ORIGINAL RESEARCH

Adiponectin has different mechanisms in type 1 and type 2 diabetes with C-peptide link

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Spomenka Ljubic¹ MD PhD

Jozo Boras² MD PhD

Anamarija Jazbec⁵ BSc PhD

Marijana Vucic Lovrencic³ BSc PhD

Vinko Vidjak⁶ MD PhD

Dubravka Jurisic Erzen⁷ MD PhD

Dean Mileta⁴ MD

¹ Department for Metabolic Medicine, ² Department of Cardiology, ³ Department of Laboratory Medicine, ⁴ Outpatient Department, Vuk Vrhovac University Hospital, Zagreb, Croatia

⁵ Faculty of Forestry, University of Zagreb, Croatia

⁶ Department of Radiology, Merkur Clinical Hospital, Zagreb, Croatia

⁷ Department of Internal Medicine, Rijeka University Hospital Center, Rijeka, Croatia

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Abstract

Purpose: Adiponectin (ApN) is considered to be responsible for reduction of inflammation and is known to be included in lipid metabolism. This study was designed to assess the role of adiponectin in patients with type 1 and type 2 diabetes and to determine parameters important in the prediction of adiponectin.

Methods: Adiponectin, high sensitive C-reactive protein, fibrinogen, homocysteine, C-peptide, and lipid panel in addition to clinical and laboratory parameters important for the definition of diabetes, obesity and the metabolic syndrome were measured in 118 patients.

Results: The best model ($R^2=0.989$) for predicting adiponectin in type 1 diabetes included fibrinogen, white blood cell count, uric acid and triglycerides. In type 2 diabetes the best model ($R^2=0.751$) included C-peptide, white blood cell count, systolic blood pressure, fasting blood glucose, glycated hemoglobin and high-density lipoprotein cholesterol. ANOVA showed among-group differences in adiponectin ($P=0.028$), body mass index ($P<0.001$), fasting blood glucose ($P<0.001$) and high-density lipoprotein cholesterol ($P=0.012$) according to the type of diabetes. Between-group differences were also observed in adiponectin ($P=0.033$) and high-density lipoprotein cholesterol

($P=0.009$) according to sex. Adiponectin correlated ($P<0.05$) with body mass index, C-peptide, pulse pressure and high-density lipoprotein cholesterol.

Conclusion: Adiponectin levels were higher in type 1 diabetes. The association between C-peptide and adiponectin is probably one of the reasons for their different respective levels in different types of diabetes. Interrelations between adiponectin and inflammation, dyslipidemia, C-peptide levels and sex appear to be important for complex adiponectin modulation and action.

Adiponectin (ApN) is considered to be responsible for reduction in inflammation, stimulation of nitric oxide production and consequently modulation of endothelial cell function.¹⁻³ Dyslipidemia in the metabolic syndrome, an important risk factor for endothelial dysfunction and atherosclerosis, is characterized by increased serum triglycerides and decreased high-density lipoprotein cholesterol (HDL-C), the latter correlating with low ApN levels.⁴ Because of their impact on dyslipidemia and inflammation, low ApN

levels are important in the onset of cardiovascular events.⁵ Increased high sensitive C-reactive protein (hs-CRP) concentrations and low circulating ApN levels are associated with an increased risk of insulin resistance in patients with impaired glucose tolerance, type 2 diabetes mellitus and obesity. Anti-inflammatory properties of ApN, however, are not associated with overall obesity.^{3,6-8} Some studies have suggested that, in healthy people, serum ApN might be associated with vascular function independently of insulin resistance.⁹ Nevertheless, owing to its insulin sensitizing action ApN seems to be important in both insulin resistance and vascular protection.¹⁰

ApN may act as an anti-inflammatory mediator and thus play a role in the prevention of diabetic microangiopathy. On the other hand, in type 1 diabetic patients diabetic nephropathy has been shown to correlate with increased ApN levels.^{11,12} Genetic factors cannot be ignored when ApN is involved, as family history of diabetes has been shown to be associated with hypoadiponectinemia. Gonzales-Sanchez and co-workers have thus demonstrated that higher incidence of impaired glucose tolerance, and ApN and tumour necrosis factor (TNF)- α levels are genetically determined.¹³

The present study was designed to investigate the role of ApN in diabetes and the parameters important for the prediction of ApN in patients with type 1 diabetes on intensive insulin treatment, those with type 2 diabetes and the control patients.

Subjects and Methods

The patients received both written and oral information about the study and signed a written informed consent. The study protocol was approved by the hospital's ethics committee.

Patients

A total of 118 outpatients were included in the study: 28 with type 1 diabetes and 49 with type 2 diabetes:

the control group consisted of 41 non-diabetic patients. Each subject was fasted for 12 hr before blood sampling. Patients with type 2 diabetes were receiving oral hypoglycemic drugs and/or diet, while patients with type 1 diabetes had received intensive insulin treatment for more than one year before the study. Diabetes mellitus was defined according to the American Diabetes Association classification.¹⁴ Distinction between patients with type 1 and type 2 diabetes was made based on time to initiation of insulin therapy after diagnosis, and on 2 positive results out of 3 analyzed autoantibodies, i.e. islet cell autoantibodies, autoantibodies to glutamic acid decarboxylase (GAD), and autoantibodies to the tyrosine phosphatase (IA-2). Patients with malignancies, immunologic and infectious inflammatory diseases, pregnant women, and patients receiving corticosteroids or cytostatics, were not included in the study.

Clinical and laboratory parameters important for the definition of diabetes, obesity and metabolic syndrome included age, diabetes duration, body mass index (BMI), ApN, hs-CRP, fibrinogen (FIB), homocysteine (HCY), C-peptide, white blood cell count (WBC), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (fBG), glycated hemoglobin (HbA1c), uric acid (UA), gamma-glutamyl transpeptidase (GGT), HDL-C, LDL-cholesterol (LDL-C), and triglycerides (TG). A 24-hr albumin excretion rate (AER) was also measured in patients with diabetes. Type 2 diabetic patients were assigned to groups according to BMI (BMI<25 – group A, BMI 25-30 – group B, BMI>30 – group C). Pulse pressure (PP) was calculated from SBP and DBP. Blood pressure was measured three times in each patient after 5 minutes of supine rest. Mean values of three measurements were used in statistical analysis.

Laboratory tests

Serum ApN was measured by sandwich ELISA (DRG, Marburg, Germany), plasma FIB by the Clauss

TABLE 1. Clinical and laboratory data of the patients included in the study

	Control group			Type 1 DM			Type 2 DM		
	N	Mean	SD	N	Mean	SD	n	Mean	SD
Age (yr)	41	46.11	13.92	28	39.36	13.89	49	54.49	11.13
Diabetes duration (yr)	41	-	-		6.24	5.10	47	7.23	7.61
ApN ($\mu\text{g/ml}$)	38	7.85	6.47	27	12.23	6.69	48	6.87	5.42
hs-CRP (mg/L)	40	3.73	3.14	28	2.17	2.88	48	3.19	3.01
Leukocyte ($10^9/\text{L}$)	40	6.14	1.96	28	6.51	2.37	45	6.75	1.72
SBP (mmHg)	41	130.79	22.13	28	124.64	16.92	48	135.00	20.89
DBP (mmHg)	41	87.1	8.38	28	77.85	9.74	48	87.28	11.48
PP (mmHg)	41	43.68	19.35	28	46.78	13.53	48	46.7	17.2
fBG (mmol/L)	41	4.71	0.96	28	5.54	2.71	49	8.37	3.31
HbA1c (%)	41	5.21	0.43	28	6.99	1.53	49	7.30	1.37
AER (mg/24h)	38			27	74.37	63.82	47	30.49	24.74
FIB (g/L)	39	3.71	0.89	28	3.44	0.82	47	3.76	1.01
HCY ($\mu\text{mol/ml}$)	39	14.54	4.79	28	11.71	2.53	47	13.70	4.09
BMI (kg/m^2)	41	30.16	7.55	28	24.74	3.12	49	29.08	5.27
Uric acid ($\mu\text{mol/L}$)	38	297.39	56.78	28	234.00	60.34	49	282.23	87.76
LDL-C (mmol/L)	41	4.33	1.79	28	2.69	0.98	49	2.98	0.79
HDL-C (mmol/L)	41	1.43	0.49	28	1.67	0.55	49	1.42	0.41
GGT (U/L)	41	39.42	34.12	27	22.31	17.68	49	32.64	21.52
Triglycerides (mmol/L)	41	2.18	1.30	28	1.19	0.52	49	2.33	2.14
C-peptide	18	3.73	3.14	28	0.21	0.24	42	1.02	0.54

ApN – Adiponectin, hs-CRP – high sensitive C-reactive protein, SBP – systolic blood pressure, DBP – diastolic blood pressure, PP – pulse pressure, fBG – fasting blood glucose, HbA1c – glycated hemoglobin, FIB – fibrinogen, HCY – homocysteine, BMI – body mass index, LDL-C – LDL-cholesterol, HDL-C – HDL-cholesterol, GGT – gamma-glutamyl transpeptidase

method, and hs-CRP by an immunoturbidimetric assay on the Olympus AU600 analyzer (Olympus Optical Co., Tokyo, Japan). HCY in EDTA plasma was measured by an automated chemiluminescence assay (Advia Centaur, Siemens Medical Solutions, Germany). Cholesterol, TG, UA and glucose were analyzed using standard enzymatic procedures, and HDL-C using a homogenous assay on an automated analyzer (Olympus AU600, Olympus Optical Co., Tokyo, Japan).

Data analysis

Descriptive statistics (N, mean and standard deviation) were estimated for all analyzed variables (age, diabetes duration, ApN, hs-CRP, WBC, SBP, fBG, HbA1c, FIB, HCY, BMI, UA, LDL-C, HDL-C, TG, GGT and C-peptide). In the results, type I error (α) of

0.05 was considered statistically significant. Associations between ApN as dependent variables and the age, sex, duration, WBC, SBP, HbA1c, hs-CRP, FIB, HCY, C-peptide, BMI, fBG, UA, AER, GGT, HDL-C, LDL-C, and TG as independent variables were analyzed using multiple stepwise regression. Univariate regression was also performed for all selected variables in the stepwise procedure. Differences between ApN, hs-CRP, BMI, fBG and HDL according to the type of DM, sex and type*sex interaction were tested using analysis of variance (ANOVA). Tukey-Kramer multiple comparison post hoc test was used to determine which group interactions were significant.¹⁵ Differences in ApN, hs-CRP, FIB, fBG, HDL-C and TG values in type 2 diabetes according to BMI (<25, 25-30, >30) were tested with Kruskal-Wallis non-parametric test, because the assumption of homogeneity of variance for all tested variables was not met.

TABLE 2. Results of multiple stepwise regression analysis and univariate regression for selected variables for ApN as a dependent variable for both types of diabetes

		Univariate				Multivariate (stepwise procedure)			
		Parameter estimate	SE	P	R ²	Parameter estimate	SE	p	Model R ²
Type 1 diabetes	FIB	1.530	2.323	0.5237	0.038	0.938	0.347	0.0427	0.989
	Leukocyte	-0.749	0.796	0.3671	0.074	0.217	0.123	0.137	
	Uric acid	-0.086	0.024	0.0039	0.546	-0.063	0.005	<0.0001	
	Triglycerides	-6.764	3.172	0.0564	0.292	-7.081	0.587	<0.0001	
Type 2 diabetes	C-peptide	1.834	1.444	0.2116	0.041	2.269	0.882	0.0157	0.751
	Leukocyte	0.668	0.494	0.1830	0.043	-0.509	0.307	0.1086	
	SBP	0.074	0.038	0.0582	0.081	0.092	0.025	0.0010	
	fBG	0.481	0.235	0.0468	0.087	0.557	0.201	0.0098	
	HbA1c	0.038	0.598	0.9490	0.0001	-1.608	0.446	0.0012	
	HDL-C	8.009	1.559	<0.0001	0.375	8.243	1.673	<0.0001	

***bold** - statistically significant

Differences in ApN, HDL-C, and BMI according to age groups (<50, ≥50) and sex were tested using ANOVA. All statistical analyses were conducted using STATISTICA 7.1 (descriptive statistics and ANOVA) and SAS 8.1 statistical package (regression analysis). Graphs were produced using STATISTICA 7.1.¹⁵⁻¹⁷

Results

Descriptive statistics of laboratory data according to the type of diabetes are presented in Table 1.

The best model (R²=0.989) for predicting ApN in type 1 diabetes obtained using stepwise multivariate regression included FIB, WBC, UA and TG. In type 2 diabetes, the best model for ApN (R²=0.751) included C-peptide, WBC, SBP, fBG, HbA1c and HDL-C. Univariate regression analysis showed that only UA was statistically significant for ApN (R²=0.546) in DM1, while fBG and HDL-C were statistically significant in DM2 (Table 2).

The differences between ApN, hs-CRP, BMI, fBG and HDL-C according to the type of diabetes and sex were tested by ANOVA (Table 3), which showed among-group differences in ApN, HDL, BMI and fBG according to the type of diabetes (Table 3). Tukey post hoc test showed a difference in ApN between type 1 diabetes and CG, and between type 1 and type 2 dia-

TABLE 3. Results of ANOVA for the differences between ApN, CRP, BMI, fBG and HDL-C according to the type of diabetes, sex and their interaction.

		df	F	P	post hoc
ApN	Intercept	1	139.079	<0.001	
	type	2	3.774	0.028	(0,2)(1)
	sex	1	4.745	0.033	(f)(m)
	type*sex	2	1.094	0.340	
	Error	69			
CRP	Intercept	1	73.600	<0.001	
	type	2	1.914	0.153	
	sex	1	1.889	0.172	
	type*sex	2	0.054	0.947	
	Error	95			
BMI	Intercept	1	2067.296	<0.001	
	type	2	8.438	<0.001	(0,2)(1)
	sex	1	0.765	0.384	
	type*sex	2	2.099	0.128	
	Error	93			
fBG	Intercept	1	354.1001	<0.001	
	type	2	9.5824	<0.001	(0)(1,2)
	sex	1	0.4780	0.491	
	type*sex	2	0.0622	0.940	
	Error	81			
HDL-C	Intercept	1	1076.637	0.000	
	type	2	4.602	0.012	(0,2)(1)
	sex	1	7.058	0.009	(f)(m)
	type*sex	2	0.311	0.733	
	Error	95			

***bold** - statistically significant

betes (Table 3). Between-group differences were also observed in ApN and HDL according to sex. No statistical differences were observed in the interaction between the type of diabetes and sex and in hs-CRP. ApN correlated significantly ($P < 0.05$) with BMI ($r = -0.26$), C-peptide ($r = -0.49$), PP ($r = 0.24$) and HDL-C ($r = 0.51$).

As there were no women with BMI > 30 nor men with BMI < 25 among the patients with type 1 diabetes, analysis of the tested variables could only be performed in type 2 diabetic patients. The differences in ApN, hs-CRP, FIB, fBG, HDL-C and TG in type 2 diabetes according to BMI (< 25 , $25-30$, > 30) were tested, with difference observed in hs-CRP ($P = 0.001$), fBG ($P = 0.005$) but not in HDL-C ($P = 0.054$) and ApN ($P = 0.167$). Comparison of ApN values according to AER ($< 30\text{mg}/24\text{hr}$ and $\geq 30\text{mg}/24\text{hr}$) did not reveal differences in either type 1 ($P = 0.49$) or type 2 diabetes ($P = 0.07$).

Total ApN and HDL-C levels were lower in men than in women. ApN was decreased in women, but not men, > 50 yr of age. HDL-C decreased with age in women, and increased in men, but not significantly (Table 4). There was no sex-related difference in BMI, which was increased in women ≥ 50 yr of age, but not decreased in men of the same age. C-peptide was found to be increased only in women ≥ 50 yr of age.

Discussion

Levels of ApN in this study were shown to be significantly higher in women as compared with men, which is in agreement with the literature.¹² There was no difference in CRP values between men and women, suggesting that a mechanism other than interrelation between inflammatory markers and ApN was responsible for ApN changes. It has been proposed that in men ApN is suppressed by sex androgens.¹⁸ A comparison of ApN and HDL-C levels between women and men according to age (50 yr was the borderline age) revealed a decrease in ApN and HDL-C in women ≥ 50 yr, and an increase in men of the same age. This cor-

responds to reports on lower values of ApN in climacteric women.¹⁹ On the other hand, higher ApN levels have been observed in the elderly, leading to a conclusion that this could be attributed to "ApN resistance".³ It can be concluded that in women the impact of estrogens is more dominant than that of age. Although women with diabetes have increased protective ApN levels, diabetes in women is associated with a higher risk of cardiovascular diseases when compared with men, which could be explained by estrogen variation during life.²⁰

Yaturu and co-workers have found decreased ApN levels in patients with prediabetes and type 2 diabetes.²¹ We found differences in ApN levels among patients with type 1 diabetes, type 2 diabetes and the control patients, with lower values observed in type 2 diabetes and the control group in comparison with

TABLE 4. Results of ANOVA for the differences between ApN, HDL-C, BMI, C-peptide according to age (< 50 , ≥ 50 yr), sex and their interaction.

		df	F	p	post hoc
ApN	Intercept	1	265.457	< 0.001	
	Age group	1	0.765	0.384	
	sex	1	8.262	0.005	(f)(m)
	Age group*sex	1	0.625	0.431	
	Error	96			
HDL-C	Intercept	1	1080.242	< 0.001	
	Age group	1	0.506	0.478	
	sex	1	9.842	0.002	(f)(m)
	Age group*sex	1	2.209	0.140	
	Error	96			
BMI	Intercept	1	2124.881	< 0.001	
	Age group	1	2.909	0.091	
	sex	1	0.286	0.594	
	Age group*sex	1	6.358	0.013	
	Error	93			
C-peptide	Intercept	1	117.644	< 0.001	
	Age group	1	4.562	0.038	(< 50)(≥ 50)
	sex	1	2.019	0.162	
	Age group*sex	1	2.126	0.151	
	Error	48			

***bold** = statistically significant

type 1 diabetes. Nevertheless, no difference was found between type 2 diabetic patients and the controls. Moreover, the studied groups were also different with regard to fBG levels and BMI.

ApN has been shown to have a negative correlation with BMI among non-diabetics, but not among type 1 diabetic patients, whereas in Japanese men no correlation has been observed whatsoever between the two parameters.^{11,22} A study of the association of ApN with insulin resistance and dyslipidemia has shown that ApN did not correlate with overall obesity, but with subcutaneous abdominal fat.²³ Furthermore, the impact of overall obesity on ApN production has also been found to be lesser than that of epicardial fat.³ In our study, ApN was found to correlate with HDL-C, PP, C-peptide and BMI, whereas changes in CRP in type 2 diabetic patients correlated with BMI, suggesting an impact of obesity on inflammation. Although ApN and inflammatory factors are inversely correlated,⁷ in this study, despite significant among-group differences in ApN no such difference in CRP, FIB, and HCY among the three studied groups was observed according to the type of diabetes. This suggests that other reasons besides inflammation are responsible for different ApN values in different types of diabetes.

Important predictors of ApN in patients with type 2 diabetes were WBC, SBP, fBG, HbA1c, HDL-C, and C-peptide. Recent literature has pointed to an interrelation between C-peptide levels and ApN production in adipocytes.²⁴ GAD-positive women have been reported to have higher concentrations of ApN than GAD negative women, which could be due to a lower C-peptide concentration. A positive correlation between ApN and GAD and a negative correlation with β -cell function in type 1 diabetes have also been reported.²⁵ In the present study increased ApN levels in patients with type 1 diabetes could be explained by the lack of inhibitory effect of C-peptide on adipocytes. The relationship between intensive insulin treatment and ApN is not clear. ApN concentration in non-obese subjects has been shown to correlate better

with β -cell function than with insulin sensitivity.²⁶ The DIGAMI study demonstrated that diabetic patients with acute myocardial infarction on intensive insulin treatment had better prognosis and an absolute reduction in mortality.²⁷ A possible explanation could be the effect of intensive insulin treatment on inflammation and ApN levels.²⁸ Several authors have observed that intensive insulin treatment normalized elevated serum C-peptide and increased circulating ApN level, improving insulin sensitivity.^{29,30} In this study, diabetic patients receiving intensive insulin treatment had increased ApN and decreased CRP levels, but the former did not correlate with the duration of insulin treatment. Although reduced C-peptide levels in type 1 diabetes correlated with an increase in ApN, account should be taken of insulin resistance in type 2 diabetes which correlates with ApN levels.²⁴ As no difference in ApN levels between patients with type 2 diabetes and the controls was observed, it could be presumed that in type 2 diabetes C-peptide levels are not as reduced as in type 1 diabetes, but are similar to those in the control group.

ApN is associated with endothelial protection.^{8,9} The presumed protective role of ApN is not in agreement with the finding of increased ApN in type 1 diabetes, especially in patients with advanced stages of nephropathy. Increased ApN level has been reported to be associated with the onset of microalbuminuria.¹¹ A possible explanation could be that ApN can change the integrity of endothelial junctions and induce nitric oxide (NO) production, which might have an effect on hyperfiltration.^{31,32} Increased ApN levels in patients with renal failure and proteinuria could be attributed to increased ApN production and reduced clearance in renal failure.³³ Based on the findings of Delporte and Pannacciulli, Behre and co-workers have hypothesized that in subjects with renal failure, anorexia nervosa, type 1 diabetes or weight loss increased ApN helps against malnutrition and starvation and could be considered as a marker of cachexia and catabolism.³⁴ Finally, an explanation for increased ApN in nephropathy could also be that ApN belongs to the soluble

collagen family.³ The lack of correlation between AER and ApN levels in this study could be due to the sample size, and to the fact that the majority of patients had microalbuminuria and normal creatinine clearance, while only few had macroalbuminuria. A previous observation about a positive correlation between increased ApN and albuminuria suggests a need for further investigation, particularly because of the protective effects of renin-angiotensin system inhibitors on AER and ApN.³⁵

We found SBP to be among the main predictors of ApN in type 2 diabetes. Low ApN level could be considered as a marker predicting arterial hypertension and stiffness as a result of impaired vasodilation due to decreased production of NO in insulin resistant patients.³⁶ Correlation between epicardial adipose tissue expression, low ApN and hypertension has been reported in the literature.³

The main predictors of ApN in type 2 diabetes in our study were C-peptide, WBC, SBP, fBG, HbA1c and HDL-C, whereas in type 1 diabetes the main predictors were FIB, WBC, UA and TG. WBC was found to be the only common predictor of ApN levels in both types of DM in this study. Together with other known predictors such as FIB, TG, HDL-C and UA, it is also considered to be responsible for the development of atherosclerosis.³⁷ Higher UA levels seem to be a protective compensatory mechanism in a state of elevated total antioxidant capacity.³⁸ Our finding that HDL-C correlated negatively with ApN levels corresponds to reports hypothesizing that low ApN might be a trigger for dyslipidemia³⁹, demonstrating that HDL-C is an important predictor of ApN levels in type 2 diabetes. HCY is a risk factor for cardiovascular diseases, but it seems that its association with nephropathy is even more important. We did not find any correlation between HCY and ApN levels in the present study.

Conclusions

In this study ApN levels were found to be similar in patients with type 2 diabetes and in those without diabetes, but higher in the type 1 diabetic patients. Serum ApN concentration was associated with HDL-C, C-peptide, PP and obesity. Intensive insulin treatment might be protective, as it improves insulin sensitivity by increasing ApN levels and normalizing C-peptide, while ApN could also provide protection by affecting dyslipidemia and inflammation. The association between C-peptide and ApN might be important, among other reasons responsible for their different respective levels in different types of diabetes. Interrelations between ApN and inflammation, dyslipidemia, C-peptide levels and sex appear to be important for complex ApN modulation and its action.

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Correspondence to:

Spomenka Ljubic, MD, PhD,
 Department for Metabolic Medicine, Vuk Vrhovac
 University Hospital,
 Zagreb, Croatia, Dugi Dol 4A;
 e-mail: spomenka.ljubic@gmail.com