

Possible Impairment of Transcardiac Utilization of Adiponectin in Patients With Type 2 Diabetes

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OBJECTIVE — Adiponectin, an adipocyte-derived protein, has been suggested to enhance insulin sensitivity and prevent atherosclerosis. Circulating adiponectin levels are reduced in states of insulin resistance such as type 2 diabetes. We examined transcardiac utilization of adiponectin in patients with and without type 2 diabetes.

RESEARCH DESIGN AND METHODS — A total of 17 male type 2 diabetic patients and 17 male nondiabetic patients were investigated. Venous blood samples were taken to measure glucose and lipid variables. Blood samples for the measurement of adiponectin were collected simultaneously from the aortic root and coronary sinus. Angiographic semiquantitative stenosis score of coronary artery was also evaluated.

RESULTS — The adiponectin levels in both the aortic root and coronary sinus in the diabetic patients were significantly lower than those in the nondiabetic patients. The adiponectin level was significantly lower in the coronary sinus than in the aortic root in the nondiabetic patients, but there was no significant difference between adiponectin levels in the aortic root and coronary sinus in the diabetic patients. The total stenosis score, as an index of severity of coronary artery stenosis, was significantly higher in the diabetic patients than in the nondiabetic patients. The stenosis score was correlated with the degree of transcardiac utilization of adiponectin from the aortic root to coronary sinus in the nondiabetic patients but not in the diabetic patients.

CONCLUSIONS — Diabetic patients not only have a decreased adiponectin level in the basal state compared with nondiabetic patients but also have impaired utilization of adiponectin in the coronary artery and/or the heart, which may promote the development of atherosclerosis.

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Adipose tissue was once thought to be simply a reservoir for energy storage in the form of triglyceride. However, it is now known that adipocytes secrete a variety of proteins, such as tu-

mor necrosis factor (TNF)- α , plasminogen activator inhibitor-1, leptin, resistin, and adiponectin. These proteins are thought to be involved in a wide range of biological effects. Adiponectin, an adipo-

cyte-derived protein referred to as Acrp30, apM1, AdipoQ, and GBP28, has been independently identified and characterized by several groups (1–5). In humans, adiponectin is one of the most abundant gene transcript proteins in adipocytes, accounting for 0.01% of all proteins (2). In contrast with other adipocyte-derived proteins, the circulating adiponectin level is reduced in patients with coronary artery disease and in states of insulin resistance such as obesity and type 2 diabetes (6–8). We have previously shown that hypoadiponectinemia is related to insulin resistance in essential hypertension (9). Animal experiments have also suggested that adiponectin enhances insulin sensitivity and prevents atherosclerosis (10,11).

It has also been suggested that adiponectin modulates endothelial function and has an inhibitory effect on vascular smooth muscle cell proliferation (12,13). Moreover, adiponectin has been shown to accumulate in an injured artery from the plasma and to suppress macrophage-to-foam cell transformation in vitro and in vivo (11,14,15). We previously showed that adiponectin concentration was negatively correlated with pulse-wave velocity (PWV), which was measured as an index of atherosclerosis, and that adiponectin was a significant determinant of PWV in multiple regression analysis (16).

Taken together, the results of previous studies suggest that the concentration of adiponectin is decreased and utilization of adiponectin in an atherosclerotic lesion is impaired in patients with diabetes who are known to be at high risk for development of atherosclerosis. We therefore examined transcardiac utilization of adiponectin, including that in the coronary artery and/or the heart, in patients with and without type 2 diabetes.

RESEARCH DESIGN AND

METHODS — We enrolled 17 male patients with type 2 diabetes and 17 age- and BMI-matched male patients without diabetes, all of whom underwent cardiac catheterization for evaluation of sus-

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Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; PWV, pulse-wave velocity; TNF, tumor necrosis factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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pected or known coronary artery diseases. The subjects with diabetes were defined as those who were being treated with hypoglycemic agents, those with fasting plasma glucose ≥ 7 mmol/l or plasma glucose ≥ 11.1 mmol/l 2 h after a 75-g oral glucose tolerance test, or those with random plasma glucose ≥ 11.1 mmol/l. These criteria were confirmed by repeating the test on another day. Patients who were being treated with thiazolidinediones, peroxisome proliferator-activated receptor- γ agonists, or glimepiride, a third-generation sulfonylurea agent, were excluded because these drugs have been reported to increase the circulating level of adiponectin (17–19). The nondiabetic patients were also identified as those with fasting plasma glucose < 5.6 mmol/l (normal fasting glucose) and a glucose level < 7.8 mmol/l (normal glucose tolerance) 2 h after a 75-g oral glucose tolerance test. Patients with renal dysfunction, acute coronary syndrome, such as unstable angina and acute myocardial infarction, or inflammatory diseases, such as collagen diseases, advanced liver diseases, malignant diseases, and arthritis, or infectious diseases, were also excluded.

Venous blood samples were taken from the antecubital vein early in the morning to measure glucose and lipid variables. All of the patients then rested in the supine position for > 20 min. Following the rest periods, left- and right-sided cardiac catheterizations were performed through the right radial artery and antecubital vein, respectively. Blood samples for measurement of adiponectin were collected simultaneously from the aortic root and coronary sinus. A 4Fr catheter for blood sampling was positioned in the coronary sinus, and the position of the catheter was confirmed by injection of contrast medium after sampling. Angiographic semiquantitative score of coronary stenosis was also evaluated by coronary angiography using a contrast medium just after blood sampling. This study was performed with the approval of the institutional ethics committee, and informed consent was obtained from all of the patients.

Semiquantitative coronary angiography

Coronary angiography was performed by multiprojections of whole coronary arterial segments; the right coronary artery

had three projections performed and the left artery seven. A 75% luminal narrowing in the major coronary artery was defined as significant stenosis. Angina pectoris was defined as significant stenosis of the coronary artery and predominant ST-segment deviation during exercise testing, with or without scintigraphic perfusion imaging regardless of chest pain. Furthermore, stenosis score as an index of severity of coronary artery stenosis was evaluated by the method recommended by the American Heart Association. The scores are as follows: 5 points in 99–100% stenosis, 4 in 90%, 3 in 75%, 2 in 50%, and 1 in 25% (in each segment). All scores were added in 15 segments, according to the method recommended by the American Heart Association. All of the data were evaluated by two experienced angiography specialists who were unaware of the purpose of this study.

Laboratory investigations

Serum adiponectin levels were measured using a commercially available sandwich enzyme-linked immunosorbent assay kit (Otsuka Pharmaceuticals, Tokushima, Japan) as previously reported (5). The intra- and interassay coefficients of variations (CVs) were 3.3 and 7.4%, respectively. Fasting plasma glucose was determined by the glucose oxidase method. Fasting plasma insulin was measured by a radioimmunoassay method (Insulin RIA bead; Dainabot, Tokyo, Japan). A homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by fasting plasma insulin (mU/l) \times fasting plasma glucose (mmol/l)/22.5. HbA_{1c} was determined by high-performance liquid chromatography. Serum creatinine and serum lipid variables, including total, HDL, and LDL cholesterol and triglyceride, were estimated by enzymatic methods.

Statistical analysis

Numeric variables are expressed as means \pm SE. The Mann-Whitney *U* test was used for comparisons between two unpaired variables. The difference between two paired variables was analyzed by Wilcoxon's signed-rank test. Spearman's rank correlation test was used for analysis of correlations between two variables. A *P* value < 0.05 was considered statistically significant.

RESULTS—As shown in Table 1, the nondiabetic and diabetic patients were matched for age, BMI, and waist circumference. The duration of diabetes was 8.4 ± 1.4 years. There were no significant differences among levels of total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, and creatinine or among kinds of medication, except for hypoglycemic drugs in the two groups. The incidences of prior myocardial infarction, angina pectoris, and smoking habits were not significantly different between the nondiabetic and diabetic patients. The diabetic patients had significantly higher levels of fasting glucose, insulin, HbA_{1c}, and HOMA-IR than the nondiabetic patients.

The serum adiponectin levels in both the aortic root and coronary sinus in the diabetic patients were significantly lower than those in the nondiabetic patients (Fig. 1). In the nondiabetic patients, the adiponectin level was significantly lower in the coronary sinus than in the aortic root. In contrast, there was no significant difference between the adiponectin levels in the aortic root and coronary sinus in the diabetic patients.

In all of the patients, the adiponectin concentrations in both the aortic root and coronary sinus were negatively correlated with BMI (aortic root: $r = -0.50$, $P < 0.01$; coronary sinus: $r = -0.43$, $P < 0.05$), fasting glucose ($r = -0.52$, $P < 0.01$; $r = -0.48$, $P < 0.01$), HbA_{1c} ($r = -0.39$, $P < 0.05$; $r = -0.31$, $P < 0.05$), insulin level ($r = -0.53$, $P < 0.01$;

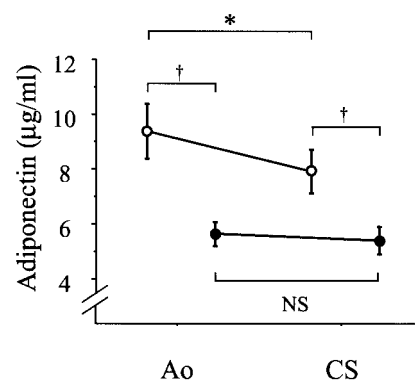


Figure 1—Serum adiponectin levels in the aortic root (Ao) and coronary sinus (CS) in patients with and without diabetes. ○, nondiabetic patients; ●, diabetic patients. The differences between unpaired and paired variables were analyzed by the Mann-Whitney *U* test and Wilcoxon's signed-rank test, respectively. * $P < 0.01$; † $P < 0.05$.

Table 1—Characteristics of the 34 studied male patients

	Nondiabetic subjects	Diabetic subjects
<i>n</i>	17	17
Age (years)	65.4 ± 2.6	64.5 ± 2.5
BMI (kg/m ²)	24.2 ± 0.5	24.9 ± 0.5
Waist circumference (cm)	87.7 ± 1.5	88.0 ± 1.6
Biochemical data		
Total cholesterol (mmol/l)	5.0 ± 0.3	4.5 ± 0.2
HDL cholesterol (mmol/l)	1.2 ± 0.1	1.2 ± 0.1
LDL cholesterol (mmol/l)	3.1 ± 0.2	2.7 ± 0.2
Triglyceride (mmol/l)	1.4 ± 0.2	1.2 ± 0.1
Fasting glucose (mmol/l)	5.0 ± 0.1	6.5 ± 0.4*
HbA _{1c} (%)	5.0 ± 0.1	7.2 ± 0.4*
Fasting insulin (pmol/l)	37.2 ± 3.9	53.6 ± 5.2†
HOMA-IR	1.38 ± 0.14	2.60 ± 0.32*
Creatinine (μmol/l)	76.0 ± 3.6	77.0 ± 4.7
Prior myocardial infarction	9 (52.9)	8 (47.1)
Angina pectoris	5 (29.4)	4 (23.5)
Smoking	8 (47.1)	5 (29.4)
Medication		
ACE inhibitors	6 (35.3)	9 (52.9)
Angiotensin II receptor blockers	8 (47.1)	7 (41.2)
Calcium channel blockers	4 (23.5)	8 (47.1)
β-Blockers	5 (29.4)	8 (47.1)
α-Blockers	1 (5.9)	2 (11.8)
Diuretics	2 (11.8)	4 (23.5)
Nitrates/nicorandil	10 (58.8)	9 (52.9)
Antiplatelet drugs	13 (76.5)	15 (88.2)
Statins	7 (41.2)	6 (35.3)
Insulin	—	6 (35.3)
Sulfonylurea agents	—	8 (47.1)
α-Glucosidase inhibitors	—	3 (17.6)

Data are means ± SE or *n* (%). **P* < 0.01 vs. nondiabetic subjects. †*P* < 0.05 vs. nondiabetic subjects.

$r = -0.48$, $P < 0.01$), and HOMA-IR ($r = -0.60$, $P < 0.01$; $r = -0.55$, $P < 0.01$).

The total stenosis score was significantly higher in the diabetic patients than in the nondiabetic patients (Fig. 2). The

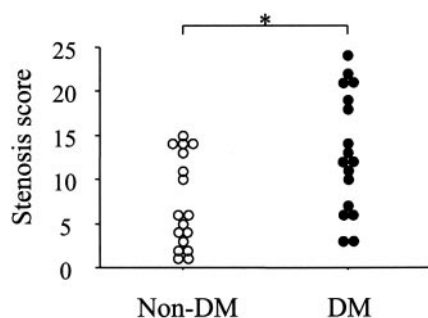


Figure 2—Total stenosis scores in nondiabetic and diabetic patients. The difference between two unpaired variables was analyzed by the Mann-Whitney U test. **P* < 0.05.

total stenosis score showed a significant positive correlation with the transcatheter gradient of adiponectin from aortic root to coronary sinus in the nondiabetic patients (Fig. 3A) but not in the diabetic patients (Fig. 3B). Regardless of diabetes, the total stenosis score was not correlated with the level of adiponectin in the aortic root or coronary sinus.

CONCLUSIONS— Serum adiponectin was significantly lower in the coronary sinus than in the aortic root in patients without diabetes, suggesting that adiponectin is used across the heart. We demonstrated for the first time that utilization of adiponectin across the heart occurred in patients without diabetes. Adiponectin has been reported to have the ability to bind to collagens I, III, and V, which are abundant in the vascular intima, and to accumulate in the vascular

subendothelial space when the endothelial barrier is damaged (14). It has also been suggested that adiponectin targets injured atherosclerotic plaque, resulting in its consumption in the circulating plasma (20). Adiponectin inhibits the expression of vascular cell adhesion molecule-1, endothelial leukocyte adhesion molecule-1, and intracellular adhesion molecule-1, which are detected in human atherosclerotic lesions, through inactivation of TNF- α (6). Adiponectin also suppresses the activity of human monocyte macrophages, including TNF- α production and foam cell formation (15). Therefore, adiponectin may be involved in the inflammation- and tissue-repairing processes in vascular walls. We found a positive correlation between the transcatheter extraction of adiponectin and the stenosis score in the coronary artery in patients without diabetes, indicating that the transcatheter extraction of adiponectin may, at least partly, prevent atherosclerosis in the coronary artery. Moreover, there was no significant difference between adiponectin levels in the aortic root and the coronary sinus in patients with diabetes, suggesting that impaired utilization of adiponectin in patients with diabetes may be related to the development of atherosclerosis.

Recently, Yamauchi et al. (21) reported the cloning of complementary DNAs encoding adiponectin receptor-1 and -2 (AdipoR1 and AdipoR2). AdipoR1 is expressed ubiquitously, with the most abundant expression occurring in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver. It has been reported that AdipoR1 is a high-affinity receptor for globular adiponectin and also a low-affinity receptor for full-length adiponectin and that AdipoR2 is an intermediate-affinity receptor for full-length and globular adiponectin. Although there is no information about the role of adiponectin in the heart, AdipoR1 and AdipoR2 have been shown to be expressed in the heart. Taken together, our results suggest that one mechanism of impaired utilization of adiponectin in patients with diabetes is the decreased receptor-binding ability of adiponectin in cardiomyocytes. It has been reported that hydroxylation and glycosylation of the four lysines (residues 68, 71, 80, and 104) in the collagenous domain of adiponectin might be critical for the three-dimensional structure required for the full

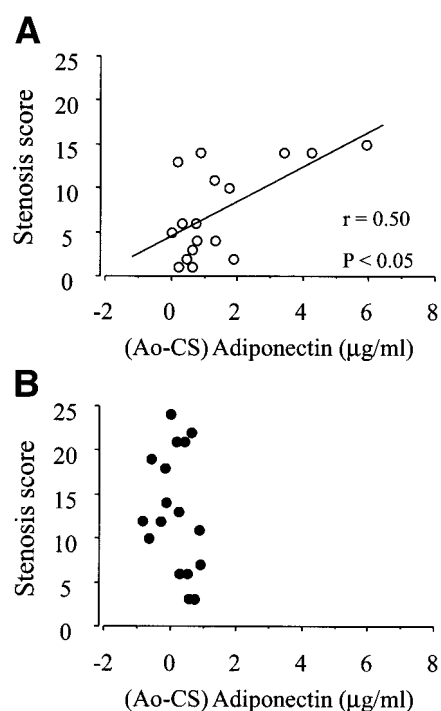


Figure 3—Correlation between stenosis score and transcardiac gradient of adiponectin from the aortic root (Ao) to coronary sinus (CS) [(Ao-CS)Adiponectin] in the nondiabetic (A) and the diabetic (B) patients. The correlation between the two variables was analyzed by Spearman's rank test.

biological activity of the adiponectin molecule and that the ability of adiponectin is significantly attenuated when the four glycosylated lysines are substituted with arginines (22). The decreased receptor-binding ability of adiponectin may be due to impaired hydroxylation or glycosylation of the four lysine residues of adiponectin in the diabetic patients. Since it has also been reported that adiponectin increases the amounts of phosphorylation of AMP kinase, acetyl coenzyme A carboxylase and p38 mitogen-activated protein kinase, peroxisome proliferator-activated receptor- α ligand activity, fatty acid oxidation, and glucose uptake mediated by adiponectin receptors AdipoR1 and AdipoR2 (21,23), another possible mechanism is impaired signal transduction of second messengers.

Most of the patients in this study were being treated with drugs. Medications, except for thiazolidinediones and glimepiride, were not restricted in the present study. The blockage of the renin-angiotensin system by ACE inhibitors or angiotensin II receptor blockers has been

shown to increase adiponectin concentration (9), although the degree of increase (~15–30% increase) is small compared with that induced by thiazolidinediones (~100–200% increase) or glimepiride (~50% increase) (17–19). In the present study, there was no difference between the proportions of subjects being treated with ACE inhibitors and angiotensin II receptor blockers: a total of 14 (82.3%) of 17 nondiabetic patients and 16 (94.1%) of 17 diabetic patients. Additional studies using subjects not on medication are needed.

One limitation of this study was the small number of subjects enrolled. Prospective studies using a larger number of subjects are needed to determine whether impaired utilization of adiponectin is a major determinant of subsequent development of atherosclerosis. Furthermore, it has been shown that adiponectin concentration is sex related, being higher in female than in male subjects (8,24). Although we enrolled only male subjects in the present study to adjust for confounding factors, it is important to confirm our findings by studies using female subjects. The transcardiac gradient of adiponectin is thought to be indirect evidence of accumulation of adiponectin in the coronary artery and binding to adiponectin receptors in the heart. However, our data may be clinically important because of consistency with results of previous studies indicating that coronary artery atherosclerosis is one of the important targets for adiponectin. We cannot deny that significant stenosis and previous infarct areas might reduce coronary flow and perfusion. To verify the effect of adiponectin extraction under such complex conditions, further studies are needed. Inability to measure the total amount of adiponectin extraction because the coronary sinus flow was not measured is also a limitation of this study.

In conclusion, type 2 diabetic patients not only have a decreased level of adiponectin in the basal state compared with nondiabetic patients but also have impaired utilization of adiponectin in the coronary artery and/or the heart, which may promote the development of atherosclerosis in diabetes.

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