Tohoku J. Exp. Med., 2007, **213**, 71-77

# Coronary Artery Bypass Grafting Ameliorates the Decreased Plasma Adiponectin Level in Atherosclerotic Patients

Zeynep Eyileten,[1] Mahmut Ilker Yilmaz,[2] Kaan Kaya,[3] A. Ruchan Akar,[1] Dogan Kahraman,[1] Sezin Bingol,[4] Adnan Uysalel[1] and Umit Ozyurda[1]

[1]Department of Cardiovascular Surgery, Ankara University School of Medicine, Ankara, Turkey
[2]Department of Nephrology, Gulhane School of Medicine, Ankara, Turkey
[3]Department of Cardiovascular Surgery, Umut Hospital, Ankara, Turkey
[4]Department of Biochemistry, Numune Hospital, Ankara, Turkey

Eyileten, Z., Yilmaz, M.I., Kaya, K., Akar, A.R., Kahraman, D., Bingol, S., Uysalel, A. and Ozyurda, U. *Coronary Artery Bypass Grafting Ameliorates the Decreased Plasma Adiponectin Level in Atherosclerotic Patients*. Tohoku J. Exp. Med., 2007, **213** (1), 71-77 —— Adiponectin functions as an anti-inflammatory and anti-atherogenic factor, and the decreased plasma adiponectin is a risk factor for coronary disease. The aim of this study was to determine the changes in plasma levels of adiponectin, a potential parameter for atherosclerosis, in patients underwent surgical revascularization. We included forty patients with atherosclerosis (age, 58 ± 9 years; body mass index [BMI] 26.93 ± 2.3 kg/m$^2$) undergoing coronary artery bypass grafting (CABG). Control group consisted of 40 healthy volunteers, matched for age, gender and BMI (age, 56 ± 6 years; BMI, 26.78 ± 2.3 kg/m$^2$). We measured various parameters, including high sensitive C-reactive protein (hsCRP), homeostasis model assessment-insulin resistance (HOMA-IR) indexes, and adiponectin. The baseline profile of the patients before CABG showed higher levels of serum hsCRP (13.15 ± 2.40 mg/l vs 3.97 ± 1.07 mg/l) and HOMA-IR (1.86 ± 0.30 vs 1.26 ± 0.33) and lower plasma adiponectin levels (7.02 ± 2.01 $\mu$g/ml vs 25.46 ± 3.9 $\mu$g/ml), compared to controls ($p < 0.001$ for each parameter). Plasma adiponectin level was increased one month after CABG from the baseline level to 8.67 ± 2.05 $\mu$g/ml ($p < 0.001$), although the level was still lower than the control value. Thus, postoperative adiponectin level might be helpful for evaluating the progression of atherosclerosis. Moreover, CABG significantly decreased hsCRP to 7.25 ± 1.89 mg/l and HOMA-IR to 1.59 ± 0.33, although these levels were higher than the controls. These results suggest that CABG decreases the cardiac risk factors in atherosclerotic patients. ———— adiponectin; coronary artery bypass grafting; insulin sensitivity; oxidative stress
© 2007 Tohoku University Medical Press

Adiponectin, an adipocyte-derived protein, was discovered through complementary DNA (cDNA) cloning techniques in the mid-1990s (Maeda et al. 1996). Previous studies indicated that adiponectin has a protective effect against atherosclerosis, due to anti-inflammatory and

Received April 6, 2007; revision accepted for publication July 31, 2007.
Correspondence: Zeynep Eyileten, M.D., Department of Cardiovascular Surgery, Heart Center, Ankara University School of Medicine, Dikimevi, Ankara, Turkey.
e-mail: zeynepb1965@yahoo.com

anti-atherogenic features (Arita et al. 2002; Matsuda et al. 2002) and may be a predictor of cardiovascular events (Ouchi et al. 1999, 2001, 2003b). Circulating adiponectin levels were found to be associated with other cardiovascular risk factors, including hyperlipidemia, high blood pressure and C-reactive protein (CRP) levels (Ouchi et al. 2003b, c; Iwashima et al. 2004).

Hypoadiponectinemia has been demonstrated in patients with obesity, type 2 diabetes mellitus, insulin resistance and coronary artery disease (Shimabukuro et al. 2003; Yilmaz et al. 2004). Endothelial dysfunction is fundamental for the development, progression, and manifestation of atherosclerosis (Davignon and Ganz 2004). Adiponectin appears to modulate endothelial function (Chen et al. 2003; Ouchi et al. 2003a).

Adiponectin exhibits favorable effects on vascular remodeling through modulation of signaling cascades in cells of the vasculature. Well-developed coronary collateral arteries in patients with coronary artery disease limit myocardial infarct size with prevention of ventricular aneurysm formation, improve ventricular function, preserve viability, and reduce future cardiovascular events (Ishihara et al. 2005). In a prospective study, men with high plasma adiponectin levels without previous coronary vascular disease were found to have lower risk of myocardial infarction. This association was independent of traditional cardiovascular risk factors (Pischon et al. 2004).

Adiponectin ameliorates cardiac injury through modulation of pro-survival reactions, cardiac energy metabolism and inhibition of hypertrophic remodeling (Shibata et al. 2005). Adiponectin also inhibits apoptosis in cardiac myocytes and fibroblasts that are exposed to hypoxia-reoxygenation stress and has a protective action against myocardial ischemia-reperfusion injury (Shibata et al. 2004).

We therefore designed the present study to investigate the changes in plasma level of adiponectin, following surgical revascularization.

## Subjects and Methods

The subjects were selected from 300 patients who received a medical check-up at the outpatient clinics of Cardiovascular Surgery Department of Ankara University, where 1,500 cases of open heart surgery are being performed annually. Forty patients and 40 healthy volunteers were included in this study. Patients were evaluated by standard physical examination, chest x-ray, baseline electrocardiogram, two-dimensional echocardiography, and routine clinical laboratory tests, including liver and kidney function tests.

The exclusion criteria were as follows: hypertension (systolic blood pressure $\geqq$ 140 mmHg and/or diastolic blood pressure $\geqq$ 90 mmHg); body mass index (BMI) > 30 kg/m$^2$; elevated liver enzymes (aspartate transaminase [AST] or alanine transaminase [ALT] levels $\geqq$ 40 U/l); renal failure (serum creatinine levels $\geqq$ 1.3 mg/dl). Patients with diabetes mellitus, and those receiving $\beta$-blokers, thiazolidinedione derivatives, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II type-2 (AT2)-receptor-blockers, statins, and/or fibrates were also excluded.

Forty patients with atherosclerotic heart disease underwent coronary artery bypass grafting (CABG) under cardiopulmonary bypass. One month after the operation the blood sampling for the same laboratory examination was repeated. None of the patients were under calorie restricted diet before and for one month after the operation. Diet of 1,600-1,800 calorie/day was applied. There was no weight loss in any patient following the operation. And also none of the patients received any antilipid medication until one month after the operation.

Forty healthy volunteers (mean age, 56 ± 6, BMI, 26.78 ± 2.3), selected from the check-up program, were enrolled into the study as control group. They underwent comprehensive physical and laboratory evaluation to ascertain that they had no hypertension, metabolic, hepatic or renal disease and diabetes. Written informed consent was obtained from all participants. The study protocol was approved by the local ethics committee of Ankara University School of Medicine.

### *Laboratory procedures*

A morning fasting (12 hrs) blood sample was drawn from the antecubital vein from all subjects. We separated the blood sample into plasma and cells through centrifugation. The plasma and serum samples were stored at –20°C, until biochemical analyses and adiponectin assay were performed. Besides the metabolic panel, plasma adiponectin concentrations, basal insulin value and insulin resistance score homeostasis model assessment-

insulin resistance (HOMA-IR) were measured. Plasma adiponectin concentrations were measured in duplicate by radioimmunoassay (RIA) (Human Adiponectin RIA Kit; Linco Research, Inc., St. Charles, MO, USA). All samples were run in the same assay.

*Biomarker assessment*

The detection interval for high sensitive C reactive protein (hsCRP) is 0.05-35 mg/l. Serum samples were diluted with a ratio of 1/101 with the diluents solution. Calibrators, kit controls and serum samples were all added on each micro-well with an incubation period of 30 min. After 3 washing intervals 100 $\mu$l enzyme conjugate (peroxidase labeled anti-CRP) was added on each micro well for additional 15 min incubation in room temperature in dark. The reaction was stopped with a stop solution and photometric measurement was performed at the 450 nm wavelength. The amount of serum samples was calculated as mg/l with a graphic that was made by noting the absorbance levels of the calibrators.

*Biochemical analysis*

Serum total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides, plasma glucose, serum urea, creatinine and total protein were determined by an enzymatic colorimetric method with an Olympus AU 600 auto analyzer using reagents from Olympus Diagnostics, GmbH (Hamburg, Germany). Low-density lipoprotein (LDL) cholesterol was calculated by the formula of Friedewald et al. (1972).

The serum basal insulin value was determined by the coated tube method (D.P.C., Los Angeles, CA, USA). In particular, a HOMA-IR was computed with the formula: HOMA-IR = fasting plasma glucose (mg/dl) × immunoreactive insulin ($\mu$U/ml)/405.

*Data analysis*

All statistical analyses were performed with Statistical Package for the Social Sciences (SPSS 10.1). Results are reported as the means ± S.D. The Levene's test was used to evaluate the distribution characteristics of variables. Differences between study and control groups were recorded by the *t*-test, Mann Whitney's U-test and chi-square test. The relationship between variables was analyzed by Pearson's correlation. Differences and correlations were considered significant at $p < 0.05$.

## Results

Clinical and laboratory data of the patient and control groups are shown in Table 1. There was a statistically significant difference in HOMA-IR indexes between the patients and controls (1.86 ± 0.30 vs 1.26 ± 0.33; $p < 0.001$). Basal plasma adiponectin levels were significantly lower in patients than the control subjects (7.02 ±

Table 1. Comparison of clinical and laboratory characteristics between two groups.

| | Patients ($n = 40$) | Controls ($n = 40$) | $p$ * |
|---|---|---|---|
| Age (yr) | 58 ± 9 | 56 ± 6 | 0.203† |
| Sex (M/F) | 20/20 | 20/20 | 0.776‡ |
| Total Cholesterol (mg/dl) | 153.50 ± 33.93 | 162.37 ± 17.37 | 0.301† |
| Triglyceride (mg/dl) | 125.20 ± 34.80 | 119.32 ± 15.14 | 0.292† |
| LDL Cholesterol (mg/dl) | 85.90 ± 29.46 | 92.92 ± 13.47 | 0.113† |
| HDL Cholesterol (mg/dl) | 40.05 ± 9.15 | 42.67 ± 4.44 | 0.150† |
| BMI (kg/m$^2$) | 26.93 ± 2.37 | 26.78 ± 2.32 | 0.756† |
| hsCRP (mg/l) | 13.15 ± 2.40 | 3.97 ± 1.07 | < 0.001† |
| Insulin ($\mu$IU/ml) | 8.54 ± 1.08 | 6.08 ± 1.42 | < 0.001† |
| HOMA-IR | 1.86 ± 0.30 | 1.26 ± 0.33 | < 0.001† |
| FPG (mg/dl) | 88.45 ± 10.84 | 84.40 ± 9.47 | 0.072§ |
| Adiponectin ($\mu$g/ml) | 7.02 ± 2.01 | 25.46 ± 3.91 | < 0.001† |

BMI, body mass index; FPG, fasting plasma glucose; HOMA, homeostasis model assessment. * These variables are matched for in analyses. †*t*-test, §Mann-Whitney's U-test and ‡Chi-Square test.

2.01 $\mu$g/ml vs 25.46 ± 3.91 $\mu$g/ml, $p < 0.001$). Conversely, HOMA-IR and hsCRP levels were higher in the patients compared with the control group both before and after surgery (r = –0.516, $p = 0.001$; r = –0.295, $p = 0.046$, respectively) (r = –0.451, $p = 0.004$; r = –0.488, $p = 0.001$, respectively) (Fig. 1).

There was a statistically significant difference between preoperative and postoperative adiponectin levels (7.02 ± 2.01 $\mu$g/ml; 8.67 ± 2.05 $\mu$g/ml, respectively; $p < 0.001$). Plasma adiponectin levels of all patients were increased following the operation. There was also a significant difference between preoperative and postoperative hsCRP levels (13.15 ± 2.40 mg/l; 7.25 ± 1.89 mg/l, respectively) ($p < 0.001$). One month after the operation, hsCRP was decreased. Furthermore HOMA-IR levels, which reflect insulin resistance, were significantly decreased after the operation (1.86 ± 0.30 vs 1.59 ± 0.33) ($p < 0.001$) (Table 2).

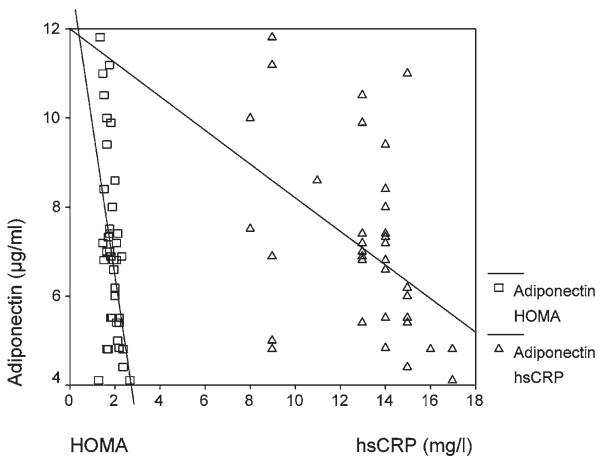



Fig. 1. The relationship between plasma adiponectin levels with both HOMA-IR and hsCRP levels before the operation.

## Discussion

The results of our study suggest that patients with coronary artery disease have lower adiponectin levels compared with control subjects. One month after surgical revascularization, significant increase in plasma adiponectin concentration and reduction in hsCRP levels and HOMA indexes were detected in the patients.

Decreased concentrations of adiponectin may be associated with a higher risk of coronary artery disease (Shimabukuro et al. 2003). Plasma adiponectin levels were recently shown to be declined after myocardial ischemia-reperfusion injury (Kojima et al. 2003). Ischemia-reperfusion injury syndrome commonly occurs after achievement of adequate coronary recirculation (Bulkley and Hutchins 1977; Ferreira et al. 1990). Besides regional reperfusion injury, as a consequence of blood flow to ischemic areas of the myocardium via coronary bypass grafts, the use of cardiopulmonary bypass with cardioplegic arrest introduces a mechanism of further myocardial injury caused by global reperfusion. Reperfusion of ischemic myocardium is necessary for the salvage of cells and cardiac function. It has been suggested that myocardial reperfusion injury occurs during the early period after reperfusion (Romaschin et al. 1987). The short half-life of antioxidants is well-known and they only seem to be generated in the

Table 2. Comparison of cardiovascular risk factors in patients before and after surgery.

| | Control | Preoperative | Postoperative |
|---|---|---|---|
| hsCRP (mg/l) | 3.97 ± 1.07 | 13.15 ± 2.40* | 7.25 ± 1.89**,# |
| Insulin ($\mu$IU/ml) | 6.08 ± 1.42 | 8.54 ± 1.08* | 7.42 ± 1.29**,# |
| HOMA-IR | 1.26 ± 0.33 | 1.86 ± 0.30* | 1.59 ± 0.33**,# |
| Adiponectin ($\mu$g/ml) | 25.46 ± 3.91 | 7.02 ± 2.01* | 8.67 ± 2.05**,# |

* *t*-test was used for the comparison of patients between control and preoperative ($p < 0.001$).
** *t*-test was used for the comparison of patients between control and postoperative ($p < 0.001$).
# *t*-test was used for the comparison of patients between preoperative and postoperative ($p < 0.001$).

early stage of reperfusion (Hashimoto et al. 1994).

Extracorporeal circulation could be another potential sources of oxidative stress. The interaction between the human body and the cardiopulmonary bypass apparatus is very complex and involves several oxidative stress sources (Edmunds 1998), which may increase the activity of antioxidants. Conversion of pulsatile flow to laminar flow, direct contact of the blood with the artificial bypass surface, global cold ischemia with cardioplegic protection, hypothermia, activation of some proteins by the operation itself, aortic cross-clamping time, and myocardial reperfusion affect the magnitude of inflammatory response comprising the activation of endothelial cells as well as leucocytes (Hiesmayr et al. 1999; Rothenburger et al. 2002), platelets (Rothenburger et al. 2002), the complement system (Chenoweth et al. 1981) and the coagulation cascade (Despotis and Joist 1999). The production of oxygen free radicals and adhesion molecules and alterations in intracellular $Ca^{2+}$ handling appear to cause further damage in ischemic myocardium (Braunwald and Kloner 1985; Kloner 1993; Sheridan et al. 1996). On the other hand, the trauma derived from the surgical procedure -sternotomy itself also has pro inflammatory effect. The activation of inflammatory system, during either vascular or noncardiac thoracic surgery, was less marked than that of cardiopulmonary bypass (Rothenburger et al. 2002).

Inflammation contributes to cardiovascular disease and hsCRP is a reliable marker of systemic inflammation (Blake and Ridker 2001), while adiponectin is an adipocyte-derived hormone that shows anti-inflammatory and antiatherogenic effects (Qi et al. 2006). Emerging evidence suggests that hsCRP may be directly involved in atherogenesis and that arterial plaque can produce hsCRP (Blake and Ridker 2002). Recently the reciprocal association of adiponectin and hsCRP levels in both human plasma and adipose tissue was demonstrated (Ouchi et al. 2003b; Baker et al. 2006). Thus adiponectin may directly or indirectly affect the hsCRP levels in plasma and adipose tissue by modulating the inflammatory cascades. The present study also confirmed this association. Furthermore, we demonstrated the significant reductions in hsCRP levels and increases in adiponectin levels at 1-month follow-up after surgery. It is not unreasonable to speculate that successful revascularization may be responsible for these changes. In this context, high plasma adiponectin levels have been recognized to be associated with a lower risk of myocardial infarction (Pischon et al. 2004).

*Study limitations*

This study has several limitations. First, our protocol does not contemplate measurements of endothelial function, which could provide useful elements for better interpretation of our data. Second, levels of adiponectin should have been checked at different time points, during the course of postoperative period (1st day, 30th day, 3 months later). Third, it is unclear why adiponectin increases following coronary bypass surgery operations. Further investigation is necessary to clarify this question.

In conclusion, plasma adiponectin may be a useful non-invasive predictor of coronary endothelial dysfunction. Higher plasma adiponectin levels may have a protective role against initiation and progression of atherosclerosis in either native coronary arteries or vascular grafts in atherosclerotic patients underwent surgical revascularization.

## References

Arita, Y., Kihara, S., Ouchi, N., Maeda, K., Kuriyama, H., Okamoto, Y., Kumada, M., Hotta, K., Nishida, M., Takahashi, M., Nakamura, T., Shimomura, I., Muraguchi, M., Ohmoto, Y., Funahashi, T. & Matsuzawa, Y. (2002) Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation*, **105**, 2893.

Baker, A.R., Silva, N.F., Quinn, D.W., Harte, A.L., Pagano, D., Bonser, R.S., Kumar, S. & McTernan, P.G. (2006) Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease. *Cardiovasc. Diabetol.*, **5**, 1-7.

Blake, G.J. & Ridker, P.M. (2001) Novel clinical markers of vascular wall inflammation. *Circ. Res.*, **89**, 763-771.

Blake, G.J. & Ridker, P.M. (2002) Inflammatory bio-markers and cardiovascular risk prediction. *J. Intern. Med.*, **252**, 283-294.

Braunwald, E. & Kloner, R.A. (1985) Myocardial reperfusion: a double edge sword? *J. Clin. Invest.*, **76**, 1713-1719.

Bulkley, B.H. & Hutchins, G.M. (1977) Myocardial conse-

quences of coronary artery bypass grafting surgery, the paradox of necrosis in areas of revascularization. *Circulation*, **56**, 906-913.

Chen, H., Montagnani, M., Funahashi, T., Shimomura, I. & Michael, J.Q. (2003) Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J. Biol. Chem.*, **107**, 671-674.

Chenoweth, D.E., Cooper, S.W., Hugli, T.E., Stewart, R.W., Blackstone, E.H. & Kirklin, J.W. (1981) Complement activation during cardiopulmonary bypass: evidence for generation of C3a and C5a anaflatoxins. *N. Engl. J. Med.*, **304**, 497-503.

Davignon, J. & Ganz, P. (2004) Role of endothelial dysfunction in atherosclerosis. *Circulation*, **109**, 11127-11132.

Despotis, G.J. & Joist, J.H. (1999) Anticoagulation reversal with cardiac surgery involving cardiopulmonary bypass: an update. *J. Cardiothorac. Vasc. Anesth.*, **13**, 18-29.

Edmunds, L.H., Jr. (1998) Inflammatory response to cardiopulmonary bypass. *Ann. Thorac. Surg.*, **66**, 12-16.

Ferreira, R., Burgos, M., Milei, J., Llesuy, S., Molteni, L., Hourquebie, H. & Boveris, A. (1990) Effect of supplementing cardioplegic solution with deferoxamine on reperfused human myocardium. *J. Thorac. Cardiovasc. Surg.*, **100**, 708-714.

Friedewald, W.T., Levy, R.I. & Fredrickson, D.S. (1972) Estimation of the distribution of low density lipoprotein cholesterol in plasma, without the use of a preparative centrifuge. *Clin. Chem.*, **18**, 499-502.

Hashimoto, Y., Itoh, K., Nishida, K., Okano, T., Miyazawa, Y. & Okinaga, K. (1994) Rapid superoxide production by endothelial cells and their injury upon reperfusion. *J. Surg. Res.*, **57**, 693-697.

Hiesmayr, M.J., Splitter, A., Lassnigg, A., Berger, R., Laufer, G., Kocher, A., Artemiou, O., Boltz-Nitulescu, G. & Roth, E. (1999) Alterations in the number of circulating leukocytes, phenotype of monocyte and cytokine production in patients undergoing cardiothoracic surgery. *Clin. Exp. Immunol.*, **115**, 315-323.

Ishihara, M., Inoue, I., Kawagoe, T., Shimatani, Y., Kurisu, S., Hata, T., Mitsuba, N., Kisaka, T., Nakama, H. & Kijima, Y. (2005) Comparison of the cardioprotective effect of prodromal angina pectoris and collateral circulation in patients with a first anterior wall acute myocardial infarction. *Am. J. Cardiol.*, **95**, 622-625.

Iwashima, Y., Katsuya, T., Ishikawa, K., Ouchi, N., Ohishi, M., Sugimoto, K., Fu, Y., Motone, M., Yamamoto, K., Matsuo, A., Ohashi, K., Kihara, S., Funahashi, T., Rakugi, H., Matsuzawa, Y. & Ogihara, T. (2004) Hypoadiponectinemia is an independent risk factor for hypertension. *Hypertension,* **43**, 1318-1323.

Kloner, R.A. (1993) Does reperfusion injury exist in humans? *J. Am. Coll. Cardiol.*, **32**, 537-545.

Kojima, S., Funahashi, T., Sakamoto, T., Miyamoto, S., Soejima, H., Hokamaki, J., Kajiwara, I., Sugiyama, S., Yoshimura, M., Fujimoto, K., Miyao, Y., Suefuji, H., Kitagawa, A., Ouchi, N., Kihara, S., Matsuzawa, Y. & Ogawa, H. (2003) The variation of plasma concentrations of a novel, adipocyte derived protein, adiponectin, in patients with acute myocardial infarction. *Heart,* **89**, 667-668.

Maeda, K., Okubo, K., Shimomura, I., Funahashi, T., Matsuzawa, Y. & Matsubara, K. (1996) cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem. Biophys. Res. Commun.*, **221**, 286-289.

Matsuda, M., Shimomura, I., Sata, M., Arita, Y., Nishida, M., Maeda, N., Kumada, M., Okamoto, Y., Nagaretani, H., Nishizawa, H., Kishida, K., Komura, R., Ouchi, N., Kihara, S., Nagai, R., Funahashi, T. & Matsuzawa, Y. (2002) Role of adiponectin in preventing vascular stenosis: The missing link of adipo-vascular axis. *J. Biol. Chem.*, **277**, 37487-37491.

Ouchi, N., Kihara, S., Arita, Y., Maeda, K., Kuriyama, H., Okamoto, Y., Hotta, K., Nishida, M., Takahashi, M., Nakamura, T., Yamashita, S., Funahashi, T. & Matsuzawa, Y. (1999) Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation*, **100**, 2473-2476.

Ouchi, N., Kihara, S., Arita, Y., Nishida, M., Matsuyama, A., Okamoto, Y., Ishigami, M., Kuriyama, H., Kishida, K., Nishizawa, H., Hotta, K., Muraguchi, M., Ohmoto, Y., Yamashita, S., Funahashi, T. & Matsuzawa, Y. (2001) Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation*, **103**, 1057-1063.

Ouchi, N., Ohishi, M., Kihara, S., Funahashi, T., Nakamura, T., Nagaretani, H., Kumada, M., Ohashi, K., Okamoto, Y., Nishizawa, H., Kishida, K., Maeda, N., Nagasawa, A., Kobayashi, H., Hiraoka, H., Komai, N., Kaibe, M., Rakugii, H., Ogihara, T. & Matsuzawa, Y. (2003a) Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension*, **42**, 231-234.

Ouchi, N., Kihara, S., Funahashi, T., Nakamura, T., Nishida, M., Kumada, M., Okamoto, Y., Ohashi, K., Nagaretani, H., Kishida, K., Nishizawa, H., Maeda, N., Kobayashi, H., Hiraoka, H. & Matsuzawa, Y. (2003b) Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation*, **107**, 671-674.

Ouchi, N., Kihara, S., Funahashi, T., Matsuzawa, Y. & Walsh, K. (2003c) Obesity, adiponectin and vascular inflammatory disease. *Curr. Opin. Lipidol.*, **14**, 561-566.

Pischon, T., Girman, C.J., Hotamisligil, G.S., Rifai, N., Hu, F.B. & Rimm, E.B. (2004) Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA*, **291**, 1730-1737.

Qi, L., Doria, A., Manson, J.E., Meigs, J.B., Hunter, D., Mantzoros, C.S. & Hu, F.B. (2006) Adiponectin genetic variability, plasma adiponectin, and cardiovascular risk in patients with type 2 diabetes. *Diabetes*, **55**, 1512-1516.

Romaschin, A.D., Rebeyka, I., Wilson, G.J. & Mickle, D.A. (1987) Conjugated dienes in ischemic and reperfused myocardium an in vivo chemical signature of oxygen free radical mediated injury. *J. Mol. Cell. Cardiol.*, **19**, 289-302.

Rothenburger, M., Trosch, F., Markewitz, A., Berendes, E., Schmid, C., Scheld, H. & Tjan, T.D.T. (2002) Leucocyte activation and phagocytotic activity in cardiac surgery and infection. *Cardiovasc. Surg.*, **10**, 470-475.

Sheridan, F.M., Cole, P.G. & Ramage, D. (1996) Leucocyte adhesion to the coronary microvasculature during ischemia and reperfusion in an in vivo canine model. *Circulation,* **93**, 1784-1787.

Shibata, R., Ouchi, N., Ito, M., Kihara, S., Shiojima, I., Pimentel, D.R., Kumada, M., Sato, K., Schiekofer, S., Ohashi, K., Funahashi, T., Colucci, W.S. & Walsh, K. (2004) Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nat. Med.*, **10**, 1384-1389.

Shibata, R., Sato, K., Pimentel, D.R., Takemura, Y., Kihara, S.,

Ohashi, K., Funahashi, T., Ouchi, N. & Walsh, K. (2005) Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK and Cox-2-dependent mechanisms. *Nat. Med.*, **11**, 1096-1103.

Shimabukuro, M., Higa, N., Asahi, T., Oshiro, Y., Takasu, N., Tagawa, T., Ueda, S., Shimomura, I., Funahashi, T. & Matsuzawa, Y. (2003) Hypoadiponectinemia is closely linked to endothelial dysfunction in men. *J. Clin. Endocrinol. Metab.*, **88**, 3236-3240.

Yilmaz, M.I., Sonmez, A., Acikel, C., Celik, T., Bingol, N., Pinar, M., Bayraktar, Z. & Ozata, M. (2004) Adiponectin may play a role in the pathogenesis of diabetic retinopathy. *Eur. J. Endocrinol.*, **151**, 135-140.