CHAPTER 3

Reduced Plasma Adiponectin is Associated with Extent, Degree and Morphology of Coronary Artery Disease in Asymptomatic Patients with Type 2 Diabetes

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ABSTRACT

Objective

Reduced plasma adiponectin has been linked to coronary artery disease (CAD). However, little is known concerning this relation in type 2 diabetes. We explored the relation between plasma adiponectin and the parameters of coronary atherosclerosis in asymptomatic patients with type 2 diabetes.

Methods

Prospectively, multi-slice CT angiography (CTA) of the coronaries was performed in 103 asymptomatic patients with type 2 diabetes. The following parameters of atherosclerosis were assessed: presence of any atherosclerosis and obstructive atherosclerosis, atherosclerotic plaque burden (number of plaques), and plaque phenotype (number of non-calcified, mixed, calcified). Parameters of atherosclerosis were studied in relation to traditional cardiovascular risk factors, high sensitivity C-reactive protein and plasma adiponectin.

Results

An independent inverse relation was observed between plasma adiponectin and the presence of any atherosclerosis (P=0.02) and obstructive atherosclerosis (P=0.003). Accordingly, an adiponectin cut-off value of <4.5 mcg/ml resulted in a sensitivity and specificity of 80% and 71% for predicting obstructive atherosclerosis. An independent inverse relation was also observed between plasma adiponectin and the atherosclerotic plaque burden (P=0.02). Further analysis demonstrated low plasma adiponectin to be independently associated with the quantity of non-calcified (P=0.04), but not with mixed or calcified plaques.

Conclusion

Low plasma adiponectin was independently related with the parameters of coronary atherosclerosis in asymptomatic patients with type 2 diabetes. A predominant association was observed with the presence of non-calcified plaques, which have been associated with unstable disease. Assessment of plasma adiponectin conveys the potential to augment risk for CAD in asymptomatic patients with type 2 diabetes.

INTRODUCTION

Early identification of patients prone to develop coronary artery disease (CAD) and prompt initiation of appropriate therapy has become a main focus in the last decades. In consequence, prognosis of CAD has improved in the general population [1]. However, in patients with diabetes, excess CAD mortality and morbidity persist despite preventive guidelines [1]. Improved ability to gauge risk in the individual patient with diabetes, may motivate physicians and patients to adhere preventive therapy, and define those who may benefit from more aggressive risk-reduction strategies and further screening for CAD.

Nevertheless, the optimal approach for the identification of the high risk patient with diabetes remains unclear. In contrast with the general population, risk assessment methods based on the traditional clinical cardiovascular risk factors have shown limited incremental value in diabetes [2,3]. As a result, the use of surrogate markers of atherosclerosis and novel serum biomarkers has gained recent attention for this purpose [4,5]. Validated novel serum biomarkers may convey the additional advantage of reflecting on an individual's predisposition to develop CAD in an early stage, prior to evident atherosclerosis, enabling successful preventive therapy.

The serum biomarker adiponectin is the most abundant adipocytokine secreted by the adipose tissue cells [6]. Synthesis of adiponectin is reduced in obesity, insulin resistance, and type 2 diabetes [6,7]. Low levels of serum adiponectin have also been related to the presence of atherosclerosis [6]. In line with these observations, genetic and experimental studies suggest adiponectin to have an array of anti-atherosclerotic effects [8]. However, based on existing epidemiological data in the general population, the prognostic value of adiponectin remains controversial [9-11]. Interestingly, cross-sectional studies in general patient populations with manifest CAD suggest adiponectin to be related with atherosclerosis only below a certain serum threshold [12,13]. Hence, adiponectin may be most strongly related with CAD in the presence of type 2 diabetes where low ranges of the biomarker are reported [14]. Thus far, only limited information is available on the association of plasma adiponectin and CAD in patients with type 2 diabetes [14-16].

In the current study, we explored the relation between plasma adiponectin and parameters of coronary atherosclerosis as assessed by multi-slice CT, in asymptomatic patients with type 2 diabetes. The association between plasma adiponectin and the presence of any atherosclerosis and obstructive atherosclerosis, atherosclerotic plaque burden, and plaque phenotype was determined.

METHODS

Study Design and Patient Characteristics

The study population comprised of 103 asymptomatic patients with type 2 diabetes. Patients were included prospectively from an ongoing registry at the diabetes outpatient clinic. Diagnosis and classification of diabetes was performed using the American Diabetes Association criteria [17]. Accordingly, presence of type 2 diabetes (prominent insulin resistance) was distinguished from primary insulinopenia and immune destruction of beta pancreas cells by determining plasma levels of C-peptide and auto-antibodies to islet cells, insulin and glutamic-acid-decarboxylase. Consecutive patients with type 2 diabetes were referred to the cardiology outpatient clinic for risk stratification and cardiovascular screening. Anginal symptoms were ruled out using a self-completed questionnaire for encountered chest pain [18]. Patients underwent clinical and laboratory evaluation. Non-invasive multi-slice CT angiography (CTA), of the coronaries was performed as part of clinical work up. Concurrently, plasma was collected and stored for later analysis in a study setting, approved by the institutional review committee of the Leiden University Medical Center, Leiden. All patients gave written consent. Patients not eligible for CTA due to arrhythmia or contraindications for the use of iodinated contrast media were excluded.

Cardiovascular risk factors

Presence of cardiovascular risk factors was defined as: 1. smoking (current smoking or smoking in the last 2 years), 2. positive family history of CAD (CAD in first degree family members <55 years in men or <65 years of age in women), 3. body mass index (BMI) as kg/m^2, 4. hypertension (blood pressure >140/90 mmHg or treatment with antihypertensive medication), 5. hypercholesterolemia (total cholesterol level >5.0 mmol/L or use of lipid lowering medication), and 5. glycosylated hemoglobin A1c (HbA1c) as a measure of glycemic control, 6. micro-albuminuria (urine albumin/creatinine ratio ≥3.5 mg/mmol).

MSCT data acquisition

Imaging was performed using a 64-slice multi-slice CT scanner (Aquilion64, Toshiba Medical Systems, Japan). If necessary and tolerated, oral beta-blockers (metoprolol 50 mg or 100 mg) were provided 1 hour preceding the scan to achieve a heart rate <65 beats per minute. First, a non-enhanced prospective electrocardiographically gated scan, triggered at 75% of the R-R interval with 4 x 3.0 mm collimation was obtained to determine the start and end position of the helical scan.

Second, CTA was performed using the following parameters: collimation 64 x 0.5 mm, tube rotation time 400, 450 or 500 ms depending on the heart rate, tube current 300 or 350 mA, tube voltage 120 kV. Non-ionic contrast material was administered in the antecubital vein at a flow rate of 5 ml/L and the amount of 90–105 ml (depending on the total scan time), followed by 50 ml of saline solution flush. Automated bolus-tracking in the aortic root was applied for the timing of the scan. Images were acquired with simultaneous ECG registration during a single breath hold of approximately 10 seconds. Segmental reconstruction algorithm was applied to generate a single image from the data of one, two or three consecutive heartbeats. Images were reconstructed in the cardiac phase showing least motion artifacts. In general, the end-diastolic phase was used. However, additional reconstructions were made throughout the entire cardiac cycle if necessary to improve image quality. Subsequently, the images were transferred to a remote workstation (Vitrea 2, Vital Images, Minnetonka, USA) for post-processing.

CTA data analysis

All CTA's were interpreted by two experienced observers blinded to patient characteristics. Discrepancies in interpretation were immediately resolved by consensus. The presence of coronary atherosclerosis was evaluated by scrolling through axial images, followed by visual assessment of curved multiplanar reconstructions in at least two orthogonal planes. Coronary plaques were defined as structures of >1 mm^2 within and/or adjacent to the coronary artery lumen, which could be vividly discriminated from the vessel lumen and the surrounding pericardial tissue [19].

Initially, the presence of any atherosclerosis (≥1 plaque in the coronary tree), and that of obstructive atherosclerosis (luminal narrowing ≥50%) were evaluated in each patient. Thereafter, plaque burden was obtained by determining the total number of atherosclerotic plaques and obstructive plaques per patient [19].

Finally, plaques were classified according to phenotype: 1. non-calcified plaques (plaques with lower density than contrast-enhanced lumen), 2. calcified plaques (plaques with higher density than contrast-enhanced lumen), and 3. mixed plaques (plaques with components of low- and high density plaques) [19].

Laboratory Analysis

From all patients, blood and urine were collected after fasting overnight. Plasma total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, HbA1c and urine albumin/creatinine ratio were determined in the hospital laboratory, following clinical evaluation.

Blood samples collected for later analysis of high sensitivity C-reactive protein (hsCRP) and adiponectin in study setting were centrifuged. Separated plasma was stored at

-80ºC until assayed. All subsequent laboratory analyses were performed blind to patient characteristics.
Plasma levels of hsCRP were determined using the Tina-quant immunoassay (Roche Diagnostics, United Kingdom) by a Cobas Integra 800 analyzer. The method has a detection limit of 0.15 mg/L and a functional sensitivity of 0.3 mg/L. The inter-assay and intra-assay coefficients of variation are <3%.
Plasma adiponectin was measured with a commercially available radioimmunoassay (Linco Research, Inc, St Charles, Missouri, USA). The sensitivity cut-off was 1 ng/ml. The intra-assay and the inter-assay coefficients of variability were 6.21% and 6.90%, for mid-range concentrations of adiponectin respectively. Plasma adiponectin results are reported in micrograms per millimeter.

Statistical Analysis
Variables were expressed as means ± standard deviation, medians (lower quartile – upper quartile) or as numbers (percentages) if categorical.
First, the correlation between plasma adiponectin and traditional cardiovascular risk factors as well as hsCRP was determined using the Pearsons correlation.
Second, the relations between plasma adiponectin and the presence and degree of coronary atherosclerosis were evaluated. For this purpose the median plasma adiponectin levels were determined and compared in patients with no atherosclerosis, non-obstructive atherosclerosis and obstructive coronary atherosclerosis. The Mann-Whitney U test was applied to evaluate the difference in plasma adiponectin levels between the groups.
Thereafter, the potential predictors of the presence of any coronary atherosclerosis and obstructive coronary atherosclerosis on CTA were studied in univariate logistic regression models. All baseline traditional cardiovascular risk factors, as well as plasma hsCRP and adiponectin levels were included in the analyses. Variables with a P value <0.05 were included in a multivariate logistic regression model to identify the independent predictors.
Similarly, the predictors of extent of atherosclerosis represented by the number of atherosclerotic plaques, and the predictors of obstructive plaques on CTA were determined using univariate- and subsequent multivariate linear regression analyses.
Finally, the predictors of plaque phenotype (number of non-calcified, mixed and calcified plaques) on CTA were evaluated using univariate- and subsequent multivariate linear regression analyses.
Statistical analyses were performed using SPSS software (version 16.0, Inc., Chicago, Illinois). P values <0.05 were considered statistically significant.

Table 1. Characteristics of the patient population (N=103)

Age (years)	54 ± 11
Male sex	53 (51%)
Diabetes mellitus duration (years)	9 ± 7
Smoking	23 (22%)
Family history of CAD	50 (49%)
BMI (kg/m2)	28 (25-34)
Hypertension	69 (67%)
Anti-hypertensive medication use	58 (56%)
Systolic blood pressur (mmHg)	130 (125-145)
Diastolic blood pressure (mmHg)	80 (79-85)
HbA1c (mmol/L)	8.3 (7.3-9.5)
Fasting glucose (mmol/L)	9.6 (7.6-12.6)
Hypercholesterolemia	79 (77%)
Statin use	55 (53%)
Total cholesterol (mmol/L)	4.6 (3.8-5.5)
LDL-cholesterol (mmol/L)	2.9 (2.3-3.6)
HDL-cholesterol (mmol/L)	1.2 (1.0-1.5)
Triglycerides (mmol/L)	1.4 (1.0-2.5)
Micro-albuminuria	31 (30%)

Data are averages ± standard deviation, median (lower quartile – upper quartile) or number of patients (%).CAD: coronary artery disease, BMI: body mass index, HbA1c: glycosylated hemoglobin A1c, LDL: Low-density lipoprotein, HDL: high-density lipoprotein.

RESULTS

Study Population

In total, 103 asymptomatic patients with type 2 diabetes, with a mean age of 54±11 years were included. Fifty-three patients (51%) were men. Further baseline characteristics and traditional cardiovascular risk factors of the patient population are provided in Table 1. Diabetes treatment comprised of only diet in 5 patients (5%), oral agents in 69 patients (67%) and insulin in 65 patients (63%). At the time of referral, 69 patients (67%) had hypertension, of which 58 patients were treated with anti-hypertensive medication. Ace-inhibitors were used in 32 (31%), beta-blockers in 16 (16%), angiotensin-II receptor antagonists in 23 (22%), calcium channel blockers in 10 (10%) and diuretics in 16 patients (16%). Seventy-nine patients (77%) had hypercholesterolemia, and 55 patients (53%) were treated with statins. Furthermore, a minority of 26 patients (26%) received aspirin therapy at referral.

Assessment of coronary atherosclerosis by CTA

As shown in Appendix 1 (page 59), CTA revealed normal coronaries in 36 patients (35%), whereas the remaining 67 patients (65%) were shown to have coronary atherosclerosis. Within this group, 39 patients (38%) had non-obstructive atherosclerosis and 28 patients (27%) had obstructive atherosclerosis.

Overall, the mean number of plaques was 9.3±11.3, and the mean number of obstructive plaques was 1.5±3.2. Analysis of plaque phenotype in the total population showed a predominance of non-calcified plaques (62%), as compared to a minority of mixed (13%) and calcified plaques (25%) (Appendix 1).

Plasma hsCRP and adiponectin levels

Measurement of plasma hsCRP resulted in an overall median value of 2.3 mg/L with an inter-quartile range of 1.1 – 5.8 mg/L.

The overall median plasma adiponectin was determined to be 6.6 mcg/ml (3.8 – 11.2). The term of interaction was not significant between adiponectin and age, duration of diabetes, smoking, family history of CAD, BMI, HbA1c, total cholesterol, LDL-cholesterol or hsCRP levels. A positive significant relation was observed between plasma adiponectin levels and HDL-cholesterol (Spearman's correlation coefficient 0.45, P<0.001). An inverse relation was observed between plasma adiponectin and triglycerides (Spearman's correlation coefficient -0.16, P=0.01) as well as with the male sex (Spearman's correlation coefficient -0.25, P=0.01).

Relation of plasma adiponectin with coronary atherosclerosis on CTA

As illustrated in Appendix 2 (page 60), median plasma adiponectin decreased only modestly from 9.6 mcg/ml (5.4-13.2) in patients with no atherosclerosis to 7.5 mcg/ml (4.7-11.1) in presence of non-obstructive atherosclerosis on CTA (P=0.23). Importantly, a significant further decrease was observed in the median plasma adiponectin of patients with obstructive coronary atherosclerosis (3.8 mcg/ml (2.8-4.7)) (P<0.001) (Appendix 2).

Predictors of the presence of any atherosclerosis and obstructive atherosclerosis

Using univariate logistic regression analysis, age, a positive family history for CAD, hypertension, low HDL-cholesterol, triglycerides, micro-albuminuria and low plasma adiponectin were identified as potential predictors of the presence of any coronary atherosclerosis on CTA (Table 2). Of note, plasma adiponectin maintained a significant inverse relation with the presence of coronary atherosclerosis (P=0.02), after correction for other predictors of any coronary atherosclerosis.

Similarly, age, male sex, low HDL-cholesterol, micro-albuminuria and low plasma adiponectin were shown to have a significant relation with the presence of obstructive

Table 2. Predictors of the presence of any atherosclerosis and obstructive atherosclerosis on CTA.

	Any Atherosclerosis				Obstructive Atherosclerosis			
Variable	*Univariate*		*Multivariate*		*Univariate*		*Multivariate*	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age	1.10 (1.05-1.16)	**<0.001**	1.12 (1.04-1.19)	**0.001**	1.09 (1.03-1.14)	**0.001**	1.10 (1.04-1.17)	**0.002**
Male sex	2.18 (0.94-5.07)	0.07	-	-	3.33 (1.29-8.60)	**0.013**	1.79 (0.51-6.35)	0.37
Duration of DM	1.03 (0.97-1.10)	0.36	-	-	1.00 (0.94-1.07)	0.99	-	-
Smoker	0.75 (0.28-1.99)	0.56	-	-	1.40 (0.50-3.95)	0.53	-	-
Family history of CAD	3.65 (1.51-8.87)	**0.004**	2.71 (0.82-8.95)	0.10	1.48 (0.61-3.60)	0.39	-	-
Body mass index	1.02 (0.96-1.08)	0.59	-	-	0.97 (0.90-1.08)	0.39	-	-
Hypertension	3.80 (1.57-9.18)	**0.003**	2.09 (0.58-7.46)	0.26	2.28 (0.82-6.35)	0.12	-	-
HbA1c	1.17 (0.92-1.48)	0.20	-	-	1.27 (0.99-1.62)	0.054	-	-
HDL-cholesterol	0.29 (0.10-0.87)	**0.03**	0.78 (0.17-3.59)	0.75	0.23 (0.06-0.94)	**0.04**	1.21 (0.17-8.75)	0.85
Triglycerides	1.68 (1.04-2.71)	**0.03**	1.32 (0.76-2.31)	0.33	1.25 (0.90-1.74)	0.19	-	-
Micro-albuminuria	3.10 (1.13-8.53)	**0.03**	1.73 (0.41-7.28)	0.46	2.79 (1.11-7.02)	**0.03**	3.54 (0.96-13.0)	0.06
Plasma hsCRP	1.08 (0.98-1.19)	0.12	-	-	1.08 (0.99-1.16)	0.054	-	-
Plasma Adiponectin	0.88 (0.81-0.96)	**0.004**	0.85 (0.74-0.97)	**0.02**	0.71 (0.59-0.85)	**<0.001**	0.68 (0.53-0.88)	**0.003**

OR: odds ration, CAD: coronary artery disease, HbA1c: glycosylated hemoglobin A1c, HDL: high-density lipoprotein, hsCRP: high sensitivity C-reactive protein.

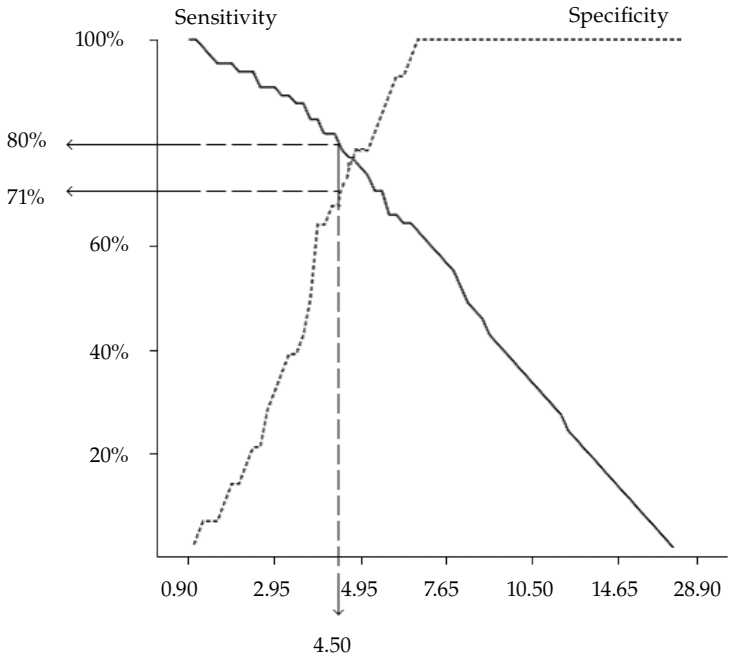

Figure 1. Predictive value of plasma adiponectin for the presence of obstructive coronary atherosclerosis. ROC curve analysis yielded a sensitivity and specificity of 80% and 71% with a plasma adiponectin cut-off value of <4.5 mcg/ml for prediction of obstructive coronary atherosclerosis.

coronary atherosclerosis (Table 2). Importantly, analysis in a multiple logistic regression model, revealed low plasma adiponectin to be an independent predictor of the presence of obstructive coronary atherosclerosis on CTA (P=0.003).

Using ROC curve analysis a cut-off value of 4.50 mcg/ml was identified for plasma adiponectin level. This cut-off value yielded a sensitivity and a specificity of 80% and 71% for predicting obstructive coronary atherosclerosis on CTA, in asymptomatic patients with type 2 diabetes (Figure 1).

Predictors of the extent of coronary atherosclerosis

Age, HbA1c, micro-albuminuria, hsCRP and low plasma adiponectin were significantly related with the extent of coronary atherosclerosis, as represented by the number of atherosclerotic plaques on CTA, in a univariate linear regression model (Table 3). The

Table 3. Predictors of the extent of atherosclerosis (number of atherosclerotic plaques and obstructive atherosclerotic plaques) on CTA.

	Number of Atherosclerotic Plaques				**Number of Obstructive Atherosclerotic Plaques**			
Variable	*Univariate*		*Multivariate*		*Univariate*		*Multivariate*	
	ß (95% CI)	P value	ß (95% CI)	P value	ß (95% CI)	P value	ß (95% CI)	P value
Age	0.32 (0.10-0.55)	**0.005**	0.38 (0.18-0.59)	**<0.001**	0.08 (0.05-0.14)	**0.003**	0.09 (0.04-0.14)	**0.001**
Male Gender	4.38 (-0.25-9.01)	0.063	-	-	1.16 (-0.08-2.40)	0.07	-	-
Duration of DM	0.26 (-0.08-0.59)	0.13	-	-	0.04 (-0.05-0.13)	0.41	-	-
Smoker	2.36 (-3.20-7.91)	0.40	-	-	1.56 (0.07-3.04)	**0.04**	1.06 (-0.39-2.51)	0.15
Family history of CAD	3.40 (-1.27-8.07)	0.15	-	-	0.76 (-0.49-2.01)	0.23	-	-
Body mass index	-0.13 (-0.48-0.21)	0.44	-	-	-0.03 (-0.12-0.07)	0.56	-	-
Hypertension	4.71 (-0.14-9.56)	0.06	-	-	1.29 (-0.02-2.60)	0.053	-	-
HbA1c	1.36 (0.09-2.62)	**0.02**	0.80 (-0.43-2.02)	0.20	0.37 (0.04-0.71)	**0.03**	0.23 (-0.11-0.57)	0.19
HDL-cholesterol	-2.27 (-8.22-3.67)	0.45	-	-	-1.64 (-3.21-(-0.08))	**0.04**	-0.47 (-2.14-1.20)	0.58
Triglycerides	0.18 (-0.47-0.84)	0.58	-	-	0.22 (0.05-0.40)	**0.01**	0.12 (-0.39-0.64)	0.63
Micro-albuminuria	4.97 (0.05-9.90)	**0.048**	1.69 (-2.95-6.33)	0.47	2.23 (0.95-3.51)	**0.001**	1.40 (-0.01-2.82)	0.05
Plasma hsCRP	0.65 (0.24-1.05)	**0.002**	0.64 (0.24-1.03)	**0.002**	0.06 (-0.05-0.17)	0.29	-	-
Plasma Adiponectin	-0.52 (-0.95-0.08)	**0.02**	-0.48 (-0.87-(-0.09))	**0.02**	-0.18 (-0.29-(-0.07))	**0.002**	-0.13 (-0.25-(-0.03)	**0.04**

CAD: coronary artery disease, HbA1c: glycosylated hemoglobin A1c, HDL: high-density lipoprotein, hsCRP: high sensitivity C-reactive protein.

Table 4. Predictors of atherosclerotic plaque phenotype (number of non-calcified, mixed and calcified plaques) on CTA.

	Number of Non-Calcified Plaques				Number of Mixed Plaques				Number of Calcified Plaques			
Variable	*Univariate*		*Multivariate*		*Univariate*		*Multivariate*		*Univariate*		*Multivariate*	
	ß (95% CI)	P value	ß (95% CI)	P value	ß (95% CI)	P value	ß (95% CI)	P value	ß (95% CI)	P value	ß (95% CI)	P value
Age	0.1 (-0.1-0.23)	0.27	-	-	0.1 (0.0-0.1)	**0.03**	0.0 (-0.0-0.1)	0.12	0.2 (0.1-0.3)	**<0.001**	0.1 (0.1-0.2)	**0.002**
Male Gender	1.8 (-1.8-5.3)	0.33	-	-	0.4 (-0.7-1.4)	0.47	-	-	2.2 (0.6-3.8)	**0.01**	0.5 (-0.4-1.4)	0.29
Duration of DM	-0.0 (-0.3-0.2)	0.84	-	-	0.1 (0.1-0.2)	**0.001**	0.1 (0.0-0.1)	0.08	0.2 (0.0-0.3)	**0.013**	0.1 (-0.0-0.2)	0.19
Smoker	1.0 (-3.2-5.3)	0.63	-	-	1.0 (-0.2-2.3)	0.09	-	-	0.3 (-1.8-2.3)	0.79	-	-
Family history of CAD	1.7 (-1.8-5.3)	0.33	-	-	0.4 (-0.6-1.4)	0.45	-	-	1.0 (-0.7-2.7)	0.23	-	-
Body mass index	-0.1 (-0.4-0.2)	0.41	-	-	-0.02 (-0.1-0.1)	0.65	-	-	-0.0 (-0.1-0.1)	0.95	-	-
Hypertension	0.2 (-3.6-3.9)	0.92	-	-	1.5 (0.5-2.5)	**0.004**	0.7 (-0.4-1.8)	0.21	3.0 (1.3-4.6)	**0.001**	0.6 (-0.5-1.7)	0.29
Hb A1c	0.5 (-0.4-1.5)	0.28	-	-	0.26 (0.0-0.5)	0.08	-	-	0.5 (-0.0-0.9)	0.05	-	-
HDL-cholesterol	-1.2 (-5.7-3.3)	0.61	-	-	-0.2 (-1.5-1.1)	0.76	-	-	-0.8 (-2.9-1.3)	0.47	-	-
Triglycerides	-0.1 (-0.6-0.4)	0.80	-	-	0.0 (-0.1-0.2)	0.54	-	-	0.2 (0.0-0.5)	0.07	-	-
Micro-albuminuria	0.8 (-3.0-4.6)	0.69			1.7 (0.7-2.8)	**0.002**	0.7 (-0.4-1.8)	0.19	2.1 (0.4-3.9)	**0.02**	0.9 (-0.2-2.0)	0.10
Plasma hsCRP	0.6 (0.3-0.9)	**<0.001**	0.6 (0.3-0.9)	**0.001**	0.1 (0.01-0.2)	**0.03**	0.1 (0.0-0.2)	0.08	-0.0 (-0.2-0.2)	0.93	-	-
Plasma Adiponectin	-0.4 (-0.7-0)	**0.03**	-0.3 (-0.60-0)	**0.04**	0.0 (-0.1-0.8)	0.79	-	-	-0.1 (-0.3-0.0)	0.12	-	-

CAD: coronary artery disease, HbA1c: glycosylated hemoglobin A1c, HDL: high-density lipoprotein, hsCRP: high sensitivity C-reactive protein.

inverse relation between plasma adiponectin with the extent of coronary atherosclerosis remained significant after correction in a multivariate linear regression model (P=0.02). Likewise, as shown in Table 3, low plasma adiponectin was shown to be independently associated with the number of obstructive coronary atherosclerotic plaques on CTA (P=0.04).

Predictors of atherosclerotic plaque phenotype

The relation of the traditional cardiovascular risk factors, hsCRP and plasma adiponectin with coronary atherosclerotic plaque phenotype is provided in Table 4. Briefly, hsCRP (P=0.001) and low plasma adiponectin (P=0.04) were shown to be independently associated with the number of non-calcified coronary atherosclerotic plaques on CTA. Age, duration of diabetes, hypertension, micro-albuminuria and hsCRP were associated with mixed atherosclerotic plaques, but all lost significance in a multi-variate linear regression model. Age was shown to be the only independent factor associated with the number of calcified coronary atherosclerotic plaques on CTA (P=0.002).

In consequence, plasma adiponectin was independently associated with the quantity of non-calcified, but not with mixed or calcified coronary atherosclerotic plaques (Table 4).

DISCUSSION

In the current study of asymptomatic patients with type 2 diabetes, an inverse relation was observed between plasma adiponectin and the presence and extent of coronary atherosclerosis. Of note, the relation between low adiponectin and coronary atherosclerosis remained significant after correction for traditional cardiovascular risk factors and hsCRP. Low adiponectin was strongly related with obstructive atherosclerosis. Accordingly, an adiponectin cut-off value of <4.5 mcg/ml resulted in a sensitivity of 80% for predicting obstructive atherosclerosis in asymptomatic patients with type 2 diabetes. Whereas no relation was observed between adiponectin and mixed or calcified coronary atherosclerotic plaques, an inverse association was shown between adiponectin and the quantity of non-calcified plaques. Thereby, in asymptomatic patients with type 2 diabetes, low plasma adiponectin predominantly contributed to the presence and extent of coronary atherosclerosis by predisposing non-calcified coronary atherosclerotic plaques.

Assessment of coronary atherosclerosis by CTA

CTA provides imaging of the structure and composition of the coronary arteries, thus allowing the evaluation of atherosclerosis. Importantly, in diabetic patients the diagnostic accuracy of CTA for the detection of obstructive atherosclerosis has been shown to be excellent, with a sensitivity of approximately 95% [20]. In addition, the technique

provides information on atherosclerotic plaque burden and plaque composition [19]. In retrospective studies, calcified coronary plaques have been associated with advanced but stable stages of atherosclerosis [21]. On the contrary, non-calcified plaques have been linked to the relatively early but more unstable stages of the disease [21]. Thus far limited studies have addressed the risk factors associated with the presence of coronary atherosclerosis and its morphology in asymptomatic patients with type 2 diabetes [22]. Due to its non-invasive nature, multi-slice CTA of the coronaries provides the possibility to assess atherosclerosis in asymptomatic patients with type 2 diabetes. Thereby, coronary atherosclerosis and its morphology can be evaluated in relation to both traditional risk factors and novel biomarkers, thus improving the understanding of pathophysiology of atherosclerosis in type 2 diabetes, as well as enabling targeted management strategies.

Relation of adiponectin with coronary atherosclerosis

Based on in vitro and animal studies, low adiponectin has been suggested to be an important causal link between adipose tissue dysfunction and atherosclerosis [23-27]. Adiponectin inhibits atherosclerosis by acting as an endogenous modulator of endothelial function through suppressing adhesion molecules [23], by inhibiting NF-κB [24], an interaction with interleukin-10 against vascular inflammation [25] and reduction of cholesterol uptake in macrophages and their transformation into foam cells [26]. In addition, adiponectin reduces vascular smooth muscle cell proliferation, migration and apoptosis [27]. A number of genetic studies confirm and extent the evidence implicating anti-atherogenic effects of adiponectin [8,28,29]. For instance, administration of recombinant adenovirus expressing human adiponectin to apoE-deficient animals caused a 30% reduction in the formation of atherosclerotic plaques [28]. Furthermore, in a meta-analysis of 827 individuals with CAD and 1887 without CAD, the adiponectin gene variant with the polymorphism +276GàT was associated with a 45% decreased risk for CAD [29].

Nevertheless, the predictive value of plasma adiponectin for cardiovascular events has been variable in the general population [9-11]. In an initial 6 years follow-up study of 18225 male participants, individuals with the highest quintile of adiponectin (24.9-56.1 mcg/mL) compared with the lowest (2.4-10.5 mcg/mL), showed a substantial decrease in risk of coronary events even after correction for other cardiovascular risk factors (RR 0.56, 95% CI 0.32-0.99) [9]. In contrast, in a prospective study and meta-analysis by Sattar and colleagues, comparison of men in the top adiponectin tertile with the lowest tertile, revealed a much more moderate association with coronary events (OR 0.84, 95% CI 0.70-1.01) [11]. However, in cross-sectional studies, low thresholds of plasma adiponectin of <4.0-5.5 mg/L have been consistently associated with CAD [12,13].

Adiponectin as a predictor of coronary atherosclerosis in type 2 diabetes

In the general population, a large variation is observed in the plasma adiponectin levels, which range up to 25 mcg/mL [11]. As a consequence, adiponectin levels may be a poor marker of coronary atherosclerosis in the general population. On the other hand, coherent to adipose tissue dysfunction, adiponectin levels are relatively low in patients with type 2 diabetes and mainly range 2-12 mcg/mL [14]. Therefore, assessment of adiponectin particularly in patients with type 2 diabetes is likely to yield a higher rate of low adiponectin levels, which predispose coronary atherosclerosis. Consequently, adiponectin may be a more effective marker of coronary atherosclerosis in type 2 diabetes.

As in the general population, the pathogenesis of atherosclerosis is multi-factorial in type 2 diabetes. However, excessive visceral adipose tissue which prompts insulin resistance, may play a more pivotal role in clustering of cardiovascular risk and development of atherosclerosis in patients with type 2 diabetes [6]. As visceral adipose tissue expands, macrophages infiltrate adipose tissue resulting in adipose tissue dysfunction [30]. This phenomenon increases the production of adipocytokines involved in glucose metabolism (e.g. resistin), lipid metabolism (e.g. cholesterol ester transfer protein), coagulation (plasminogen activator inhibitor-1) and inflammation (e.g. tumor necrosis factor-α, interleukin-6, C-reactive protein) [6]. Only the production of the adipocytokine adiponectin decreases during this process [6]. Being produced by adipocytes, a low plasma adiponectin concentration is a good marker of adipose tissue dysfunction [31].

The results of the current study indeed confirm an association between adiponectin and the presence, degree and extent of coronary atherosclerosis in asymptomatic patients with type 2 diabetes. Of note, a plasma adiponectin of <4.5 mcg/mL was associated with a sensitivity of 80% for the presence of obstructive atherosclerosis in this population of patients. These findings confirm previous observations in patients with type 2, wherein adiponectin was found to be inversely associated with coronary events [15, 16]. In a study by Schulze et al, the predictive value of adiponectin was attenuated by HDL cholesterol [15]. In contrast, in the current population of asymptomatic patients with type 2 diabetes, low adiponectin was shown to be associated with the presence, degree and extent of atherosclerosis also after correction for traditional risk factors (including low HDL cholesterol) and hsCRP.

In line with the current study results, previous studies in asymptomatic patients with type 2 diabetes have revealed a high prevalence of obstructive atherosclerosis (26-34%) and a high proportion of non-calcified coronary plaques (41-66%) [32,33]. Prognostic data suggest the presence of not only obstructive atherosclerotic plaques, but also non-calcified coronary plaques to convey an increased risk for coronary events [34].

Importantly, low adiponectin, akin to hsCRP (a marker of inflammation), was shown to be especially related with non-calcified atherosclerotic plaques.
Use of a biomarker, such as adiponectin, for the cardiovascular risk stratification of asymptomatic patients with type 2 diabetes may comprise several advantages. Potentially, patients at risk for CAD, could be distinguished at an early stage, prior to clinically manifest atherosclerosis and treated more aggressively. In addition, application of a biomarker may provide a more general estimation of atherosclerotic risk, opposing non-invasive vascular or cardiac imaging techniques which are often restricted to a certain aspect of vascular disease or a specific organ. On the other hand, clinical utility of biomarkers has often been limited by their low specificity [35]. Although several biomarkers (e.g. hsCRP) show a strong correlation with CAD in study populations, the inverse claim that elevated markers indicate a high risk has often been difficult to substantiate [35]. Remarkably however, in this study of asymptomatic patients with type 2 diabetes, low adiponectin (<4.5 mcg/mL), was not only associated with a good sensitivity, but also with a reasonable specificity of 71% for predicting obstructive coronary atherosclerosis.

Study limitations

The current study was limited to the evaluation of the relation between adiponectin and coronary atherosclerosis in patients with type 2 diabetes, and assessment did not include a non-diabetic control group. As CTA is accompanied with radiation exposure, it is not feasible to perform a similar assessment in asymptomatic subjects free of cardiovascular risk. Furthermore, the prognostic value of adiponectin was not evaluated as no follow-up data were available.

CONCLUSION

Low plasma adiponectin was independently related with the presence, degree and extent of coronary atherosclerosis in asymptomatic patients with type 2 diabetes. A predominant association was observed with the presence of non-calcified plaques. Assessment of plasma adiponectin conveys the potential to augment risk for CAD in asymptomatic patients with type 2 diabetes.

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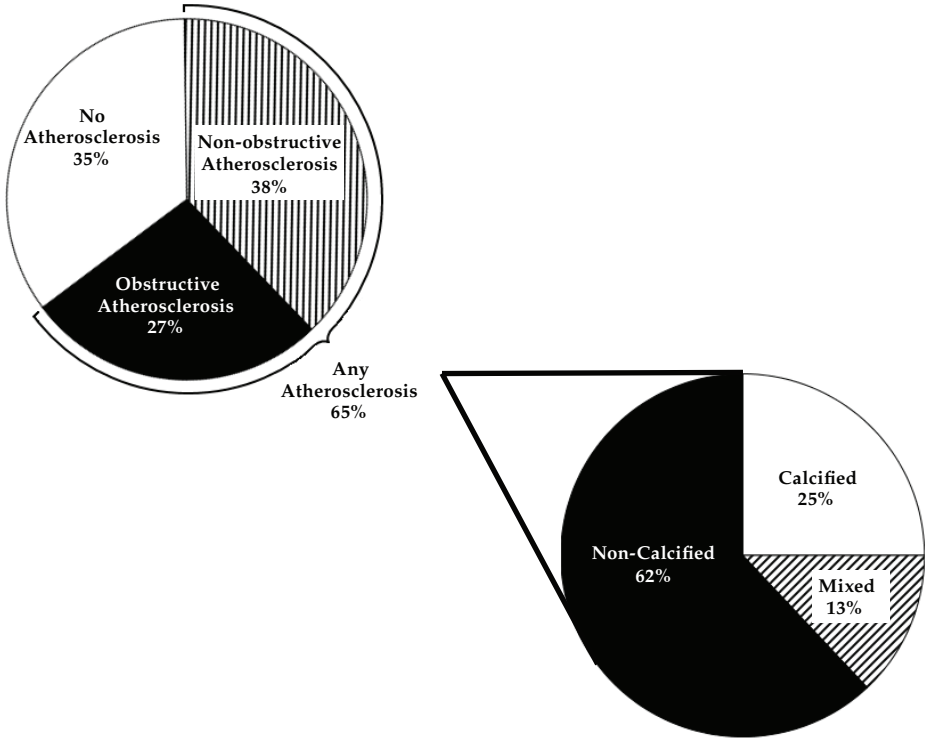
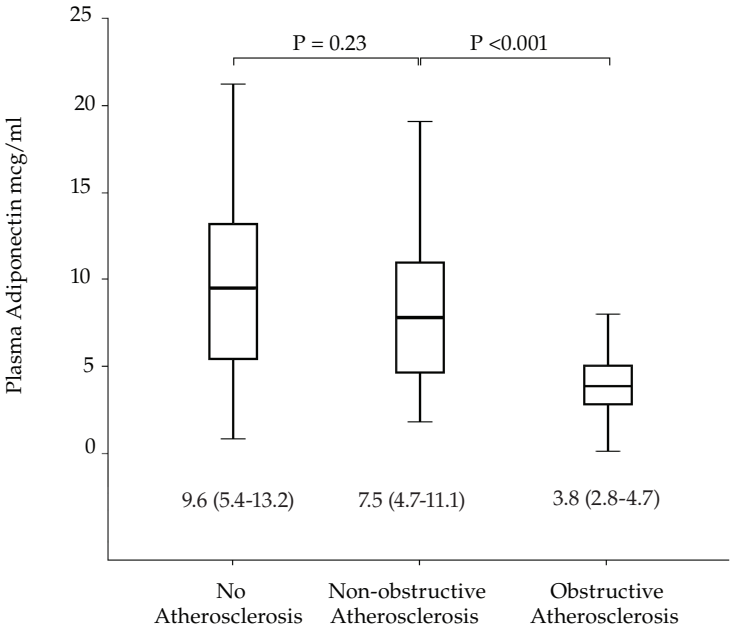
SUPPLEMENTARY FIGURES



Appendix 1. Results of CTA in asymptomatic patients with type 2 diabetes. Pie-chart on the top left presents the results of CTA at patient level. Pie-chart on the bottom right presents the distribution of plaque phenotype in patients with atherosclerotic plaques.



Appendix 2. Relation of plasma adiponectin with coronary atherosclerosis as assessed by CTA. Plasma adiponectin decreased with the presence and degree of coronary atherosclerosis.