathy ( $n = 30$ ;  $6.30 \pm 1.57 \mu\text{g/ml}$ ,  $P = 0.001$  and  $P = 0.014$  respectively). Plasma adiponectin concentrations in the patients with PDR were not significantly lower than those in patients with NPDR ( $P = 0.584$ ; Fig. 1, Table 2).

The effect of plasma adiponectin concentrations on diabetes and diabetic retinopathy were assessed by conditional logistic regression analysis, after adjustment for the other parameters (HOMA-IR, insulin, HbA<sub>1c</sub>, adiponectin and glucose) by matching. When the presence of diabetes was defined as the final variable in the conditional logistic regression model with the adiponectin concentrations as the continuous variable, adiponectin was significantly involved in the model ( $P < 0.001$ ;

**Table 2** Characteristics of patients according to severity of retinopathy. Values are means $\pm$ s.d. or number.

	No DR ( <i>n</i> = 30)	NPDR ( <i>n</i> = 24)	PDR ( <i>n</i> = 20)	<i>P</i>
Age (years)	47.36 $\pm$ 5.1	46.29 $\pm$ 5.3	48.25 $\pm$ 3.8	0.532
Sex (M/F)	18/12	12/12	11/9	0.666
Systolic BP (mmHg)	131.66 $\pm$ 20.52	130.50 $\pm$ 4.40	127.95 $\pm$ 7.83	0.247
Diastolic BP (mmHg)	82.83 $\pm$ 3.77	84.91 $\pm$ 4.20	83.30 $\pm$ 2.49	0.263
Total cholesterol (mmol/l)	11.34 $\pm$ 1.58	10.93 $\pm$ 1.59	10.88 $\pm$ 1.99	0.246
Triglycerides (mmol/l)	7.04 $\pm$ 1.16	7.01 $\pm$ 0.82	6.85 $\pm$ 1.49	0.290
LDL cholesterol (mmol/l)	6.20 $\pm$ 0.97	6.07 $\pm$ 0.71	6.48 $\pm$ 0.92	0.434
HDL cholesterol (mmol/l)	2.40 $\pm$ 0.34	2.48 $\pm$ 0.36	2.33 $\pm$ 0.31	0.139
BMI (kg/m <sup>2</sup> )	26.61 $\pm$ 2.16	27.07 $\pm$ 2.02	26.93 $\pm$ 2.17	0.684
Insulin ( $\mu$ U/ml)	10.70 $\pm$ 3.13	12.08 $\pm$ 4.18	16.47 $\pm$ 4.59	0.076
HOMA-IR	2.96 $\pm$ 0.77	3.18 $\pm$ 0.60	3.25 $\pm$ 1.13	0.116
HbA <sub>1c</sub> (%)	8.74 $\pm$ 1.60	9.30 $\pm$ 1.57	8.66 $\pm$ 2.35	0.638
FPG (mmol/l)	7.52 $\pm$ 1.23	7.78 $\pm$ 1.38	7.68 $\pm$ 1.27	0.087
Duration of diabetes (months)	39.87 $\pm$ 6.20	45.46 $\pm$ 7.16	44.15 $\pm$ 9.31	0.818
Adiponectin ( $\mu$ g/ml)	6.30 $\pm$ 1.57†	3.97 $\pm$ 1.47‡	3.16 $\pm$ 1.83	<0.001

DR, diabetic retinopathy. Bonferroni-adjusted *t*-test: \* $P = 0.014$  compared with non-proliferative DR (NPDR); †  $P = 0.001$  compared with proliferative DR (PDR); ‡  $P = 0.585$  compared with PDR (all by one-way analysis of variance).



**Figure 1** Plasma adiponectin concentrations in patients with diabetes grouped according to the severity of diabetic retinopathy. 1, Control group; 2, no diabetic retinopathy; 3, non-proliferative diabetic retinopathy; 4, proliferative diabetic retinopathy.

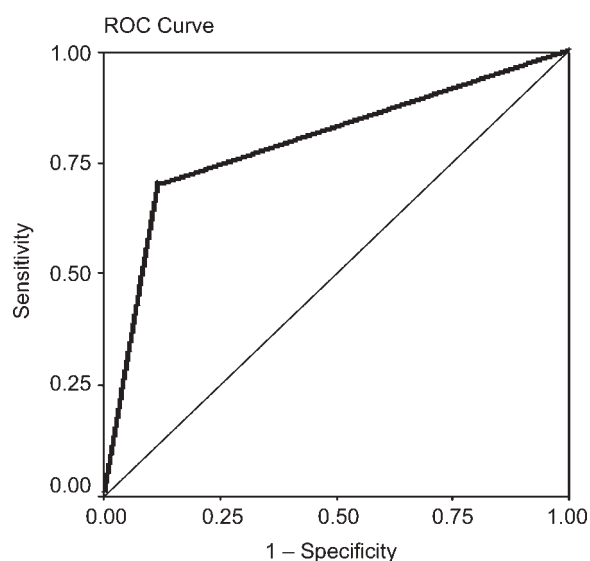
**Table 3** Odds ratios (OR) and 95% confidence intervals (CI) for associations of adiponectin with diabetes ( $n = 128$ ) and retinopathy ( $n = 74$ ) (Ankara, Turkey 2003).

	OR (95% CI)
Diabetes	0.75 (0.67 to 0.85)
Retinopathy	0.77 (0.68 to 0.88)

Table 3). ROC curve analysis was performed in order to establish a threshold adiponectin concentration for the existence of diabetic retinopathy. However, there was not a statistically significant cut-off value of plasma adiponectin for the condition (Fig. 2).

## Discussion

The protein product of the *apM1* gene (6), adiponectin, seems to be an essential modulator for metabolic and vascular diseases. Several studies have indicated that adiponectin possesses anti-inflammatory properties and thus may negatively modulate the process of atherogenesis (10, 11). Adiponectin dose-dependently suppresses the tumour necrosis factor- $\alpha$ -stimulated adherence of monocytes to cultured human endothelial cells (10). In addition, it inhibits specific binding of oxidized LDLs and their uptake by macrophages (11). Plasma concentrations are decreased in obesity, coronary disease, hypertension, insulin resistance and type 2 diabetes (7–10). Adiponectin decreases blood glucose in various animal models (17, 18), a hypoglycaemic effect that is not associated with stimulation of insulin secretion, but rather with increased insulin sensitivity. Experimental studies have shown that adiponectin reduces the plasma concentrations of free fatty acids and triglycerides and improves insulin resistance (19). Because adiponectin possesses anti-inflammatory properties and improves glucose tolerance, hypoadiponectinaemia has been suggested to be a



**Figure 2** ROC curve of plasma adiponectin concentrations for occurrence of diabetic retinopathy. The estimated cut-off value of adiponectin is  $5 \mu\text{g/ml}$ , with 88.6% sensitivity and 70% specificity for the existence of diabetic retinopathy (area under the curve = 0.610,  $P > 0.05$ ). Diagonal segments were produced by ties.

contributor to the pathogenesis of type 2 diabetes mellitus and its vascular complications (20, 21).

The major finding in the present study was that fasting plasma concentrations of adiponectin were decreased in diabetic patients, proportionate to the severity of retinopathy. Many factors are reported to affect the progression of diabetic retinopathy (2–4); however, there is general agreement that the duration of diabetes and the severity of hyperglycaemia are the major risk factors for its development (22). Nevertheless, intensive treatment regimens are only able to delay the development of diabetic retinopathy, and cannot prevent it completely (23). As indicated by the findings of the Diabetes Control and Complications Trial, susceptibility to diabetic retinopathy is influenced

by genetic factors, in addition to the well known causes of the condition (5). In recruiting the diabetic patients for this study, we were careful to ensure that all sub-groups were similar, not only in body weight, but also with respect to other factors known to contribute to the development of retinopathy, such as HbA<sub>1c</sub>, time elapsed since diagnosis of diabetes, and coexistence of other significant health problems. Thus the positive relationship between adiponectin concentrations and retinopathy observed in this study was not accompanied by any obvious difference among the retinopathy sub-groups with respect to factors promoting microangiopathy. According to the results of conditional logistic regression analysis, adiponectin is likely to be one of the chief contributors to the pathogenesis of both type 2 diabetes and diabetic retinopathy. However, ROC curve analysis did not reveal a threshold concentration of adiponectin for the existence of diabetic retinopathy and thus a predictive value cannot be assigned to adiponectin concentrations in relation to the development of the condition. Very probably, such estimates would require much larger sample sizes.

As expected, plasma adiponectin concentrations were correlated with the concentrations of immunoreactive insulin and HOMA-IR indexes in patients with diabetes. However, such an association was not present in normal individuals. Former studies showing this association between adiponectin and insulin sensitivity were all performed in patients with diabetes, dyslipidaemia or obesity (7, 8, 24) or in populations with a high prevalence of these diseases (25). There are, however, no data concerning the association of adiponectin and insulin sensitivity in healthy individuals with normal BMI and normal fasting blood glucose, total cholesterol and triglyceride concentrations. It has been demonstrated that adiponectin knockout mice develop insulin resistance, obesity, hyperglycaemia, hypertension and impaired endothelial function only if they are fed with a high-fat/high-sucrose diet (26, 27); no significant difference occurs between adiponectin knockout and wild-type mice when they are fed a normal diet. These experiments and the findings of the present study indicate that an association between fasting blood glucose, insulin sensitivity, serum lipids and adiponectin is not present in healthy individuals.

The study had several drawbacks. As most patients with type 2 diabetes are not aware of the periods in their life when they are asymptomatic or hyperglycaemic (28), the duration of diabetes could not be judged reliably. Also, the number in the study population was barely adequate to permit clear estimations to be made about the association of plasma adiponectin concentrations with the severity of the diabetic retinopathy. Most probably, with larger numbers of patients it would be possible to obtain a threshold for adiponectin concentration that is significant for the development of diabetic retinopathy. Finally, as the study was a

case-control design, it is not easy to predict exactly whether the low adiponectin concentrations preceded the retinopathy or *vice versa*. Future cohort studies will help to provide the answer.

Diabetic retinopathy is the principal cause of irreversible blindness in patients of working age in the industrialized world (1). By early detection of the condition through screening programmes, patients who receive treatment are less likely to go blind from proliferative changes and maculopathy (29, 30). Unfortunately, a screening programme is effective only if patients at risk can be identified and persuaded to attend. Thus a reliable, easily performable and inexpensive screening method is necessary for the early detection of diabetic retinopathy (22). The association of adiponectin with the severity of diabetic retinopathy may open up future projects on the early detection and prevention of the condition. However, as we were unable to establish a threshold concentration for the development of retinopathy, we cannot propose the measurement of plasma adiponectin concentrations as a candidate test for establishing the risk of developing diabetic retinopathy. Furthermore, some reports have referred to high plasma adiponectin concentrations in type 1 diabetes mellitus; these data are contradictory and necessitate further controlled study (31, 32).

In summary, the results from the present study suggest that plasma adiponectin concentrations are lower in patients with type 2 diabetes than in age-, BMI- and sex-matched controls, are lower in patients with diabetic retinopathy than in those without it, and are involved in the generation of diabetes and diabetic retinopathy. Future prospective studies with greater numbers of patient are recommended to establish a direct relationship between plasma adiponectin concentrations and the severity of vascular complications.

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