Inverse Relationship Between Adiponectin Levels and Subclinical Carotid Atherosclerosis in Patients Undergoing Coronary Artery Bypass Grafting

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SUMMARY

The purpose of this study was to examine the relation between adiponectin levels and subclinical carotid atherosclerosis in patients undergoing coronary artery bypass grafting (CABG). Serum concentrations of adiponectin and carotid intima/media thickness (IMT) were measured in 84 consecutive patients who underwent CABG. Carotid IMT both at the common carotid artery and carotid bulb level was correlated negatively and significantly ($r = -0.581$ and $r = -0.415$, respectively, $P < 0.01$) with the serum concentrations of adiponectin. Linear regression modeling identified adiponectin as the strongest predictive variable for carotid IMT both at the common carotid artery and carotid bulb level ($P < 0.001$). Stepwise regression analyses also showed that adiponectin was the strongest independent determinant of the carotid IMT both at the common carotid artery and the carotid bulb level ($F = 20.215$ and $F = 19.565$, respectively, $P < 0.001$). The mean number of diseased coronary arteries, mean number of distal anastomoses, cardiopulmonary bypass time, and aortic cross-clamping time did not significantly correlate with the serum concentrations of adiponectin. The findings indicate the presence of an inverse relationship between serum concentrations of adiponectin and subclinical carotid atherosclerosis in patients undergoing CABG. In these patients, the absence of a significant correlation between severity of coronary atherosclerosis and adiponectin might suggest that adiponectin levels may predict the early stages rather than further progression of atherosclerosis. (Int Heart J 2006; 47: 855-866)

Key words: Adiponectin, Subclinical carotid atherosclerosis, Coronary artery bypass grafting

Adipose tissue was once considered to be only a reservoir for energy storage. Now adipose tissue is known to operate as an endocrinologically active tissue that releases peptides into circulation in response to specific extracellular stimuli or
Adiponectin is a recently identified adipocytokine which has important anti-inflammatory and antiatherogenic effects. Adiponectin has an anti-inflammatory effect on endothelial cells, inhibits the proliferation of vascular smooth muscle cells, and suppresses the conversion of macrophages to foam cells. Experiments with adiponectin-deficient mice have demonstrated the acceleration of neointimal thickening and proliferation of smooth muscle cells in response to wire-injured arteries. Moreover, adiponectin reduced the progression of atherosclerotic lesions in apolipoprotein E-deficient mice, a well-established animal model of atherosclerosis.

In addition to in vivo studies, clinical observations also suggest an inverse association between serum concentrations of adiponectin and atherosclerosis. Decreased serum concentrations of adiponectin were observed in patients with coronary artery disease independently of common cardiovascular risk factors. Patients with unstable angina and myocardial infarction showed lower plasma concentrations of adiponectin. Plasma concentrations of adiponectin were also decreased and independently associated with ankle brachial index in patients with peripheral arterial occlusive disease.

Early or subclinical atherosclerosis in carotid arteries and severe or advanced atherosclerosis in coronary arteries usually may coexist in patients undergoing coronary artery bypass grafting (CABG). Although there is growing evidence that adiponectin has protective effects against atherosclerosis and that adiponectin levels are decreased in patients with CAD, the exact relation between serum concentrations of adiponectin and subclinical carotid atherosclerosis in patients undergoing CABG remains unclear in clinical practice. The purpose of this study was to determine whether there is a relation between serum concentrations of adiponectin and subclinical carotid atherosclerosis in patients undergoing CABG. For this purpose, we measured serum concentrations of adiponectin and carotid intima/media thickness (IMT) in patients undergoing CABG.

**METHODS**

**Subjects:** From January to September 2005, 84 consecutive patients who underwent CABG operations in our clinic were enrolled in this prospective study. There were 69 (82.1%) males, and the mean age of the patients was 60.81 ± 10.59 years. All patients had documented coronary artery disease defined as more than 75% stenosis in one or more principal coronary arteries by coronary angiography. Patients who had serious active infectious diseases, malignancies, chronic inflammatory diseases, or renal dysfunction were excluded from the study. The
baseline characteristics of the patients are shown in Table I. Prior written informed consent was obtained from all patients. This study was conducted in accordance with the guidelines approved by the ethics committee at our institution. The investigation conformed to the principles outlined in the Declaration of Helsinki.11)

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<tr>
<th></th>
<th>Number</th>
<th>Age (years)</th>
<th>Gender (M/F)</th>
<th>Body mass index (kg/m²)</th>
<th>Diabetes mellitus</th>
<th>Hypertension</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Diastolic blood pressure (mmHg)</th>
<th>Smoking</th>
<th>Myocardial infarction</th>
<th>Stable angina pectoris</th>
<th>Unstable angina pectoris</th>
<th>Left ventricular aneurysm</th>
<th>Coronary artery stenting</th>
<th>Coronary artery balloon angioplasty</th>
<th>Number of diseased vessels</th>
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<tr>
<td></td>
<td>84</td>
<td>60.81 ± 10.59</td>
<td>69/15</td>
<td>27.63 ± 4.97</td>
<td>26</td>
<td>59</td>
<td>127.38 ± 19.70</td>
<td>78.21 ± 11.83</td>
<td>48</td>
<td>35</td>
<td>43</td>
<td>37</td>
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**Surgical technique:** Standard anesthetic techniques and anesthetics were used in all patients by the same anesthetic team. Following median sternotomy, the ascending aorta was cannulated for arterial inflow and the right atrial appendage was cannulated with a two-stage cannula for venous uptake. A cardioplegic tack was introduced into the aortic root proximal to the aortic cannulation site for antegrade cardioplegic delivery. Heparin was given at a dose of 3 mg/kg for systemic anticoagulation, and cardiopulmonary bypass was established. Myocardial protection was maintained using initially cold (0-4°C) crystalloid cardioplegia solution followed by cold blood (10°C) cardioplegia and finally warm blood (37°C) cardioplegia. Mild systemic hypothermia (32°C) was applied. The left internal thoracic artery was anastomosed to the left anterior descending coronary artery and saphenous venous grafts were used for bypass to the other coronary arteries in all patients.

**Laboratory analysis:** Venous blood samples were collected after the patients had fasted overnight. Samples drawn from an antecubital vein were immediately transferred into glass tubes and centrifuged at 3500 rpm for 4 minutes. Serum
samples were kept at -80°C for subsequent assays. Serum concentrations of adiponectin were measured by an enzyme immunoassay method using a sandwich ELISA system (adiponectin ELISA kit, ACRP30®, Linco Research, Missouri, USA). Serum concentrations of glucose, uric acid, creatinine, blood urea nitrogen, albumin, protein, total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride were measured by photometric methods adapted to an autoanalyzer (Aeroset®, Abbott Diagnostics, Illinois, USA). Serum high sensitivity C-reactive protein (hsCRP) concentrations were measured using a chemiluminescent immunometric assay method (Immulite 2000®, Diagnostic Products Corporation, Los Angeles, USA). Diabetes mellitus was defined according to World Health Organization criteria.12) Smoking was defined as current smoker. Body mass index (BMI) was calculated as weight divided by the square of height.

Carotid artery ultrasound: A high-resolution ultrasound unit (Aplio 80®, Toshiba, Tokyo) equipped with a 6-12 MHz broad-band linear transducer was used for all examinations, which were all performed by the same radiologist. Measurements were obtained from stored digital images by an experienced reader. For the present analysis, 2 carotid segments were identified on both the left and right sides: the distal 1 cm of the common carotid artery (CCA) and the carotid bulb. The carotid IMT was measured at the far wall on each carotid segment on both sides as the distance between the interface of the lumen and intima, and the interface between the media and adventitia. Measurements on both sides were averaged to obtain the mean IMT, which defines the carotid IMT of each patient in this study. A thorough search of the CCA and the carotid bulb was made to determine the presence of focal atherosclerotic plaque. Focal atherosclerotic plaque was defined as a clearly identified area of focal increased thickness (≥ 1 mm) of the intima-media layer. In the presence of plaque in the examined carotid segments, the maximal plaque length and the maximal plaque thickness were also measured.

Statistical analysis: For continuous variables, results are presented as the mean ± standard deviation. Categorical variables are presented by frequency counts. Spearman rank correlations were used to assess the relationship between adiponectin and all other variables. Independent predictors of carotid IMT were determined by linear and stepwise regression modeling. All P values < 0.05 were interpreted as statistically significant. All calculations were performed using a standard statistical package (SPSS 13.0, SPSS Inc., Chicago, IL, USA).

Results

Serum concentrations of adiponectin and carotid atherosclerosis: Carotid IMT at the CCA level correlated negatively and significantly ($r = -0.581$, $P < 0.01$) with
the serum concentrations of adiponectin (Figure 1). Carotid IMT at the carotid bulb level also correlated negatively and significantly ($r = -0.415, P < 0.01$) with the serum concentrations of adiponectin (Figure 2). The mean serum concentration of adiponectin was $7.73 \pm 5.34 \mu g/mL$. Mean carotid IMT at the CCA and the
carotid bulb level were 0.97 ± 0.13 and 1.01 ± 0.14 mm, respectively. However, neither the maximal plaque length nor the maximal plaque thickness correlated with the serum concentrations of adiponectin ($r = 0.012$ and $r = 0.053$, respectively). Focal atherosclerotic plaque was found in 51 patients. Mean maximal plaque length and maximal plaque thickness were 6.96 ± 7.1 and 1.47 ± 1.46 mm, respectively.
Linear regression modeling using the carotid IMT as the dependent variable and BMI, glucose, cholesterol, HDL, LDL, triglycerides, systolic blood pressure, diastolic blood pressure, the number of diseased coronary arteries, hsCRP, age, and adiponectin as independent variables identified adiponectin as the strongest predictive variable for carotid IMT both at the CCA and the carotid bulb level (Table II). To examine if the association of adiponectin with carotid IMT is independent of other clinical parameters, stepwise regression analyses were performed. When BMI, glucose, cholesterol, HDL, LDL, triglycerides, systolic blood pressure, diastolic arterial blood pressure, the number of diseased coronary arteries, hsCRP, age, and adiponectin were used as variables, adiponectin was found to be the strongest independent determinant of the carotid IMT both at the CCA and the carotid bulb level (Table III).

**Serum concentrations of adiponectin and operative outcome of the patients:** The mean number of diseased coronary arteries and distal anastomoses were 2.56 ± 0.93 and 2.43 ± 0.92, respectively. Neither the number of diseased coronary arteries nor the number of distal anastomoses correlated with the serum concentrations of adiponectin ($r = -0.051$ and $r = -0.223$, respectively). Mean cardiopulmonary bypass time and aortic cross-clamping time were 113.85 ± 37.41 and 62.22 ± 22.3 min, respectively. Neither cardiopulmonary bypass time nor aortic cross-clamping time correlated with the serum concentrations of adiponectin ($r = 0.090$ and $r = 0.027$, respectively). However, cardiopulmonary bypass time correlated positively and significantly both with the mean number of diseased coronary arteries ($r = 0.313$, $P < 0.01$) and distal anastomoses ($r = 0.418$, $P < 0.01$). Similarly, aortic cross-clamping time correlated positively and significantly both with the mean number of diseased coronary arteries ($r = 0.228$, $P < 0.05$) and distal anastomoses ($r = 0.307$, $P < 0.01$). Surgical repair of concomitant left ventricular aneurysm by linear closure was performed in 6 patients. In the postoperative period, 45

<table>
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<th>Table IV. Metabolic Parameters of the Patients</th>
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<tr>
<td>Fasting blood glucose (mg/dL)</td>
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<tr>
<td>Total cholesterol (mg/dL)</td>
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<td>HDL (mg/dL)</td>
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<td>LDL (mg/dL)</td>
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<td>Triglycerides (mg/dL)</td>
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<td>Uric acid (mg/dL)</td>
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<td>Creatinine (mg/dL)</td>
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<td>Blood urea nitrogen (mg/dL)</td>
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<td>Albumin (g/dL)</td>
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<td>Protein (g/dL)</td>
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HDL indicates high density lipoproteins; LDL, low density lipoproteins; and hsCRP, high sensitivity C-reactive protein.
patients required positive inotropic support and intraaortic balloon pumping was used in 6 of them. Severe carotid artery stenosis was diagnosed ultrasonographically in 2 patients. One of these patients had bilateral carotid artery stenosis. First, he underwent left carotid artery stenting, and later, CABG to the 4 coronary arteries and then right carotid endarterectomy. The other patient had unilateral carotid artery stenosis and underwent combined carotid endarterectomy and CABG. The postoperative courses of the 2 patients with severe carotid artery stenosis were uneventful. In the whole sample, a total of 5 patients died perioperatively. Three patients died due to multiorgan failure and 2 patients died due to cardiac events.

**Serum concentrations of adiponectin and other variables:** The metabolic parameters of the patients are shown in Table IV. Serum concentrations of adiponectin correlated negatively and significantly with the serum concentrations of triglycerides and blood urea nitrogen (\(r = -0.324\) and \(r = -0.308, P < 0.05\), respectively). There were no significant correlations between the serum concentrations of adiponectin and other metabolic parameters.

**DISCUSSION**

Our findings indicate the presence of an inverse relation between serum concentrations of adiponectin and subclinical carotid atherosclerosis in patients undergoing CABG. The evidence is that the serum concentrations of adiponectin correlated negatively and significantly with the carotid IMT both at the CCA and at the carotid bulb level in the patients who underwent CABG.

Subclinical or early carotid atherosclerosis may accompany severe coronary atherosclerosis in patients undergoing CABG. Carotid IMT measured by high-resolution B-mode ultrasonography has been used as a marker of early atherosclerosis in carotid arteries in several studies. Since adiponectin has antiatherogenic properties, an inverse relation between carotid atherosclerosis and adiponectin levels might be anticipated. In our study, we found that carotid IMT was significantly and inversely related with the serum concentrations of adiponectin. Similarly, a negative correlation between adiponectin levels and carotid IMT has been reported in obese juveniles and in patients with coronary artery disease. However, in our study, adiponectin levels were not significantly correlated with either the atherosclerotic carotid plaque thickness or the plaque length. Compatible with our findings, Iglseeder, et al reported that adiponectin levels independently and inversely related with carotid IMT, but there was no relation between adiponectin levels and the presence of atherosclerotic plaque. These clinical observations suggest that adiponectin might be a useful marker for identifying atherosclerotic carotid wall thickening, a marker of early atherosclerosis, rather than plaque severity.
A growing body of evidence indicates a link between carotid atherosclerosis and metabolic syndrome. Metabolic syndrome is a clustering of cardiovascular risk factors and consists of atherogenic dyslipidemia, elevated blood pressure and plasma glucose, and a prothrombic and a proinflammatory state. The incidence and progression of subclinical carotid atherosclerosis is increased in subjects with metabolic syndrome. In addition, metabolic syndrome was reported to be significantly associated with the presence of CAD and carotid IMT in a recent large cohort study. Since adiponectin is increasingly recognized to be a potential biomarker for metabolic syndrome, it might be anticipated that serum adiponectin levels may predict subclinical carotid atherosclerosis. In our study, regression analyses identified adiponectin as the strongest predictive variable for carotid IMT. This result suggests that adiponectin levels are predictive of subclinical carotid atherosclerosis independently from the variables of metabolic syndrome.

Clinical studies have revealed that adiponectin levels are related to the variables of metabolic syndrome. Mohan, et al found that serum adiponectin concentrations were correlated negatively with BMI, fasting blood glucose and triglycerides, and positively with HDL in 100 diabetic and 100 control subjects. Similarly, Choi, et al found that serum adiponectin concentrations were correlated negatively with BMI, blood pressure (systolic and diastolic), fasting blood glucose and triglycerides, and positively with HDL in 372 elderly Koreans. We found that serum adiponectin concentrations were correlated negatively with BMI, glucose, cholesterol, triglycerides, and positively with HDL. Consistent with our findings, clinically, circulating adiponectin levels are known to decrease in patients with obesity, type 2 diabetes and dyslipidemia. Taken together, these findings support the interrelation between adiponectin, metabolic syndrome, and subclinical carotid atherosclerosis.

Severe coronary atherosclerosis, a marker of advanced atherosclerotic vascular disease, is usually present in patients requiring CABG. The severity of coronary artery disease has been estimated by the number of diseased vessels in many studies. Although decreased adiponectin levels were observed in patients with coronary artery disease, the association between adiponectin levels and severity of coronary artery disease is controversial. Some investigators have reported a negative correlation between adiponectin levels and the severity of coronary artery disease. However, in a recent study there was no significant association between adiponectin and the severity of angina pectoris. In our study, we did not find a significant correlation between the serum concentrations of adiponectin and the number of diseased coronary arteries. The absence of a significant correlation might suggest that adiponectin levels may not be related to the severity of coronary atherosclerosis.
In our study, the mean number of distal anastomoses did not significantly correlate with the serum concentrations of adiponectin. This finding can be explained by the fact that the number of diseased coronary arteries is usually associated with the number of distal anastomoses in CABG operations. Similarly, neither cardiopulmonary bypass time nor aortic cross-clamping time significantly correlated with the serum concentrations of adiponectin. A broad literature survey was undertaken, however, we could not find a study that examined the relation between adiponectin levels and aortic cross-clamping and cardiopulmonary time in CABG. The absence of a significant correlation between adiponectin and these operative variables in our study also supports the assumption that adiponectin levels may not be related to the severity of coronary artery disease. We also speculate that adiponectin levels may predict the early stages rather than further progression of atherosclerosis.

In the early stages of atherosclerosis, endothelial cell activation by various inflammatory stimuli, including TNF-α, results in the synthesis of adhesion molecules and increases the adherence of monocytes. This monocyte adhesion to the arterial endothelium is considered crucial for the development of vascular disease. In vitro evidence indicates that physiological concentrations of adiponectin inhibit TNF-α-induced monocyte adhesion and expression of adhesion molecules on the endothelium during the early stage of atherosclerosis. In addition, Takahashi, et al found that impaired expression of cardiac adiponectin may contribute to the progression of viral myocarditis through enhanced expression of TNF-α in leptin-deficient mice. They speculated that local expression of adiponectin in the damaged heart may be a compensatory phenomenon against severe inflammatory conditions such as viral myocarditis. These anti-inflammatory effects of adiponectin may contribute to its protective features against the development of atherosclerosis.

Conclusions: In conclusion, our findings indicate an inverse relation exists between serum concentrations of adiponectin and subclinical carotid atherosclerosis in patients undergoing CABG. To the best of our knowledge, this is one of the first studies to show that adiponectin levels are an independent predictor of concomitant subclinical carotid atherosclerosis in patients undergoing CABG. This finding may be a basis for further investigations to clarify the possible biomarker function of adiponectin in severe carotid stenosis in these patients.

REFERENCES

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