

ORIGINAL

## Circulating adiponectin level is associated with major adverse cardiovascular events in type 2 diabetic patients with coronary artery disease

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**Abstract.** Elevated levels of circulating adiponectin (ADPN), an anti-inflammatory and anti-oxidative peptide, are associated with unfavorable cardiovascular outcomes in patients with cardiovascular diseases. The aim of this study was to investigate whether plasma ADPN levels could help predict major adverse cardiovascular events (MACE) in patients with documented coronary artery disease (CAD). We prospectively enrolled 193 CAD patients, who underwent percutaneous coronary intervention (PCI), and/or stenting and coronary artery bypass graft (CABG) surgery. ELISA was used to measure plasma ADPN concentrations. MACE—myocardial infarction, PCI, CABG, stroke, carotid revascularization, and death—was evaluated during a follow-up period of median 15.3 months (range 5–21 months). Cox regression analysis revealed that diabetes status, waist circumference, and plasma ADPN levels were significantly associated with MACE occurrence. On stratification according to diabetes status, plasma ADPN levels helped predict MACE only in patients with type 2 diabetes mellitus (T2DM). Kaplan-Meier analysis revealed higher MACE rates in diabetic patients with high-plasma ADPN levels than in those with low-plasma ADPN levels. High ADPN plasma concentrations can independently be associated with MACE in CAD with T2DM but not in those without diabetes. This indicates that plasma ADPN may have potential roles in high risk T2DM patients with ischemic heart disease.

**Key words:** Plasma adiponectin, Major adverse cardiovascular events (MACE), Coronary artery disease, Type 2 diabetes mellitus

**ADIPONECTIN** (ADPN), an adipocytokine, is the most abundant gene product in the adipose tissue, and regulates glucose and lipid homeostasis, energy metabolism, and anti-inflammatory activity [1, 2]. Dysregulation of ADPN has been implicated in metabolic X syndrome and atherosclerosis as well as in insulin resistance, obesity, type 2 diabetes mellitus (T2DM), hypertension, coronary artery disease (CAD), and ischemic strokes [3-7]. ADPN can potentially inhibit all the molecular pathways of atherosclerosis [8], which include monocyte adhesion to endothelial cells by ad-

hesion molecules [9], oxidized LDL uptake of macrophages through scavenger receptors [10], and proliferation of migrated smooth muscle cells by the action of platelet-derived growth factors and heparin-binding epidermal growth factor-like growth factor [11].

Thus, it is highly likely that ADPN *per se* is predominantly beneficial and high levels of circulating ADPN confers vascular protection [12]; thus, ADPN may function as a therapeutic target for diabetic patients. Interestingly, recent studies have consistently shown that high ADPN levels were associated with an increased risk of cardiovascular disease and/or mortality [13-16]. However, the mechanisms responsible for the increased cardiovascular risk associated with ADPN are still unclear; whether the high plasma ADPN level in association with higher adverse car-

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diovascular outcomes is a general phenomenon or is limited to a specific group needs to be clarified. This study was performed to elucidate whether ADPN is associated with major adverse cardiovascular events (MACE) in CAD patients with or without T2DM.

## Materials and Methods

### *Subjects design*

The study population included 193 consecutive coronary artery disease (CAD) patients admitted to our institute from June 2006 to June 2008. The study population comprised 147 men and 46 women with a mean age of 65 years (range 30 to 92 years). Of the 193 patients, 113 patients had diabetes mellitus; 142 patients, hyperlipidemia; and 161 patients, hypertension. Written informed consents were obtained from the patients before enrollment. The study was in agreement with the guidelines approved by the Human Research Ethics Committee at our hospital.

### *Patients*

Demographic data, clinical characteristics, and current medications of the patients were recorded by two independent observers at admission, and special attention was paid to cardiovascular risk factors and co-morbidities. Age, sex, smoking habits, hyperlipidemia, arterial hypertension, diabetes mellitus, coronary artery disease, history of myocardial infarction (MI), and stroke were assessed. Interviews, physical examinations, and urinalysis were conducted and subjects presenting with concomitant inflammatory diseases, such as infection, sepsis, malignancy, liver and advanced stage of chronic kidney disease, collagen disease, steroid use, surgery within 1 month prior to admission, and those who refused to participate were excluded. Data were evaluated for interobserver agreement on the day of patient discharge. In case of discrepancies, the patient was re-evaluated by both investigators in consensus. To detect undiagnosed diabetes at admission, fasting blood glucose and hemoglobin A1c (HbA1c) levels were determined. During the hospital stay, blood pressure measurements were conducted repeatedly to detect undiagnosed hypertension.

### *Laboratory investigations*

All blood samples were drawn after overnight fasting before coronary angiography, and plasma samples were kept at  $-80^{\circ}\text{C}$  for subsequent assays. Levels of

plasma triglycerides, total cholesterol, low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), uric acid, creatinine, and glucose were measured, as previously described [7, 17]. High-sensitivity C-reactive protein (Hs-CRP) was measured using a high-sensitivity assay (N Latex CRP Mono; DBMG Behring, Marburg, Germany); the detection limit was 0.2 mg/L. The molecular weight of high plasma ADPN levels was determined using a commercial solid phase ELISA kit (B-Bridge International, Sunnyvale, CA). The dilution curve was parallel to the standard curve. The inter- and intra-assay coefficients of variation of the assay were 3.2–7.3% ( $n = 3$ ) and 3.1–6.2% ( $n = 4$ ), respectively. Samples were measured in duplicate in a single experiment. The hs-CRP and other biochemical markers were measured concurrently during sample collection. To describe the true CCr as closely as possible, the estimated CCr used a primary estimate of renal function. This was serum creatinine, estimated CCr calculated by the modification of estimated GFR calculated with the MDRD extended version as in our previous reports [18].

### *Study end points*

The composite study end point was the occurrence of first MACE, which constituted MI, percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG), stroke, carotid revascularization, and all-cause mortality. We analyzed the associations of ADPN with all-cause mortality by using MI, stroke, and death as secondary end points.

### *Follow-up*

Patients were clinically reevaluated at 3, 6, and 12 months after hospital discharge and then annually to record clinical conditions until June 2008. Furthermore, all participants entered a disease management program at the Cardiovascular Clinic and each patient received a follow-up questionnaire from our trained nurse during June 2008, re-evaluating the occurrence of MACE during the entire follow-up period. If patients did not respond to the questionnaire, personal telephone contact was made with them or their relatives. Further information was obtained by reviewing hospital discharge reports relating to any other re-admission during the follow-up period. The performance of PCI, CABG, or carotid revascularization was validated by reviewing original procedure protocols. Outcome was adjudicated by two indepen-

dent observers who were blinded with respect to the patients' baseline clinical and laboratory data.

### Definitions

The diagnosis of CAD was confirmed by coronary angiography in all patients. Significant coronary stenosis was defined as a luminal diameter decrease of greater than or equal to 70% in at least one of 15 coronary segments. PCI comprised either balloon angioplasty or adjust stenting independent of operator. Left main disease and/or severe triple vessel CAD suggested CABG incidence. Diabetic subjects were defined as those with currently treated diabetes, fasting plasma glucose (FPG) greater than or equal to 126 mg/dL on 3 separate days, or casual glucose greater than or equal to 200 mg/dL. Stroke was defined as a neurological deficit that persisted >24 hours, as evaluated by a neurologist according to the modified Rankin stroke scale. The diagnosis was confirmed using mandatory cranial computed tomography or, if available, magnetic resonance imaging. Arterial hypertension was diagnosed in patients with resting blood pressure values  $\geq 140/90$  mmHg, and in patients with a history of hypertension and taking anti-hypertensive drugs. Hyperlipidemia was defined as a fasting total serum cholesterol >200 mg/dL, LDL-C >130 mg/dL, or serum triglycerides >180 mg/dL; moreover, all patients receiving lipid-lowering therapy, which was routinely administered at our institution during the study period, were diagnosed with hyperlipidemia. Smoking status was classified based on history of smoking, former instances of smoking (ceased smoking for at least 1 year) or current instances of smoker. Those who had stopped smoking more than 1 year prior to the examination were considered non-smokers. Metabolic syndrome was defined according to the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (NCEP-ATP III) but with a modified definition of central obesity [19]. NCEP-ATP III-metabolic syndrome was defined as the presence of three or more of the following criteria: (1) high arterial BP  $\geq 130/85$  mmHg (2) central obesity (waist circumference, male  $\geq 90$  cm; female  $\geq 80$  cm) (3) serum triglyceride level  $\geq 150$  mg/dL (4) serum HDL-cholesterol <40 mg/dL in males or <50 mg/dL in females and (5) fasting glucose  $\geq 100$  mg/dL.

### Statistical analysis

Descriptive data were examined for all variables. For continuous variables, results were presented as mean  $\pm$  SD or median (interquartile range) as appropriate and as a percentage of the total for categorical variables. All statistical analyses were performed using SAS software, v10.0 (SAS Institute, Cary, NC). ADPN levels were defined as high with median values of 2.55  $\mu\text{g/mL}$ . Cumulative event curves were plotted using the Kaplan-Meier method and differences between the curves were compared using a log-rank test. The risks for the occurrence of MACE were assessed by Cox regression analysis models, the results of which are presented as estimated relative risks (RR) and 95% confidence intervals (CI). To assess the factors associated with the occurrence of MACE, univariate analysis was performed.

Cox proportional hazard models were used to evaluate the relationship between ADPN and MACE, adjusting for several groups of a priori defined confounding variables. Multivariable adjustment was carried out: (1) adjusted for age, and gender; (2) adjusted for age, gender, waist circumference, mean blood pressure (MBP), plasma triglycerides, total cholesterol, LDL-C, HDL-C, fasting sugar and smoking status; (3) adjusted for age, gender, waist circumference, MBP, plasma triglycerides, total cholesterol, LDL-C, HDL-C, fasting sugar, smoking status, metabolic syndrome, and anti-hypertensive therapy (ARB/ACEI), statins, and aspirin. All of the statistical analyses were two-sided and a  $p < 0.05$  was considered significant.

## Results

The mean follow-up was 15.3 months (range 5 to 21 months). During the follow-up period, of the 193 CAD patients, there were 58 MACE, of which 17 (8.8%) were due to death from all causes. The mean ADPN levels in the study were 3.7  $\mu\text{g/mL}$ . The whole cohort median values of plasma ADPN levels were 2.55  $\mu\text{g/mL}$  (interquartile range, 1.30–5.24  $\mu\text{g/mL}$ ). ADPN was elevated in patients with MACE, without hyperlipidemia and without treatment ARB, statins, and aspirin. There are no gender (male: female), previous stroke (yes: no), disease status (myocardial infarction: stable angina), procedural (coronary artery bypass graft: percutaneous coronary intervention), treatment with ACE inhibitors and TZD (yes: no), and current smoker (yes: no) differences among plasma

**Table 1** Baseline characteristics of 193 study patients with coronary artery disease

Characteristic	Total (N = 193)
Age, mean (SD), years	64.6 ± 12.2
Male, n (%)	147 (76.2)
Diabetes mellitus, n (%)	113 (58.6)
Hypertension, n (%)	161 (83.4)
Hyperlipidemia, n (%)	142 (73.6)
Smoking, n (%)	93 (48.2)
BMI (kg/m <sup>2</sup> )	25.2 ± 4.4
Waist circumference (cm)	91.4 ± 9.4
Systolic BP (mmHg)	135 ± 23
Diastolic BP (mmHg)	77 ± 12
Fasting sugar (mg/dL)	149.8 ± 72.4
Total cholesterol (mg/dL)	176.0 ± 48.0
Triglyceride (mg/dL) (media)	151.4 ± 105.1 (118.2)
HDL-cholesterol (mg/dL)	39.0 ± 9.7
LDL-cholesterol (mg/dL)	109.8 ± 38.8
Uric acid (mg/dL) (media)	7.0 ± 4.6 (6.4)
Creatinine (mg/dL) (media)	1.6 ± 1.3 (1.2)
Adiponectin (µg/mL) (media)	3.7 ± 3.1 (2.55)
MACE, n (%)	58 (30.1)
Anti-hypertensive drug use, n (%)	133 (68.9)
Anti-lipid drug use, n (%)	117 (60.6)

Data are expressed as %, mean ± SD and sometimes (median) for variables with a non-normal distribution. BMI: body mass index; BP: blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein. MACE: major adverse cardiovascular events.

adiponectin (all  $p > 0.05$ ). Additionally, our study population had contained no patient under estrogen therapy (Tables 1 and 2).

Moreover, we found that the estimated GFR value and the prevalence of proteinuria did not differ between CAD patients with or without T2DM, and the mean plasma adiponectin levels of proteinuric patients with MACE is not differ to that of proteinuric patients without MACE ( $6.5 \pm 3.3$  µg/mL vs.  $4.2 \pm 3.3$  µg/mL,  $p = 0.124$ ). This result may exclude the confounding effect of proteinuria on plasma ADPN levels and the prognosis of CVD. In addition, treatment with ACE inhibitors, statins and aspirin rates also did not differ between CAD patients with T2DM subjects and CAD with non-diabetic subjects (all  $p > 0.05$ ). Although the ADPN was elevated in patients without ARB treatment (Table 2), the prevalence of using ARB did not differ between CAD patients with or without MACE.

**Table 2** Median (interquartile range) baseline plasma concentrations of adiponectin grouped according to categorical variables

Parameter	N	Adiponectin (µg/mL)	p-value
Gender			
Male	147	2.29 (1.22–5.14)	0.076
Female	46	3.15 (1.85–7.01)	
Previous stroke			
Yes	19	2.65 (0.92–6.24)	0.649
No	174	2.91 (1.49–5.68)	
Arterial hypertension			
Yes	161	2.78 (1.36–5.24)	0.350
No	32	1.71 (1.03–5.55)	
Hyperlipidemia			
Yes	142	2.22 (1.19–4.35)	0.012
No	51	3.96 (1.66–7.59)	
Disease status			
Myocardial infarction	61	3.05 (1.25–4.98)	0.693
Stable angina	114	2.25 (1.34–5.58)	
Procedural			
Coronary artery bypass graft	14	2.25 (0.99–5.87)	0.690
Percutaneous coronary intervention	179	2.66 (1.31–5.21)	
Treatment with ACE inhibitors			
Yes	101	2.35 (1.29–5.16)	0.794
No	92	2.83 (1.25–5.36)	
Treatment with ARB			
Yes	31	1.5 (1.0–2.8)	0.016
No	162	3.1 (1.4–5.6)	
Treatment with thiazolidinediones			
Yes	8	3.3 (1.1–7.3)	0.577
No	185	2.6 (1.3–5.1)	
Treatment with statins			
Yes	117	2.0 (1.2–4.1)	0.017
No	76	3.6 (1.6–6.6)	
Treatment with aspirin			
Yes	123	2.0 (1.1–4.4)	0.004
No	70	3.3 (1.6–8.1)	
Current smoker			
Yes	93	2.35 (1.18–5.38)	0.717
No	100	2.55 (1.38–4.79)	
Major adverse cardiovascular events			
Yes	58	4.54 (1.24–8.68)	0.009
No	135	3.16 (1.33–4.63)	

Thirty one patients used ARB, among these, 11 patients with MACE, and 20 patients without MACE. The mean ADPN levels were not different between patients who used ARB with MACE and without MACE ( $2.7 \pm 2.8$  µg/mL vs.  $1.8 \pm 1.1$  µg/mL,  $p = 0.640$ ).

Among CAD patients with T2DM subjects (total of



77 patients); seventy-five diabetic patients were treated with oral hypoglycemic agents alone, 2 cases with insulin alone. Among patients with oral hypoglycemic agents treatment (total of 75 patients), 21 cases received sulfonylurea alone, 11 cases with metformin alone, 20 cases with sulfonylurea and metformin, and 8 cases with sulfonylurea and thiazolidinediones (TZD) as well as 15 cases with other oral hypoglycemic agents. The mean plasma level of ADPN in subjects receiving insulin treatment did not differ from that of patients receiving oral hypoglycemic agents. The mean plasma ADPN levels of diabetic subjects with different oral hypoglycemic agent treatment did not show any difference among the groups. Please note that the number of subjects is too small to draw any conclusion. Although previous reports had shown that TZD treatment would elevate plasma adiponectin level [20], however, in the present study, only 8 patients used TZD, among these, 4 patients with MACE, and 4 patients without MACE. The mean plasma ADPN levels of 4 patient receiving TZD with MACE is higher, yet, not statistical significance, than that of 4 patients without MACE ( $6.8 \pm 4.0 \mu\text{g/mL}$  vs.  $2.1 \pm 1.6 \mu\text{g/mL}$ ,  $p=0.083$ ). When excluded these 8 patients with TZD treatment and re-calculated the adiponectin levels between patients with and without MACE, the mean adiponectin level is still significantly elevated in patients with MACE ( $4.4 \pm 3.8 \mu\text{g/mL}$  vs.  $3.2 \pm 2.4 \mu\text{g/mL}$ ,  $p=0.037$ ). Thus, as the number of patients received TZD this study is small; we believe the drug of TZD could not be a significant confounder to affect the results of present study.

To evaluate the factors associated with the occurrence of MACE, we performed Cox regression analysis models using the following parameters: age, gender, T2DM, body mass index, waist circumference, smoking, systolic blood pressure, diastolic blood pressure, fasting glucose, HbA1C, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, creatinine, plasma ADPN and Hs-CRP levels. Univariate analysis revealed that T2DM, waist circumference, and ADPN levels were individually and significantly associated with the occurrence of MACE ( $p < 0.05$ ; Table 3). Kaplan-Meier analysis revealed that the occurrence of MACE was significantly higher in patients with T2DM ( $p=0.032$ ), versus those without T2DM (Fig 1A). Kaplan-Meier analysis also revealed that the occurrence of MACE was significantly higher in patients with higher ADPN than in patients with lower

**Table 3** Risk factors for major adverse cardiovascular events

Parameter	RR	95% CI	<i>p</i> -value
Age	1.008	0.985–1.030	0.508
Gender	0.933	0.523–1.770	0.823
Type 2 diabetes	1.810	1.044–3.280	0.034
BMI	1.020	0.959–1.092	0.542
Waist circumference	1.036	1.004–1.067	0.026
Smoking	1.513	0.893–2.609	0.125
Systolic BP	0.992	0.980–1.004	0.177
Diastolic BP	0.981	0.947–0.995	0.056
Fasting glucose	0.999	0.995–1.002	0.692
HbA1C	0.976	0.814–1.128	0.759
Total cholesterol	1.001	0.995–1.006	0.861
Triglycerides	1.000	0.997–1.003	0.957
HDL-cholesterol	0.976	0.945–1.005	0.108
LDL-cholesterol	1.000	0.993–1.007	0.951
Creatinine	1.163	0.996–1.303	0.056
Plasma adiponectin	1.108	1.019–1.199	0.018
High-sensitivity CRP	1.004	0.984–1.017	0.627

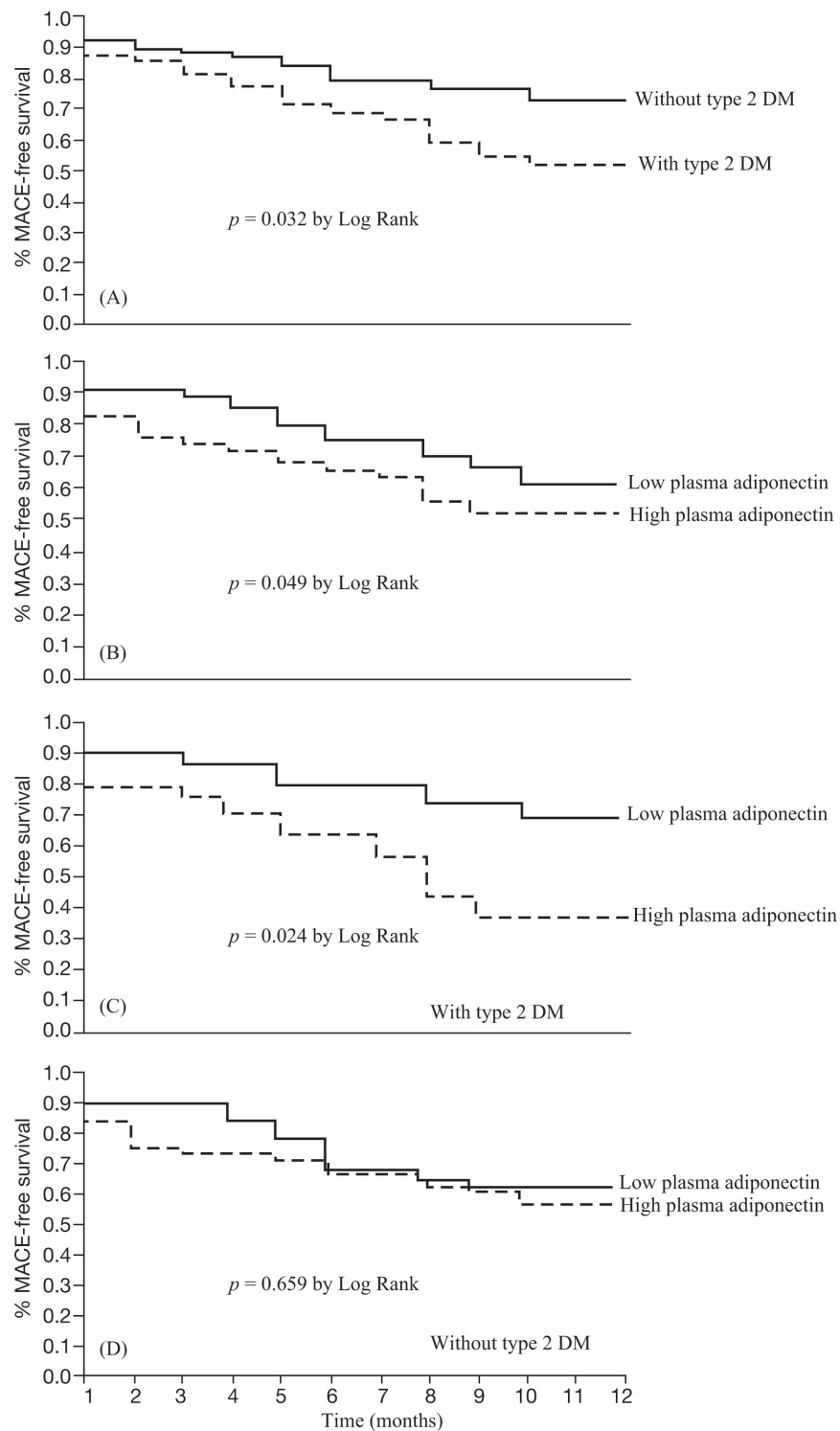
BMI: body mass index; BP: blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

ADPN ( $p=0.049$ ) (Fig. 1B).

When patients were stratified according to diabetes status, plasma ADPN levels were found to predict MACE in patients with T2DM even after adjusting for anthropometric variables, blood pressure, lipid profile, fasting sugar, smoking status, metabolic syndrome, and antihypertensive therapy (ARB/ACEI), statins, and aspirin (Table 4). The association between plasma ADPN and MACE in patients without T2DM disappeared when further adjustments were made to each variable, and no association could be detected following further adjustments (Table 4). Using Kaplan-Meier analysis demonstrated higher MACE rates in diabetic patients with high-plasma ADPN than in patients with low-plasma ADPN (Fig. 1C). However, there were insignificant differences between low and high plasma ADPN levels in CAD patients without T2DM (Fig. 1D).

## Discussion

In the present study, we demonstrated that plasma ADPN levels were associated with an increased MACE rate, even in a fully adjusted model. Furthermore, plasma ADPN levels were found to pre-



**Fig. 1** Results of the univariate Kaplan-Meier survival analysis showing the association between T2DM (A), and high plasma adiponectin levels (B) with major adverse cardiovascular events. Further, analysis of patients with and without T2DM; the log-rank tests revealed statistically significant differences between low and high plasma adiponectin levels in patients with CAD and T2DM (C). However, there were insignificant differences between low and high plasma adiponectin levels in CAD patients without T2DM (D).

**Table 4** Plasma adiponectin adjusted relative risk of major adverse cardiovascular events (MACE) according to cardiovascular disease with and without T2DM

Model adjusted for	MACE		
	RR	95% CI	<i>p</i> -value
All subjects			
Age, gender	1.117	1.018–1.218	0.021
Age, gender, waist circumference, MBP, lipid profile	1.129	1.015–1.245	0.027
Age, gender, waist circumference, MBP, lipid profile, fasting sugar, smoking status	1.134	1.010–1.263	0.034
Age, gender, waist circumference, MBP, lipid profile, fasting sugar, smoking status, metabolic syndrome, and antihypertensive therapy (ARB/ACEI), statins, and aspirin	1.161	1.005–1.333	0.043
CAD with type 2 diabetes			
Age, gender	1.166	1.026–1.316	0.020
Age, gender, waist circumference, MBP, lipid profile	1.171	1.016–1.341	0.029
Age, gender, waist circumference, MBP, lipid profile, fasting sugar, smoking status	1.185	1.008–1.389	0.040
Age, gender, waist circumference, MBP, lipid profile, fasting sugar, smoking status, metabolic syndrome, and antihypertensive therapy (ARB/ACEI), statins, and aspirin	1.182	1.002–1.440	0.048
CAD without type 2 diabetes			
Age, gender	1.070	0.920–1.219	0.363
Age, gender, waist circumference, MBP, lipid profile	1.090	0.891–1.297	0.378
Age, gender, waist circumference, MBP, lipid profile, fasting sugar, smoking status	1.109	0.886–1.351	0.349
Age, gender, waist circumference, MBP, lipid profile, fasting sugar, smoking status, metabolic syndrome, and antihypertensive therapy (ARB/ACEI), statins, and aspirin	0.884	0.600–1.297	0.521

MBP: mean blood pressure; lipid profile: including total cholesterol, triglyceride, LDL- and HDL-cholesterol.

dict MACE only in CAD patients with type 2 diabetes. The relationship established between ADPN concentration and MACE in this study is similar to the findings of previous studies showing association of high ADPN levels with significantly increased MACE in patients with ischemic heart disease [13, 16, 21–23]. However, these studies did not account for a specific group, especially in the presence of T2DM in cases of ischemic heart disease that may have obscured the effect of ADPN concentration on MACE. Our findings extend the link between high ADPN levels and MACE to patients with CAD and T2DM but not to those patients with CAD without T2DM. The current study also demonstrated that T2DM was an independent pre-

dictor for the occurrence of MACE. It is known that the prevalence of T2DM in patients with CAD is high [24, 25]. These finding—higher plasma ADPN associated with a higher risk of MACE occurrence—runs contrary to the fact that plasma ADPN levels were lower in patients with T2DM and CAD [7, 26]. The reason why diabetic patients with CAD and high plasma ADPN levels were associated with a higher rate of MACE incidence has not been elucidated thus far.

MACE incidence in patients with T2DM could be attributed to increased arterial stiffness and intimal medial thickness because of increased circulating glucose levels, thereby adversely affecting cardiovascular protection of estrogen; this may possibly result

in increased vascular tone, platelet aggregation, and enhanced vascular proliferation [27, 28]. An essential question to be answered is why coexisting CAD and CAD T2DM disease is associated with increased concentrations of ADPN and how this could predict MACE development. A more attractive option is the possibility of reverse causality, whereby silent or clinically apparent vascular disease leads to compensatory rises in ADPN. Indeed, it has been proposed that ADPN synthesis is stimulated in response to vascular inflammation to counter the atherosclerotic process [29]. Alternatively, a rise in the brain's natriuretic peptide levels may link silent ischemia or existing vascular disease to higher ADPN levels because the circulating levels of these two parameters show remarkable positive correlations in patients with and without chronic heart failure [15, 30]. Irrespective of the mechanism, a recent study suggested that high levels of ADPN in patients with acute coronary syndrome or heart failure may be a reflection of a salvage mechanism to improve insulin resistance and fatty acid metabolism [30]. In other words, elevations in ADPN represent chronic or acute compensatory mechanisms to counteract metabolic and vascular stress.

ADPN has been suggested to increase energy expenditure and induce weight loss through a direct effect on the brain [31, 32]. Therefore, it could be hypothesized that high plasma ADPN levels, in connection with increased energy expenditure, might not be beneficial in patients with established CAD. Conversely, loss in weight increases plasma ADPN levels [33] and therefore, high plasma ADPN levels in patients with CAD and those with CAD and T2DM could be indicative of the wasting process. This may explain the association between high ADPN levels and increased mortality risk in patients with CAD alone and those with CAD and T2DM. We did not measure changes in weight in the present study, and future studies in populations with established CAD and CAD with T2DM are needed to address this issue.

Previous studies confirm that high ADPN levels are associated with significantly increased mortality in those with heart failure [15]. Wannamethee and colleagues provide further support and extend the link between high ADPN levels and mortality to the general population of older men without diagnosed CVD or heart failure. ADPN levels increase with age and it is suggested that the age-related decline in renal function may contribute to this increase [34]. Aging is as-

sociated with weight loss and a loss in skeletal muscle mass and strength (sarcopenia) [35, 36], which are significant predictors of mortality in the elderly population [37]. Thus, high ADPN levels in elderly people may be a consequence of weight loss and sarcopenia associated with aging.

In another study of patients with T2DM and overt diabetic nephropathy, the increase in serum ADPN was suggested to reflect enhanced production of ADPN in the adipose tissue rather than a reduced clearance of ADPN by the kidneys [38]. It could be speculated that ADPN itself may play a compensatory role in mitigating the disease burden induced by renal disease via its anti-atherogenic and anti-inflammatory properties [1]. Hence, according to this issuer [1], ADPN indicates an increased risk of cardiovascular disease and mortality that seems to be related to the severity of the disease.

A limitation of this study is that we enrolled individuals in whom coronary angiography was clinically indicated. This population is at an intermediate-high risk of future cardiovascular events and our findings may not apply to lower risk individuals. Further, we analyzed ADPN without accounting for urine adiponectin concentration, expression in adipose tissue and the occurrence of different isoforms, which may have distinct biological functions [39]. Furthermore, the cutoff point used for ADPN in the present study population may be not applicable in other CAD populations. It would be interesting to gather serial measurements of plasma ADPN and other blood samples—such as leptin, brain natriuretic peptide and TNF- $\alpha$ —and each function of renal, cardiac, peripheral vascular, and weight change, plus other relevant factors linked to changes in ADPN levels. In addition, ACE inhibitors have been shown to increase plasma ADPN levels in non-diabetic patients with essential hypertension [40]. In the present study, plasma ADPN levels were the same between patients with and without treatment with ACE inhibitors and all analyses were adjusted for anti-hypertensive treatments.

In conclusion, our results illustrate that high ADPN plasma concentrations independently associated with MACE in CAD patients with T2DM. This indicates that plasma ADPN may play potential roles in high risk T2DM patients with ischemic heart disease.

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