71. Schulze MB, Rimm EB, Shai I, Rifai N, Hu FB (2004) Relationship between adiponectin and glycemic control, blood lipids, and inflammatory markers in men with type 2 diabetes. Diabetes Care 27:1680–1687
72. Neumeier M, Sigruener A, Eggenhofer E, Weigert J, Weiss TS, Schaeffler A, Schlitt HJ, Aslanidis C, Piso P, Langmann T, Schmitz G, Scholmerich J, Buechler C (2007) High molecular weight adiponectin reduces apolipoprotein B and E release in human hepatocytes. Biochem Biophys Res Commun 352:543–548
73. Choi KM, Lee J, Lee KW, Seo JA, Oh JH, Kim SG, Kim NH, Choi DS, Baik SH (2004) Serum adiponectin concentrations predict the developments of type 2 diabetes and the metabolic syndrome in elderly Koreans. Clin Endocrinol (Oxf) 61:75–80
74. Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, Nagai M, Matsuzawa Y, Funahashi T (2004) Adiponectin as a biomarker of the metabolic syndrome. Circ J 68:975–981
75. Ohashi K, Ouchi N, Kihara S, Funahashi T, Nakamura T, Sumitsuji S, Kawamoto T, Matsumoto S, Nagaretani H, Kumada M, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Hiraoka H, Iwashima Y, Ishikawa K, Ohishi M, Katsuya T, Rakugi H, Ogihara T, Matsuzawa Y (2004) Adiponectin I164T mutation is associated with the metabolic syndrome and coronary artery disease. J Am Coll Cardiol 43:1195–1200
76. Engeli S, Feldpausch M, Gorzelniak K, Hartwig F, Heintze U, Janke J, Mohlig M, Pfeiffer AF, Luft FC, Sharma AM (2003) Association between adiponectin and mediators of inflammation in obese women. Diabetes 52:942–947
77. 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 (2003) Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation 107:671–674
78. Krakoff J, Funahashi T, Stehouwer CD, Schalkwijk CG, Tanaka S, Matsuzawa Y, Kobes S, Tataranni PA, Hanson RL, Knowler WC, Lindsay RS (2003) Inflammatory markers, adiponectin, and risk of type 2 diabetes in the Pima Indian. Diabetes Care 26:1745–1751
79. Matsubara M, Namioka K, Katayose S (2003) Decreased plasma adiponectin concentrations in women with low-grade C-reactive protein elevation. Eur J Endocrinol 148:657–662
80. Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G (2003) Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. Diabetes 52:1779–1785
81. Yamamoto K, Kiyohara T, Murayama Y, Kihara S, Okamoto Y, Funahashi T, Ito T, Nezu R, Tsutsui S, Miyagawa JI, Tamura S, Matsuzawa Y, Shimomura I, Shinomura Y (2005) Production of adiponectin, an anti-inflammatory protein, in mesenteric adipose tissue in Crohn's disease. Gut 54:789–796
82. Senolt L, Pavelka K, Housa D, Haluzik M (2006) Increased adiponectin is negatively linked to the local inflammatory process in patients with rheumatoid arthritis. Cytokine 35:247–252
83. Kelesidis I, Kelesidis T, Mantzoros CS (2006) Adiponectin and cancer: a systematic review. Br J Cancer 94:1221–1225
84. Housa D, Housova J, Vernerova Z, Haluzik M (2005) Adipocytokines and cancer. Physiol Res 55:233–244
85. Petridou E, Mantzoros C, Dessypris N, Koukoulomatis P, Addy C, Voulgaris Z, Chrousos G, Trichopoulos D (2003) Plasma adiponectin concentrations in relation to endometrial cancer: a case-control study in Greece. J Clin Endocrinol Metab 88:993–997
86. Dal Maso L, Augustin LS, Karalis A, Talamini R, Franceschi S, Trichopoulos D, Mantzoros CS, La Vecchia C (2004) Circulating adiponectin and endometrial cancer risk. J Clin Endocrinol Metab 89:1160–1163
87. Cust AE, Kaaks R, Friedenreich C, Bonnet F, Laville M, Lukanova A, Rinaldi S, Dossus L, Slimani N, Lundin E, Tjonneland A, Olsen A, Overvad K, Clavel-Chapelon F, Mesrine S, Joulin V, Linseisen J, Rohrmann S, Pischon T, Boeing H, Trichopoulos D, Trichopoulou A, Benetou V, Palli D, Berrino F, Tumino R, Sacerdote C, Mattiello A, Quiros JR, Mendez MA, Sanchez MJ, Larranaga N, Tormo MJ, Ardanaz E, Bueno-de-Mesquita HB, Peeters PH, van Gils CH, Khaw KT, Bingham S, Allen N, Key T, Jenab M, Riboli E (2007) Plasma adiponectin levels and endometrial cancer risk in pre- and postmenopausal women. J Clin Endocrinol Metab 92:255–263
88. Mantzoros C, Petridou E, Dessypris N, Chavelas C, Dalamaga M, Alexe DM, Papadiamantis Y, Markopoulos C, Spanos E, Chrousos G, Trichopoulos D (2004) Adiponectin and breast cancer risk. J Clin Endocrinol Metab 89:1102–1107
89. Tworoger SS, Eliassen AH, Kelesidis T, Colditz GA, Willett WC, Mantzoros C, Hankinson SE (2007) Plasma adiponectin concentrations and risk of incident breast cancer. J Clin Endocrinol Metab 92:1510–1516
90. Wei E, Giovannucci E, Fuchs C, Wilett W, Mantzoros CS (2005) Plasma adiponectin levels and the risk of colorectal cancer in men. JNCI 97:1688–1694
91. Otake S, Takeda H, Suzuki Y, Fukui T, Watanabe S, Ishihama K, Saito T, Togashi H, Nakamura T, Matsuzawa Y, Kawata S (2005) Association of visceral fat accumulation and plasma adiponectin with colorectal adenoma: evidence for participation of insulin resistance. Clin Cancer Res 11:3642–3646
92. Ishikawa M, Kitayama J, Kazama S, Hiramatsu T, Hatano K, Nagawa H (2005) Plasma adiponectin and gastric cancer. Clin Cancer Res 11:466–472
93. Goktas S, Yilmaz MI, Caglar K, Sonmez A, Kilic S, Bedir S (2005) Prostate cancer and adiponectin. Urology 65:1168–1172
94. Petridou E, Mantzoros CS, Dessypris N, Dikalioti SK, Trichopoulos D (2006) Adiponectin in relation to childhood myeloblastic leukaemia. Br J Cancer 94:156–160
95. Brakenhielm E, Veitonmaki N, Cao R, Kihara S, Matsuzawa Y, Zhivotovsky B, Funahashi T, Cao Y (2004) Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proc Natl Acad Sci U S A 10:2476–2481
96. Richards JB, Valdes AM, Burling K, Perks UC, Spector TD (2007) Serum adiponectin and bone mineral density in women. J Clin Endocrinol Metab 92:1517–1523
97. Okamoto Y, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M, Igura T, Inui Y, Kihara S, Nakamura T, Yamashita S, Miyagawa J, Funahashi T, Matsuzawa Y (2000) An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. Horm Metab Res 32:47–50
98. 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 man. J Clin Endocrinol Metab 88:3236–3240
99. Tan KC, Xu A, Chow WS, Lam MC, Ai VH, Tam SC, Lam KS (2004) Hypoadiponectinemia is associated with impaired endothelium-dependent vasodilation. J Clin Endocrinol Metab 89:765–769
100. Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ (2003) Adiponectin stimulates production of nitric oxide in vascular endothelial cells. J Biol Chem 278:45021–45026
101. Motoshima H, Wu X, Mahadev K, Goldstein BJ (2004) Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. Biochem Biophys Res Commun 315:264–271
102. 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, Rakugi H, Ogihara T, Matsuzawa Y (2003) Association of hypoadiponectinemia with impaired vasoreactivity. Hypertension 42:231–234
103. 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
104. Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, Maeda K, Nagaretani H, Kishida K, Maeda N, Nagasawa A, Funahashi T, Matsuzawa Y (2004) Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. Circulation 109:2046–2049

105. 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 105:2893–2898
106. Matsuzawa Y. Funahashi T. Kihara S. Shimomura I (2004) Adiponectin and metabolic syndrome. Arterioscler Thromb Vasc Biol 24:29–33
107. Kato H. Kashiwagi H. Shiraga M. Tadokoro S. Kamae T. Ujiie H. Honda S. Miyata S. Ijiri Y. Yamamoto J. Maeda N. Funahashi T. Kurata Y. Shimomura I. Tomiyama Y. Kanakura Y (2006) Adiponectin acts as an endogenous antithrombotic factor. Arterioscler Thromb Vasc Biol 26:224–230
108. Shibata R. Sato K. Pimentel DR. 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
109. Shibata R. Ouchi N. Ito M. Kihara S. Shiojima I. Pimentel DR. Kumada M. Sato K. Schiekofer S. Ohashi K. Funahashi T. Colucci WS. Walsh K (2004) Adiponectin-mediated modulation of hypertrophic signals in the heart. Nat Med 10:1384–1389
110. Combs TP. Berg AH. Obici S. Scherer PE. Rossetti L (2001) Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J Clin Invest 108:1875–1881
111. Yamauchi T. Kamon J. Minokoshi Y. Ito Y. Waki H. Uchida S. Yamashita S. Noda M. Kita S. Ueki K. Eto K. Akanuma Y. Froguel P. Foufelle F. Ferre P. Carling D. Kimura S. Nagai R. Kahn BB. Kadowaki T (2002) Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 8:1288–1295
112. Stefan N. Stumvoll M. Vozarova B. Weyer C. Funahashi T. Matsuzawa Y. Bogardus C. Tataranni PA (2003) Plasma adiponectin and endogenous glucose production in humans. Diabetes Care 26:3315–3319
113. Fruebis J. Tsao TS. Javorschi S. Ebbets-Reed D. Erickson MR. Yen FT. Bihain BE. Lodish HF (2001) Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci U S A 98:2005–2010
114. Yamauchi T. Kamon J. Waki H. Terauchi Y. Kubota N. Hara K. Mori Y. Ide T. Murakami K. Tsuboyama-Kasaoka N. Ezaki O. Akanuma Y. Gavrilova O. Vinson C. Reitman ML. Kagechika H. Shudo K. Yoda M. Nakano Y. Tobe K. Nagai R. Kimura S. Tomita M. Froguel P. Kadowaki T (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 7:941–946
115. Tomas E. Tsao TS. Saha AK. Murrey HE. Zhang CC. Itani SI. Lodish HF. Ruderman NB (2002) Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc Natl Acad Sci U S A 99:16309–16313
116. Maeda N. Shimomura I. Kishida K. Nishizawa H. Matsuda M. Nagaretani H. Furuyama N. Kondo H. Takahashi M. Arita Y. Komuro R. Ouchi N. Kihara S. Tochino Y. Okutomi K. Horie M. Takeda S. Aoyama T. Funahashi T. Matsuzawa Y (2002) Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 8:731–737
117. Yamauchi T. Nio Y. Maki T. Kobayashi M. Takazawa T. Iwabu M. Okada-Iwabu M. Kawamoto S. Kubota N. Kubota T. Ito Y. Kamon J. Tsuchida A. Kumagai K. Kozono H. Hada Y. Ogata H. Tokuyama K. Tsunoda M. Ide T. Murakami K. Awazawa M. Takamoto I. Froguel P. Hara K. Tobe K. Nagai R. Ueki K. Kadowaki T (2007) Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nat Med 13:332–339
118. Wang C. Mao X. Wang L. Liu M. Wetzel MD. Guan KL. Dong LQ. Liu F (2007) Adiponectin sensitizes insulin signaling by reducing p70 S6 kinase-mediated serine phosphorylation of IRS-1. J Biol Chem 282:7991–7996
119. Okamoto Y. Kihara S. Ouchi N. Nishida M. Arita Y. Kumada M. Ohashi K. Sakai N. Shimomura I. Kobayashi H. Terasaka N. Inaba T. Funahashi T. Matsuzawa Y (2002) Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. Circulation 106:2767–2770
120. Ouchi N. Kihara S. Arita Y. Okamoto Y. Maeda K. Kuriyama H. Hotta K. Nishida M. Takahashi M. Muraguchi M. Ohmoto Y. Nakamura T. Yamashita S. Funahashi T. Matsuzawa Y (2000) Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation 102:1296–1301
121. Takemura Y. Ouchi N. Shibata R. Aprahamian T. Kirber MT. Summer RS. Kihara S. Walsh K (2007) Adiponectin modulates inflammatory reactions via calreticulin receptor-dependent clearance of early apoptotic bodies. J Clin Invest 117:375–386
122. Vandivier RW. Ogden CA. Fadok VA. Hoffmann PR. Brown KK. Botto M. Walport MJ. Fisher JH (2002) Role of surfactant proteins A, D, and C1q in the clearance of apoptotic cells in vivo and in vitro: calreticulin and CD91 as a common collectin receptor complex. J Immunol 169:3978–3986
123. Tsao TS. Murrey HE. Hug C. Lee DH. Lodish HF (2002) Oligomerization state-dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30kDa (Acrp30). J Biol Chem 277:29359–29362
124. Nishihara T. Matsuda M. Araki H. Oshima K. Kihara S. Funahashi T. Shimomura I (2006) Effect of adiponectin on murine colitis induced by dextran sulfate sodium. Gastroenterology 131:853–861
125. Kamada Y. Tamura S. Kiso S. Matsumoto H. Saji Y. Yoshida Y. Fukui K. Maeda N. Nishizawa H. Nagaretani H. Okamoto Y. Kihara S. Miyagawa J. Shinomura Y. Funahashi T. Matsuzawa Y (2003) Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. Gastroenterology 125:1796–1807
126. Matsumoto H. Tamura S. Kamada Y. Kiso S. Fukushima J. Wada A. Maeda N. Kihara S. Funahashi T. Matsuzawa Y. Shimomura I. Hayashi N (2006) Adiponectin deficiency exacerbates lipopolysaccharide/-galactosamine-induced liver injury in mice. World J Gastroenterol 12:3352–3358
127. Shore SA. Terry RD. Flynt L. Xu A. Hug C (2006) Adiponectin attenuates allergen-induced airway inflammation and hyperresponsiveness in mice. J Allergy Clin Immunol 118:389–395
128. Peake PW. Shen Y. Campbell LV. Charlesworth JA (2006) Human adiponectin binds to bacterial lipopolysaccharide. Biochem Biophys Res Commun 341:108–115
129. Kubota N. Terauchi Y. Yamauchi T. Kubota T. Moroi M. Matsui J. Eto K. Yamashita T. Kamon J. Satoh H. Yano W. Froguel P. Nagai R. Kimura S. Kadowaki T. Noda T (2002) Disruption of adiponectin causes insulin resistance and neointimal formation. J Biol Chem 277:25863–25866
130. Matsuda M. Shimomura I. Sata M. Arita Y. Nishida M. Maeda N. Kumada M. Okamoto Y. Nagaretani H. Nishizawa H. Kishida K. Komuro 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
131. Yu JG. Javorschi S. Hevener AL. Kruszynska YT. Norman RA. Sinha M. Olefsky JM (2002) The effect of thiazolidinedione on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. Diabetes 51:2968–2974
132. Yang WS. Jeng CY. Wu TJ. Tanaka S. Funahashi T. Matsuzawa Y. Wang JP. Chen CL. Tai TY. Chuang LM (2002) Synthetic peroxisome proliferator-activated receptor-γ agonist, rosiglitazone, increases plasma levels of adiponectin in type 2 diabetic patients. Diabetes Care 25:376–380
133. Tonelli J. Li W. Kishore P. Pajvani UB. Kwon E. Weaver C. Scherer PE. Hawkins M (2004) Mechanisms of early insulin-sensitizing effects of thiazolidinediones in type 2 diabetes. Diabetes 53:1621–1629
134. Bodles AM. Banga A. Rasouli N. Ono F. Kern PA. Owens RJ (2006) Pioglitazone increases secretion of high-molecular-weight adiponectin from adipocytes. Am J Physiol Endocrinol Metab 291:E1100–E1105
135. Hiuge A. Tenenbaum A. Maeda N. Benderly M. Kumada M. Fisman EZ. Tanne D. Matas Z. Hibuse T. Fujita K. Nishizawa H. Adler Y. Motro M. Kihara S. Shimomura I. Behar S. Funahashi T (2006) Effects of peroxisome proliferator-activated receptor

ligands. bezafibrate and fenofibrate. on adiponectin level. Arterioscler Thromb Vasc Biol 27:635-641
136. Chinetti G. Zawadski C. Fruchart JC. Staels B (2004) Expression of adiponectin receptors in human macrophages and regulation by agonists of the nuclear receptors PPARalpha. PPARgamma. and LXR. Biochem Biophys Res Commun 314:151-158
137. Iwaki M. Matsuda M. Maeda N. Funahashi T. Matsuzawa Y. Makishima M. Shimomura I (2003) Induction of adiponectin. a fat-derived antidiabetic and antiatherogenic factor. by nuclear receptors. Diabetes 52:1655–1663

Serum Adiponectin Level as an Independent Predictor of Mortality in Patients With Congestive Heart Failure

Toshihiro Tamura, MD; Yutaka Furukawa, MD; Ryoji Taniguchi, MD; Yukihito Sato, MD*; Koh Ono, MD; Hisanori Horiuchi, MD; Yoshihisa Nakagawa, MD; Toru Kita, MD; Takeshi Kimura, MD

Background Congestive heart failure (CHF) is associated with altered energy homeostasis and myocardial inflammation, hypertrophy, and fibrosis. Adiponectin, an insulin-sensitizing adipocytokine, may affect these pathogenic factors, and the circulating adiponectin level may serve as a biological marker of CHF. This study aimed to assess the significance of serum adiponectin as a prognostic marker for Japanese CHF patients.

Methods and Results The serum adiponectin levels were compared between 54 (24 ischemic and 30 non-ischemic) CHF patients with left ventricular systolic dysfunction and 55 age- and gender-matched control subjects. The CHF patients also underwent simultaneous clinical assessment and measurements for brain natriuretic peptide (BNP) and parameters of lipid or glucose metabolism. Compared with the controls, the CHF patients showed significantly increased serum adiponectin levels [6.7 (4.9–12.6) vs 14.6 (9.7–25.4) μg/ml, p<0.0001]. In the CHF patients, the log-transformed values of the serum adiponectin levels positively correlated with the log-transformed values of the plasma BNP levels (p=0.0003, r=0.48) and inversely correlated with the body mass index (p=0.0006, r=–0.46). Furthermore, an increase in the serum adiponectin level was associated with higher mortality (p<0.05), particularly in the ischemic CHF patients (p<0.005).

Conclusions An increase in the circulating adiponectin level was associated with higher mortality in the ischemic CHF patients. Adiponectin may be an informative risk marker for Japanese CHF patients. (*Circ J* 2007; **71:** 623–630)

Key Words: Adiponectin; Brain natriuretic peptide; Congestive heart failure; Mortality

Despite recent improvements in survival, the 5-year mortality rate for patients with congestive heart failure (CHF) ranges between 40% and 60%[1,2] The discovery of risk markers for CHF and their appropriate use have contributed to improved screening, prevention, diagnosis and treatment of CHF. Because the pathophysiology of CHF is complex, a variety of markers associated with different pathophysiologic conditions may be used in the management of CHF.

Adiponectin is an insulin-sensitizing adipocytokine that regulates energy metabolism by increasing free fatty acid oxidation in skeletal myocytes and gluconeogenesis in the liver[3–5] In addition to its beneficial effects on both lipid and glucose metabolism, adiponectin has antiinflammatory properties[6] It reduces the endothelial expression of cell adhesion molecules and the monocyte/macrophage production of inflammatory cytokines; however, it induces antiinflammatory cytokines[7–10] Moreover, in a mouse model of pressure overload adiponectin had antihypertrophic effects on cardiac myocytes, and its deficiency exacerbated CHF[11,12] Myocardial expression of adiponectin receptor 1 and adiponectin receptor 2 suggest possible effects of adiponectin on cardiac myocytes[13,14] Adiponectin can also inhibit growth factor-mediated fibroblast proliferation and prevent fibrosis[15] Because these factors, namely, myocardial inflammation, hypertrophy and fibrosis, and dysregulation of myocardial lipid/glucose metabolisms can either cause or exacerbate CHF[16,17] adiponectin may play a protective role in CHF.

Increased levels of circulating adiponectin and the prognostic potential of adiponectin have been reported in Caucasian CHF patients[18,19] and very recently, an increase in the circulating adiponectin level and an association of this with the severity of CHF have been shown in Japanese CHF patients[20] Nevertheless, it remains uncertain whether adiponectin has prognostic potential in Asians, and the difference in the circulating adiponectin levels of Caucasian and Asian patients suggests that the prognostic potential of

(Received June 26, 2006; revised manuscript received January 29, 2007; accepted February 9, 2007)

*Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, *Department of Cardiology, Hyogo Prefectural Amagasaki Hospital, Amagasaki, Japan*

Mailing address: Yutaka Furukawa, MD, Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8397, Japan. E-mail: yutakaf@kuhp.kyoto-u.ac.jp

Table 1 Background Characteristics of the Study Subjects

	Control (n=55)	*CHF (n=54)*	*p value*
Age (years)	*59.0±17.3*	*63.2±15.1*	*NS*
Male gender, n (%)	*39 (71)*	*42 (78)*	*NS*
BMI (kg/m²)	*23.5±3.4*	*22.6±4.3*	*NS*
DM, n (%)	*14 (25)*	*20 (37)*	*NS*
TC (mg/dl)	*192.6±31.3*	*184.6±38.7*	*NS*
TG (mg/dl)	*105.0 (84.0–142.0)**	*96.5 (66.5–154.3)**	*NS*

*CHF, congestive heart failure; NS, not significantly different; BMI, body mass index; DM, diabetes mellitus; TC, total cholesterol; TG, triglyceride. Values are means±SD or *median value (interquartile range).*

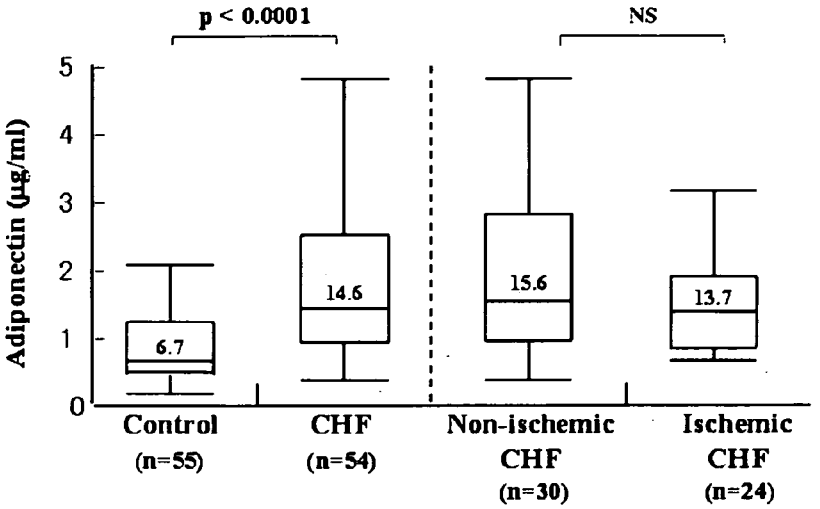

Fig 1. Serum adiponectin concentrations in control (n=55) and congestive heart failure (CHF) (n=54) subjects. Data are expressed as median value with interquartile and 10–90% range. NS, not significantly different.

Table 2 Clinical Information and Biochemical Marker Levels at Baseline in CHF Patients

	Overall (n=54)	*Adiponectin levels*		*p value*
		≤14.6 μg/ml (n=27)	*>14.6 μg/ml (n=27)*	
Age (years)	*63.2±15.1*	*59.8±15.6*	*66.6±14.1*	*NS*
Male gender, n (%)	*42 (78)*	*24 (89)*	*18 (67)*	*NS*
BMI (kg/m²)	*22.6±4.3*	*23.9±5.2*	*21.3±2.7*	*<0.05*
NYHA class III/IV, n (%)	*15 (28)*	*6 (22)*	*9 (33)*	*NS*
Ischemic CHF, n (%)	*24 (44)*	*13 (48)*	*11 (41)*	*NS*
DM, n (%)	*20 (37)*	*8 (30)*	*12 (44)*	*NS*
Hypertenstion, n (%)	*30 (56)*	*16 (59)*	*14 (52)*	*NS*
TC (mg/dl)	*184.6±38.7*	*185.3±39.2*	*184.0±39.0*	*NS*
TG (mg/dl)	*96.5 (66.5–154.3)**	*115.0 (69.0–160.0)**	*80.0 (56.0–134.0)**	*NS*
Creatinine (mg/dl)	*1.1±0.5*	*1.1±0.3*	*1.1±0.7*	*NS*
Hemoglobin A1c (%)	*5.9±0.9*	*5.9±0.9*	*5.9±0.9*	*NS*
BNP (pg/ml)	*281.8 (90.0–559.0)**	*192.7 (37.2–320.0)**	*403.1 (167.2–690.7)**	*<0.005*
LVEF (%)	*30.3±9.0*	*31.2±8.8*	*29.3±9.2*	*NS*
LVDd (mm)	*62.8±10.4*	*61.3±10.9*	*64.2±10.0*	*NS*
Medication, n (%)				
β-blocker	*43 (80)*	*23 (85)*	*20 (74)*	*NS*
ACEI/ARB	*48 (89)*	*25 (93)*	*23 (85)*	*NS*
Digoxin	*18 (33)*	*10 (37)*	*8 (30)*	*NS*
Statin	*21 (39)*	*13 (48)*	*8 (30)*	*NS*
Diuretics	*44 (81)*	*22 (81)*	*22 (81)*	*NS*

NYHA, New York Heart Association; BNP, brain natriuretic peptide; LVEF, left ventricular ejection fraction; LVDd, left ventricular diastolic dimension; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker. Other abbreviations see in Table 1.

*Values are mean±SD or *median value (interquartile range).*

the adiponectin level may possibly differ among ethnic groups[21,22] In addition, it needs to be analyzed whether the increase in the adiponectin level is similar in both ischemic and non-ischemic CHF because it has been reported that, compared with controls, patients with coronary artery disease exhibited significantly lower circulating adiponectin levels[23,24]

In this study, we compared the serum adiponectin levels between CHF patients and control subjects with normal cardiac function and between ischemic and non-ischemic CHF patients; all the study subjects were Japanese. We also analyzed the predictive potential of adiponectin with regard to mortality and the association between adiponectin and other parameters of CHF.

Methods

This study was approved by the Institutional Review Board of Kyoto University Graduate School and Faculty of Medicine. Informed written consent was given by all study subjects in accordance with the Declaration of Helsinki.

Study Subjects

Fifty-four Japanese CHF patients (24 ischemic CHF, 30 non-ischemic CHF) with left ventricular (LV) systolic dysfunction who were admitted to hospital during 2003–2005 were retrospectively analyzed. LV systolic dysfunction was defined as LV ejection fraction (LVEF) of <50%. LVEF was calculated from 2-dimensional echocardiography images using the apical biplane modified Simpson's method. The 55 control subjects with normal cardiac function included healthy volunteers, patients with controlled essential hypertension, and those with paroxysmal supraventricular arrhythmias who had not experienced arrhythmic events for ≥48 h before blood sample collection. Patients with end-stage renal dysfunction were excluded from the study

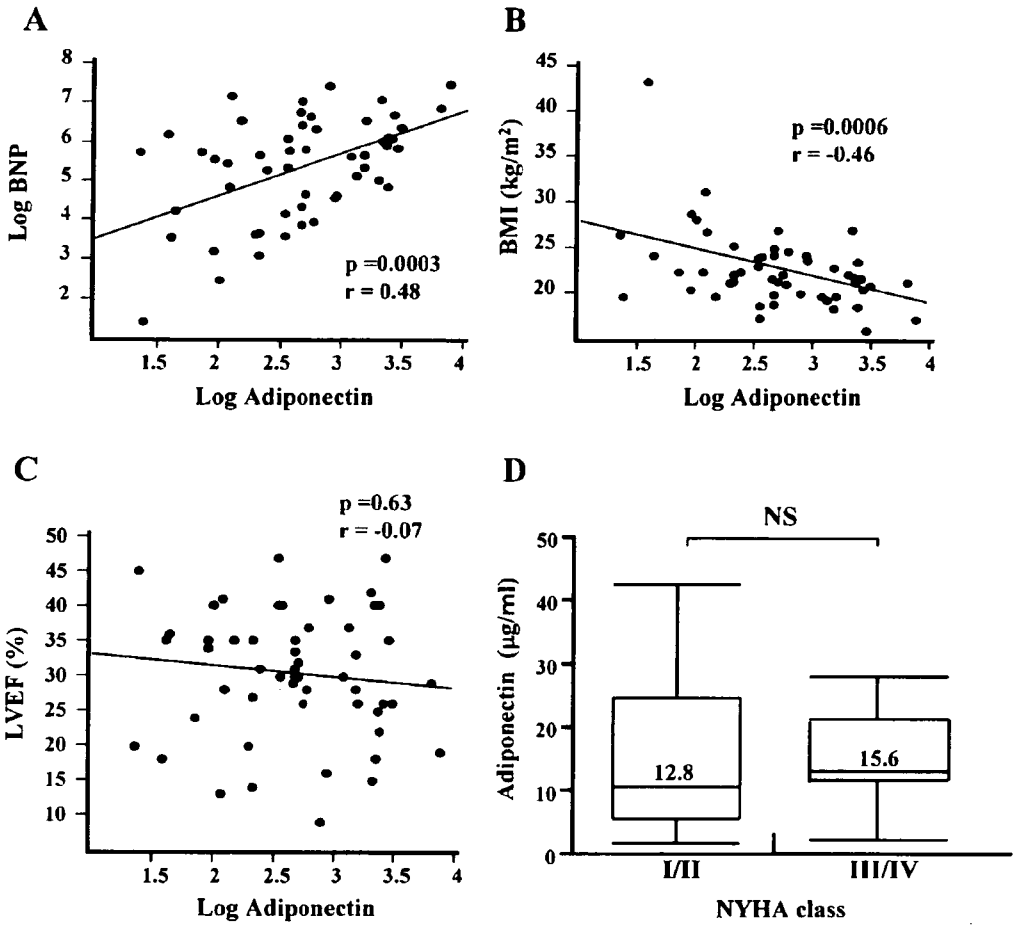

Fig 2. Scatter plots of the association between log-transformed values of serum adiponectin levels (Log Adiponectin) and log-transformed values of plasma brain natriuretic peptide (BNP) levels (Log BNP) (A), body mass index (BMI) (B) and left ventricular ejection fraction (LVEF; C), and comparison of serum adiponectin levels between the patients with high and low New York Heart Association (NYHA) functional classes (D) in congestive heart failure (CHF) patients. Log Adiponectin positively correlated with Log BNP and inversely with BMI. There was no significant association between Log Adiponectin and LVEF, and no significant difference in serum adiponectin concentrations between NYHA class I/II and class III/IV. Data are expressed as median value with interquartile and 10–90% range in panel (D). NS, not significantly different.

because they reportedly have an increase in the circulating adiponectin level.[25] Clinical and analytical information of the CHF patients obtained at baseline included the New York Heart Association (NYHA) functional classification, body mass index (BMI) calculated from measurements of height and weight, conditions such as diabetes mellitus and hypertension, blood biochemistry, and echocardiography. Diabetes mellitus was defined according to whether the patients were receiving active treatment with antidiabetic drugs or insulin. Patients on dietary treatment alone who met the diagnostic criteria listed in the "Report of the Committee of Japan Diabetes Society (JDS) on the Classification and Diagnostic Criteria of Diabetes Mellitus" were considered to have diabetes.[26] The CHF patients were followed up with regard to mortality for a median of 1.8 years (range, 0.2–2.3 years). All deaths were confirmed by medical records or telephone interview with the patients' families.

Measurements of Adiponectin and BNP Levels

All blood samples were collected by venipuncture. The sera and plasma were prepared by centrifugation of the blood samples and stored at −80°C until use. The serum adiponectin concentrations were measured using a commercially available enzyme-linked immunosorbent assay kit (Otsuka Life Science Initiative, Tokyo, Japan). The plasma brain natriuretic peptide (BNP) levels were measured using enzyme immunoassay (Tosoh Corporation, Tokyo, Japan).

Statistical Analysis

The serum adiponectin, plasma BNP, and serum triglyceride levels are expressed as medians (interquartile ranges). All the other continuous variables are expressed as means± SD. Statistical significance with regard to the differences in subject demographics between the CHF and control subjects or between the subgroups of CHF patients was assessed by Student's t test because the values were distributed parametrically. The Mann-Whitney U-test was used for comparison of nonparametric values (ie, serum adiponectin, plasma BNP, and serum triglyceride levels) between 2 groups. Pearson's correlation coefficient was used to assess the correlations between the log-transformed values of the serum adiponectin levels (Log Adiponectin) and the log-transformed values of the plasma BNP levels (Log BNP), BMI,

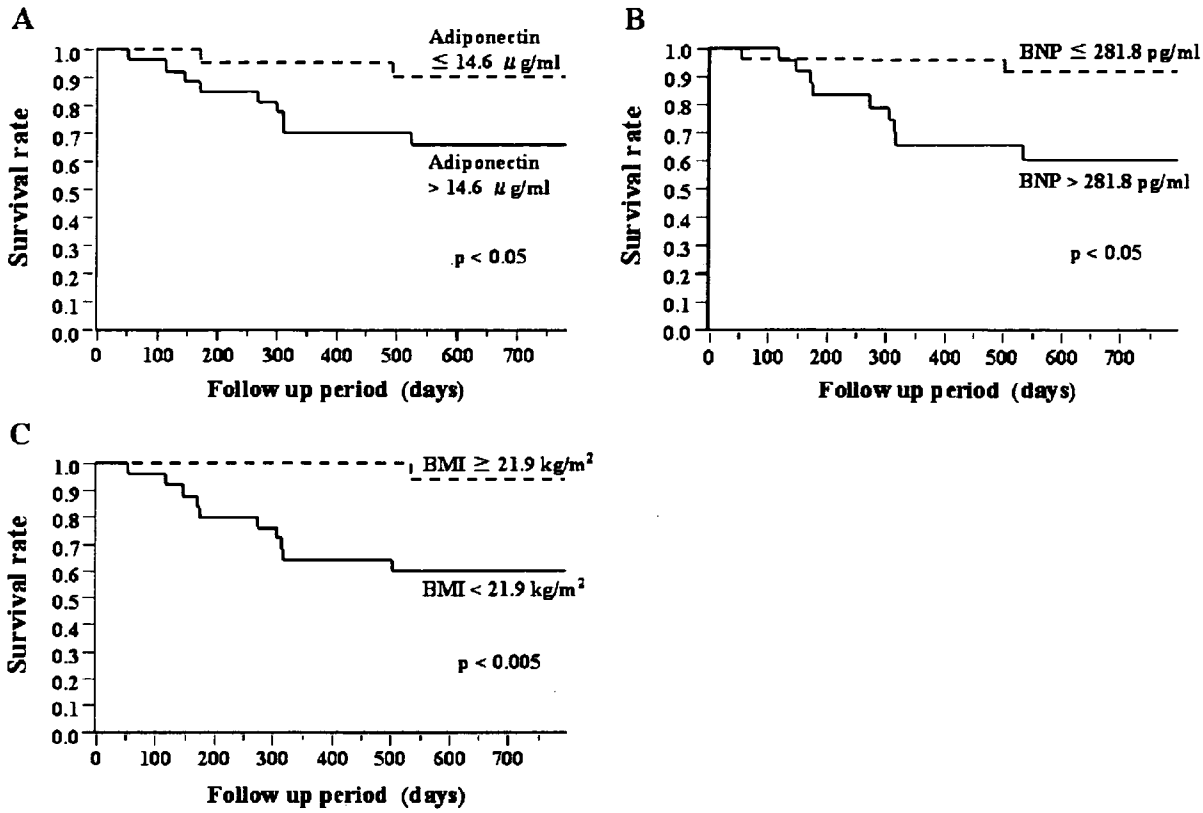

Fig 3. Kaplan-Meier analysis of cumulative rates of survival in patients with congestive heart failure (CHF) stratified into 2 groups by the median of serum adiponectin (A), plasma brain natriuretic peptide (BNP) (B), and body mass index (BMI) (C).

or LVEF in the CHF patients.

Kaplan-Meier analysis compared the cumulative survival rates between the 2 groups of CHF patients stratified according to the median serum adiponectin levels, plasma BNP levels, or BMI. Differences between the survival curves were tested using a log-rank test for these factors. Kaplan-Meier analysis was also used to compare the cumulative survival rates between the 2 groups that were stratified according to the median serum adiponectin level in the ischemic or non-ischemic CHF patients. Univariate analysis with the Cox proportional hazards model was used to assess the association of each variable with patient survival. Multivariate analysis with the Cox proportional hazards model was used to assess the independence of the predictors of mortality. The covariates included: (1) all the parameters with $p<0.05$ in the univariate analysis (age, BMI, plasma BNP level, serum adiponectin level) and (2) the established predictors of mortality in CHF patients, such as gender, LVEF, NYHA classification, renal dysfunction (serum creatinine ≥1.5 mg/dl), and ischemic etiology[27-31] All analyses were performed using SAS Institute Inc JMP version 5 (Cary, NC, USA).

Results

Subject Demographics and Serum Adiponectin Levels

We studied a total of 54 CHF patients and 55 control subjects (Table 1). There were no significant differences in age, gender distribution, BMI, or serum total cholesterol and triglyceride levels. Compared with the control subjects, the CHF patients showed significantly increased serum adiponectin levels [6.7 (4.9–12.6) vs 14.6 (9.7–25.4) μg/ml; Fig 1]. Significant difference was not observed with regard to the serum adiponectin level between the ischemic and non-ischemic CHF patients. Detailed clinical information and baseline circulating biomarker levels in the CHF patients are presented in Table 2. With the exception of BMI and the plasma BNP levels, all other parameters were comparable between the high- and low-adiponectin level groups. Thiazolidinediones, which increase the circulating adiponectin level, were not administered to any patients at the time of blood sample collection.

Correlations Between Serum Adiponectin Levels and Established Clinical and Biochemical Markers of Mortality in CHF

In the CHF patients, Log Adiponectin was positively associated with Log BNP ($p=0.0003$, $r=0.48$; Fig 2A) and inversely associated with BMI ($p=0.0006$, $r=-0.46$; Fig 2B). There were no significant correlations between Log Adiponectin and LVEF (Fig 2C), and the serum adiponectin levels were comparable between patients with higher (III/IV) and lower (I/II) NYHA functional class (Fig 2D).

Cumulative Survival of CHF Patients With High and Low Serum Adiponectin Levels

During the follow-up period (median, 1.8 years; range, 0.2–2.3 years), 11 of the 54 patients (20.4%) died: 6 cardiac deaths, including 4 from CHF and 2 sudden deaths, and 5 non-cardiac deaths. All the cardiac deaths occurred in the high serum adiponectin level group. The Kaplan-Meier

Table 3 Univariate Analysis for Predictors of Mortality

	Odds ratio	Lower 95%CI	Upper 95%CI	chi-square	p value
Age (years)	1.17	1.07	1.32	16.5	<0.0001
BMI (kg/m^2)	0.62	0.44	0.81	13.4	0.0003
BNP (pg/ml)	1.001	1.0003	1.02	6.3	0.01
Adiponectin (μg/ml)	1.07	1.01	1.12	5.7	0.02
LVEF (%)	0.94	0.88	1.01	3.2	0.07
TG (mg/dl)	1.008	1.00	1.02	1.9	0.16
High NYHA class (III/IV)	1.26	0.64	2.29	0.5	0.47
TC (mg/dl)	1.006	0.99	1.02	0.5	0.48
Gender (male)	0.85	0.46	1.82	0.2	0.64
Hemoglobin A1c (%)	1.14	0.56	2.08	0.2	0.70

CI, confidence interval. Other abbreviations see in Tables 1, 2.

Table 4 Multivariate Analysis for Predictors of Mortality

	Odds ratio	Lower 95%CI	Upper 95%CI	chi-square	p value
Age (years)	1.31	1.11	1.66	14.1	0.0002
BMI (kg/m^2)	0.45	0.23	0.77	8.9	0.003
LVEF (%)	0.80	0.66	0.94	8.3	0.004
Adiponectin (μg/ml)	1.12	1.01	1.27	4.3	0.037
High NYHA class (III/IV)	5.43	1.03	38.9	4.0	0.046
BNP (pg/ml)	1.00	0.99	1.00	3.6	0.058
Gender (male)	2.06	0.50	10.73	1.0	0.31
Ischemic etiology	0.77	0.36	1.58	0.5	0.48
Renal dysfunction (serum creatinine ≥1.5 mg/dl)	0.78	0.24	2.44	0.2	0.67

Abbreviations see in Tables 1–3.

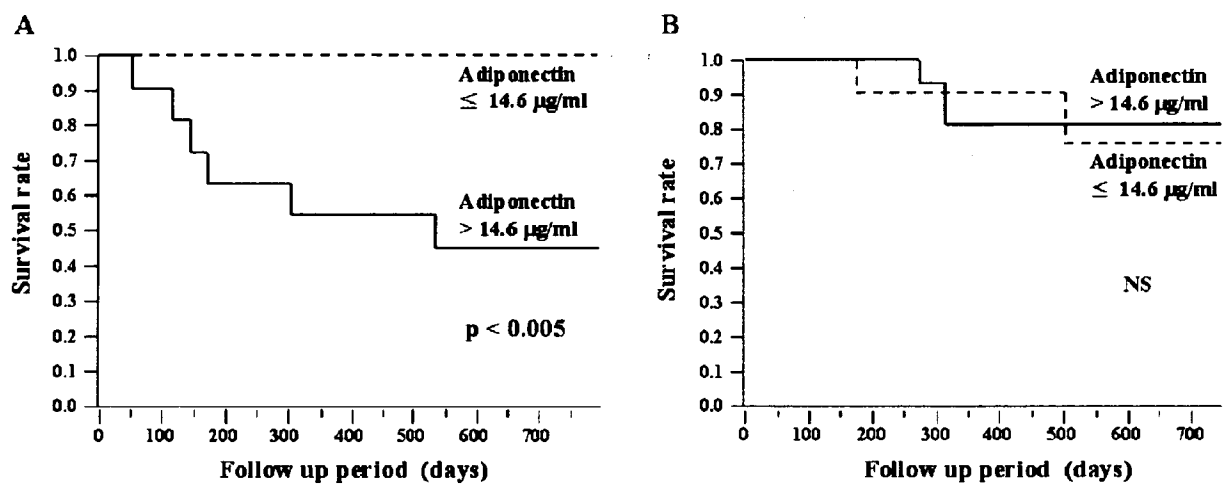

Fig 4. Kaplan-Meier analysis of cumulative rates of survival in patients with congestive heart failure (CHF) of ischemic etiology (A) and non-ischemic etiology (B) stratified into 2 groups by the median of serum adiponectin. NS, not significantly different.

survival method and log-rank analyses comparing the 2 groups stratified according to the median serum adiponectin levels (14.6 μg/ml) indicated that the unadjusted overall mortality risk was significantly elevated in the high serum adiponectin level group ($p<0.05$; Fig 3A). The unadjusted overall mortality risk was also significantly elevated in the high plasma BNP level ($p<0.05$; Fig 3B) and low BMI ($p<0.005$; Fig 3C) groups.

Predictive Potential of Serum Adiponectin Level for Mortality in CHF Patients

Univariate analysis with the Cox proportional hazards model showed that old age (chi-square=16.5, $p<0.0001$), low BMI (chi-square=13.4, $p=0.0003$), high plasma BNP level (chi-square=6.3, $p=0.01$), and high serum adiponectin level (chi-square=5.7, $p=0.02$) were significant predictors of mortality (Table 3). Multivariate analysis with the Cox proportional hazards model used the parameters showing $p<0.05$ in the univariate analysis and the established predictors of mortality in CHF, such as gender, LVEF, NYHA classification, ischemic etiology, and renal dysfunction. As shown in Table4, our results indicate that old age (chi-square=14.1, $p=0.0002$), BMI (chi-square=8.9, $p=0.003$), LVEF (chi-square=8.3, $p=0.004$), serum adiponectin level (chi-square=4.3, $p=0.037$), and NYHA class (chi-square=4.0, $p=0.046$) were independent predictors of mor-

tality in the CHF patients.

Impact of High Serum Adiponectin Level on Cumulative Survival in Ischemic vs Non-Ischemic CHF Patients

The Kaplan-Meier survival method and log-rank analyses of 2 groups stratified according to the median serum adiponectin level (14.6 μg/ml) were performed separately for the ischemic CHF patients (n=24) and non-ischemic CHF patients (n=30). The number of deaths in the ischemic (6 deaths) and non-ischemic (5 deaths) CHF groups was similar. The results indicated that the unadjusted overall mortality risk was significantly elevated in the high serum adiponectin level group of ischemic CHF patients (p=0.0026; Fig 4A) but not in the non-ischemic CHF patients (p=0.83; Fig 4B).

Discussion

In the present study, we observed that the serum adiponectin levels were significantly higher in Japanese CHF patients than in age- and gender-matched Japanese control subjects. In the CHF patients, the circulating adiponectin level positively correlated with the plasma BNP level and negatively correlated with BMI. Higher serum adiponectin level, lower LVEF, severity of CHF as assessed by the NYHA classification, old age, and lower BMI were predictors of overall mortality in the CHF patients. The predictive potential of a high serum adiponectin level was based on its effect in ischemic CHF patients.

Adiponectin, BNP, and BMI as Prognostic Markers of Mortality in CHF Patients

The serum adiponectin levels clearly and positively correlated with the plasma BNP levels. Despite the apparently positive correlation of the serum adiponectin level with the plasma BNP level, multivariate Cox hazards analysis that included BNP as a covariate revealed adiponectin as an independent prognostic marker of CHF. These observations were in agreement with recent reports indicating a positive correlation of adiponectin with the N-terminal prohormone BNP,[18,19] thus confirming the significance of the circulating adiponectin level as a prognostic marker of CHF. The reasons for the positive relationship between these 2 molecules cannot be explained by the results of our study. Considering that adiponectin was a predictor of overall mortality in CHF independent of BNP and that the main sources of these 2 molecules are different, the circulating adiponectin and BNP levels may be differently regulated.

In this study, a low BMI was also a significant predictor of overall mortality. An inverse association between adiponectin and BMI has been reported in obese or diabetic subjects, and this association was very recently extended to CHF patients.[18,19,32,33] Interestingly, the inverse association of BMI with mortality in CHF has been also shown by many other groups.[34–37] The mortality rate remains high in CHF patients with a low BMI, even after excluding cachexic patients.[35,37] It is possible that cardiac cachexia caused an increase in the circulating adiponectin levels in the present study, although these levels, as well as the BMIs, were comparable between the 4 patients who died from CHF and the 2 patients who died suddenly. Further studies are required to clarify the rational explanation for these observations.

Adiponectin in Ischemic vs Non-Ischemic CHF Patients

Because lower circulating adiponectin levels are reported in patients with coronary artery disease, including vasospastic angina,[23,24,38] we hypothesized that the circulating adiponectin levels might be decreased in the ischemic CHF patients and that increased levels might have a better predictive potential for mortality in non-ischemic CHF patients. Unexpectedly, the circulating adiponectin levels were paradoxically increased in patients with coronary artery disease complicated with CHF. Moreover, the Kaplan-Meier analyses of the subgroups of ischemic and non-ischemic CHF patients indicated that a higher adiponectin level was a significant predictor of overall mortality in the ischemic CHF patients but not in the non-ischemic CHF patients. Although the small sample size may preclude demonstration of the prognostic potential of the serum adiponectin level in non-ischemic CHF, this potential appeared to be derived from its effects in ischemic CHF and may attenuate the importance of the circulating adiponectin levels in the non-ischemic CHF subgroup, despite the fact that these levels were higher in the non-ischemic CHF patients than in the control subjects. One possible link between the biological activities of adiponectin and these observations is the role of adiponectin in myocardial ischemia and angiogenesis. Adiponectin inhibits the effects of angiogenic growth factors, such as the basic fibroblast growth factor, and it may exhibit anti-angiogenic effects.[15] Thus, the adiponectin levels may influence the severity of ischemia via the effects of adiponectin on angiogenesis. However, possible angiogenic effects of adiponectin have been reported, and the clinical implications of these contrary observations remain uncertain.[39,40]

Ethnic Differences in Circulating Adiponectin Levels

Ethnic differences in the circulating adiponectin levels have been reported between Caucasians and Asians as well as between Caucasians and African Americans.[21,22] Although previous reports have indicated the predictive potential of high circulating adiponectin levels for mortality in Caucasians, who have relatively higher adiponectin levels,[18,19] it is important to study the significance of these levels in other ethnic groups that have lower adiponectin levels. Our observations in Japanese CHF patients were concordant with previous reports in Caucasians, thus strengthening the importance of adiponectin as a risk marker for CHF.

Study Limitations

Several limitations should be noted in the interpretation of these results. First, the study design might limit the strength of the conclusion. The present study was a single-center study analyzing a small number of subjects; therefore, a study on a larger scale is warranted to confirm the relationship between worse prognosis and increased adiponectin levels in ischemic CHF patients. In this study, multivariate analysis did not present BNP as an independent predictor of mortality, although it was a significant predictor of mortality according to the univariate analysis, and is definitely recognized as an established prognostic predictor for CHF. Because BNP showed a significant correlation with many covariates, such as adiponectin, BMI, LVEF, NYHA class, and age, in the multivariate analysis, the analysis could not indicate the independence of BNP in our setting of a small number of subjects.

Second, the influences of drugs on the serum adiponectin levels should be considered. Most of the present CHF patients were being treated with optimal therapy when their blood samples were collected. Because the use of angiotensin-converting enzyme inhibitors, angiotensin-II receptor blockers, and β-blockers can improve the survival of CHF patients[41] these drugs were routinely prescribed to the patients (Table 2). In addition, statins were also prescribed to 39% of the CHF patients. Drugs such as angiotensin-II receptor blockers, statins, or their combination, have been reported to raise the circulating adiponectin levels[42-44] Thus, increased adiponectin levels in CHF patients should be carefully assessed although there were no significant differences in the use of such drugs between the high and low serum adiponectin level groups of CHF patients in the present study (Table 2).

Conclusion

The circulating adiponectin level is increased, positively correlates with the plasma BNP level, and is associated with overall mortality in Japanese CHF patients. The impact of a high serum adiponectin level on mortality was significant in the ischemic CHF patients but not in the non-ischemic CHF patients. Circulating adiponectin may be a useful marker to predict the outcomes of CHF patients.

Acknowledgments

We would like to thank Mr Tomohiko Shimizu and Ms Kanako Takahashi, Kimie Itoh, and Yuka Yoshikawa for their skillful assistance.

This work was supported in part by a Grant for Clinical Research for Evidence Based Medicine from the Ministry of Health, Labour and Welfare in Japan to T. K.

References

1. Levy D, Kenchaiah S, Larson MG, Benjamin EJ, Kupka MJ, Ho KK, et al. Long-term trends in the incidence of and survival with heart failure. *N Engl J Med* 2002; **347:** 1397–1402.
2. Roger VL, Weston SA, Redfield MM, Hellermann-Homan JP, Killian J, Yawn BP, et al. Trends in heart failure incidence and survival in a community-based population. *JAMA* 2004; **292:** 344–350.
3. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci USA* 2001; **98:** 2005–2010.
4. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; **8:** 1288–1295.
5. Wolf G. Adiponectin: A regulator of energy homeostasis. *Nutr Rev* 2003; **61:** 290–292.
6. Matsuzawa Y. Adiponectin: Identification, physiology and clinical relevance in metabolic and vascular disease. *Atheroscler Suppl* 2005; **6:** 7–14.
7. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Novel modulator for endothelial adhesion molecules: Adipocyte-derived plasma protein adiponectin. *Circulation* 1999; **100:** 2473–2476.
8. Wulster-Radcliffe MC, Ajuwon KM, Wang J, Christian JA, Spurlock ME. Adiponectin differentially regulates cytokines in porcine macrophages. *Biochem Biophys Res Commun* 2004; **316:** 924–929.
9. Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. *Biochem Biophys Res Commun* 2004; **323:** 630–635.
10. Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* 2005; **115:** 911–919; quiz 920.
11. Shibata R, Ouchi N, Ito M, Kihara S, Shiojima I, Pimentel DR, et al. Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nat Med* 2004; **10:** 1384–1389.
12. Liao Y, Takashima S, Maeda N, Ouchi N, Komamura K, Shimomura I, et al. Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism. *Cardiovasc Res* 2005; **67:** 705–713.
13. Steinmetz M, Quentin T, Poppe A, Paul T, Jux C, Changes in expression levels of genes involved in fatty acid metabolism: Upregulation of all three members of the PPAR family (alpha, gamma, delta) and the newly described adiponectin receptor 2, but not adiponectin receptor 1 during neonatal cardiac development of the rat. *Basic Res Cardiol* 2005; **100:** 263–269.
14. Ding ST, Liu BH, Ko YH. Cloning and expression of porcine adiponectin and adiponectin receptor 1 and 2 genes in pigs. *J Anim Sci* 2004; **82:** 3162–3174.
15. Wang Y, Lam KS, Xu JY, Lu G, Xu LY, Cooper GJ, et al. Adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization-dependent manner. *J Biol Chem* 2005; **280:** 18341–18347.
16. Paolisso G, Gambardella A, Galzerano D, D'Amore A, Rubino P, Verza M, et al. Total-body and myocardial substrate oxidation in congestive heart failure. *Metabolism* 1994; **43:** 174–179.
17. Taylor M, Wallhaus TR, Degrado TR, Russell DC, Stanko P, Nickles RJ, et al. An evaluation of myocardial fatty acid and glucose uptake using PET with [18F]fluoro-6-thia-heptadecanoic acid and. *J Nucl Med* 2001; **42:** 55–62.
18. Kistorp C, Faber J, Galatius S, Gustafsson F, Frystyk J, Flyvbjerg A, et al. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. *Circulation* 2005; **112:** 1756–1762.
19. George J, Patal S, Wexler D, Sharabi Y, Peleg E, Kamari Y, et al. Circulating adiponectin levels predict outcome in patients with severe congestive heart failure. *Heart* 2006.
20. Nakamura T, Funayama H, Kubo N, Yasu T, Kawakami M, Saito M, et al. Association of hyperadiponectinemia with severity of ventricular dysfunction in congestive heart failure. *Circ J* 2006; **70:** 1557–1562.
21. Hulver MW, Saleh O, MacDonald KG, Pories WJ, Barakat HA. Ethnic differences in adiponectin levels. *Metabolism* 2004; **53:** 1–3.
22. Valsamakis G, Chetty R, McTernan PG, Al-Daghri NM, Barnett AH, Kumar S. Fasting serum adiponectin concentration is reduced in Indo-Asian subjects and is related to HDL cholesterol. *Diabetes Obes Metab* 2003; **5:** 131–135.
23. Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, et al. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol* 2003; **23:** 85–89.
24. Ohashi K, Ouchi N, Kihara S, Funahashi T, Nakamura T, Sumitsuji S, et al. Adiponectin I164T mutation is associated with the metabolic syndrome and coronary artery disease. *J Am Coll Cardiol* 2004; **43:** 1195–1200.
25. Zoccali C, Mallamaci F, Tripepi G. Inflammatory proteins as predictors of cardiovascular disease in patients with end-stage renal disease. *Nephrol Dial Transplant* 2004; **19**(Suppl 5): V67–V72.
26. Kuzuya T, Nakagawa S, Satoh J, Kanazawa Y, Iwamoto Y, Kobayashi M, et al. Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. *Diabetes Res Clin Pract* 2002; **55:** 65–85.
27. Ho KK, Anderson KM, Kannel WB, Grossman W, Levy D. Survival after the onset of congestive heart failure in Framingham Heart Study subjects. *Circulation* 1993; **88:** 107–115.
28. Bart BA, Shaw LK, McCants CB Jr, Fortin DF, Lee KL, Califf RM, et al. Clinical determinants of mortality in patients with angiographically diagnosed ischemic or nonischemic cardiomyopathy. *J Am Coll Cardiol* 1997; **30:** 1002–1008.
29. Johnson G, Carson P, Francis GS, Cohn JN. Influence of prerandomization (baseline) variables on mortality and on the reduction of mortality by enalapril: Veterans Affairs Cooperative Study on Vasodilator Therapy of Heart Failure (V-HeFT II): V-HeFT VA Cooperative Studies Group. *Circulation* 1993; **87:** VI-32–VI-39.
30. Tsutamoto T, Wada A, Maeda K, Hisanaga T, Maeda Y, Fukai D, et al. Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure: Prognostic role of plasma brain natriuretic peptide concentration in patients with chronic symptomatic left ventricular dysfunction. *Circulation* 1997; **96:** 509–516.
31. Cohen N, Gorelik O, Almoznino-Sarafian D, Alon I, Tourovski Y, Weissgarten J, et al. Renal dysfunction in congestive heart failure, pathophysiological and prognostic significance. *Clin Nephrol* 2004; **61:** 177–184.
32. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; **257:** 79–83.
33. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000; **20:** 1595–1599.
34. Horwich TB, Fonarow GC, Hamilton MA, MacLellan WR, Woo

MA, Tillisch JH. The relationship between obesity and mortality in patients with heart failure. *J Am Coll Cardiol* 2001; **38**: 789–795.
35. Davos CH, Doehner W, Rauchhaus M, Cicoira M, Francis DP, Coats AJ, et al. Body mass and survival in patients with chronic heart failure without cachexia: The importance of obesity. *J Card Fail* 2003; **9**: 29–35.
36. Gustafsson F, Kragelund CB, Torp-Pedersen C, Seibaek M, Burchardt H, Akkan D, et al. Effect of obesity and being overweight on long-term mortality in congestive heart failure: Influence of left ventricular systolic function. *Eur Heart J* 2005; **26**: 58–64.
37. Bozkurt B, Deswal A. Obesity as a prognostic factor in chronic symptomatic heart failure. *Am Heart J* 2005; **150**: 1233–1239.
38. Maruyoshi H, Kojima S, Otsuka F, Funahashi T, Kaikita K, Sugiyama S, et al. Hypoadiponectinemia is associated with coronary artery spasm in men. *Circ J* 2005; **69**: 1154–1156.
39. Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, et al. Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells. *J Biol Chem* 2004; **279**: 1304–1309.
40. Shibata R, Ouchi N, Kihara S, Sato K, Funahashi T, Walsh K. Adiponectin stimulates angiogenesis in response to tissue ischemia through stimulation of amp-activated protein kinase signaling. *J Biol Chem* 2004; **279**: 28670–28674.
41. Matsumura Y, Takata J, Kitaoka H, Kubo T, Baba Y, Hoshikawa E, et al. Long-term prognosis of dilated cardiomyopathy revisited: An improvement in survival over the past 20 years. *Circ J* 2006; **70**: 376–383.
42. Koh KK, Quon MJ, Han SH, Chung WJ, Ahn JY, Seo YH, et al. Additive beneficial effects of losartan combined with simvastatin in the treatment of hypercholesterolemic, hypertensive patients. *Circulation* 2004; **110**: 3687–3692.
43. Fujimoto M, Masuzaki H, Tanaka T, Yasue S, Tomita T, Okazawa K, et al. An angiotensin II AT1 receptor antagonist, telmisartan augments glucose uptake and GLUT4 protein expression in 3T3-L1 adipocytes. *FEBS Lett* 2004; **576**: 492–497.
44. Koh KK, Quon MJ, Han SH, Chung WJ, Ahn JY, Seo YH, et al. Additive beneficial effects of fenofibrate combined with atorvastatin in the treatment of combined hyperlipidemia. *J Am Coll Cardiol* 2005; **45**: 1649–1653.

Original Article

Polymorphisms of Apolipoprotein E and Methylenetetrahydrofolate Reductase in the Japanese Population

Hidenori Arai[1], Akira Yamamoto[2], Yuji Matsuzawa[3], Yasushi Saito[4], Nobuhiro Yamada[5], Shinichi Oikawa[6], Hiroshi Mabuchi[7], Tamio Teramoto[8], Jun Sasaki[9], Noriaki Nakaya[10], Hiroshige Itakura[11], Yuichi Ishikawa[12], Yasuyoshi Ouchi[13], Hiroshi Horibe[14], Tohru Egashira[15], Hiroaki Hattori[15], and Toru Kita[16]

[1]Department of Geriatric Medicine, Kyoto University Graduate School of Medicine, Japan.
[2]National Cardiovascular Center, Japan.
[3]Sumitomo Hospital, Japan.
[4]Department of Clinical Cell Biology and Medicine, Chiba University, Japan.
[5]Department of Internal Medicine, University of Tsukuba, Japan.
[6]Department of Internal Medicine, Nippon Medical School, Japan.
[7]Department of Laboratory Science, Kanazawa University, Japan.
[8]Department of Internal Medicine, Teikyo University, Japan.
[9]International University of Health and Welfare, Japan.
[10]Fussa Hospital, Japan.
[11]Ibaraki Christian University, Japan.
[12]Faculty of Health Sciences, Kobe University, Japan.
[13]Department of Geriatric Medicine, University of Tokyo, Japan.
[14]Keisen Clinic, Japan.
[15]Department of Advanced Technology and Development, BML, Inc., Japan.
[16]Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Japan.

Aim: The aim of this study is to analyze the effect of apolipoprotein E (apo E) and methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphisms on serum lipid and homocysteine levels in the general Japanese population.
Methods: We analyzed the polymorphisms in individuals randomly selected from among participants of Serum Lipid Survey 2000.
Results: The frequency of the ε2, ε3, and ε4 alleles of *APOE* was 4.2, 85.3, and 10.5%, respectively. Individuals with the genotype ε4/ε4 had the highest total and low-density lipoprotein (LDL) cholesterol levels, while those with ε2/ε2 had the lowest. Individuals with the ε2/ε2 and ε2/ε4 genotypes had higher remnant-like particles (RLP)-cholesterol levels than those with ε2ε3, ε3ε3, and ε3ε4. There was a trend for individuals with the ε2/ε4 and ε2/ε2 genotypes to have higher triglyceride levels, although the difference was not significant. The presence of the T allele in a *MTHFR* polymorphism (C667T) was associated with higher homocysteine levels, which is more prominent in men than in women.
Conclusion: Thus in our large-scale analysis we have shown that RLP-cholesterol is better associated with *APOE* genotype than triglyceride and the effect of the T allele on *MTHFR* polymorphism (C667T) homocysteine levels is more prominent in men than in women among Japanese.

J Atheroscler Thromb, 2007; 14:167-171.

Key words; Hyperlipidemia, Polymorphism, Apolipoprotein E, *MTHFR*, Homocysteine

Address for correspondence: Hidenori Arai, Department of Geriatric Medicine, Kyoto University School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.
E-mail: harai@kuhp.kyoto-u.ac.jp
Received: January 22, 2007
Accepted for publication: March 14, 2007

Introduction

Apolipoprotein E (apo E) is an important structural constituent of serum chylomicrons, very low-density lipoproteins, and high-density lipoproteins (HDL) and plays a critical role in lipoprotein metablism, where it can facilitate the clearance of remnant lipoprotein and cellular efflux of cholesterol[1]. Apo E has three polymorphisms, ε2, ε3, and ε4, which affect lipoprotein metabolism and atherosclerosis[2]. The ε4 allele is associated with higher low-density lipoprotein (LDL) cholesterol levels than the other alleles and with a higher incidence of coronary heart disease[3]. Apo E4 is also shown to be involved in the development of Alzheimer's disease[4], while homozygosity for apo E2 is associated with the development of type Ⅲ hyperlipidemia[5].

We also studied the *MTHFR* gene because its polymorphisms affect serum homocysteine levels and homocysteine is also associated with cardiovascular disease and Alzheimer's disease[6-9]. An elevated homocysteine level is associated with coronary heart disease and the C677T polymorphism in the *MTHFR* gene results in reduced *MTHFR* enzyme activity and reduced methylation of homocysteine to methioneine resulting in mild hyperhomocysteinemia[10]. Although several studies have examined the incidence of *APOE* and *MTHFR* polymorphisms[8, 11], these has been no large-scale study to determine the incidence of *APOE* and *MTHFR* polymorphisms and their association with lipoprotein profiles and homocysteine levels in the general Japanese population. In 2000, we conducted a lipid survey in the Japanese population, 12,839 people all over the country. In this survey, we examined *APOE* and *MTHFR* gene polymorphisms to determine the incidence of each and its relationship with lipid profiles and homocysteine levels in the Japanese.

Methods

Design and Data Collection

This work is part of Serum Lipid Level Survey 2000 from various parts of Japan. The Ethics committee, Graduate School and Faculty of Medicine, Kyoto University approved the study protocol and all subjects provided written informed consent for participation in the gene analysis. The handling of DNA samples followed the guidelines from the Ministry of Health, Labor, and Welfare. In Serum Lipid Survey 2000, a total of 12,839 subjects were recruited at 36 hospitals across the country. The subjects in the present study were participants in the survey at 9 hospitals from whom informed content for genotyping was sought. Of the 12,839 subjects, 2,267 (17.7%) with no lipid-altering medication were randomly selected for the present study. Among the 2,267 participants, we examined serum homocysteine levels and *MTHFR* gene polymorphisms in 505 participants.

Laboratory Methods

All serum and blood samples were obtained in the fasting state. All lipid and other analyses were conducted on venous blood samples within one week of collection at BML (Saitama, Japan). Serum cholesterol and TG levels were measured by enzymatic assay. HDL-cholesterol and LDL-cholesterol levels were measured enzymatically with a kit from Daiichi Kagaku Co. Ltd. (Tokyo, Japan). The results of lipid analyses in the four surveys were indirectly standardized according to the criteria of the CDC Lipid Standardization Program[12]. The serum homocysteine level was assayed by high performance liquid chromatography with fluorescent detection as described by Ubbink *et al.*[13]. DNA was extracted with a QIAamp DNA blood kit (Qiagen, Hilden, Germany).

Detection of gene Mutations by Invader® Assay

We used the Invader® assay to screen for mutations of the *APOE* and *MTHFR* genes, as previously described. In brief, the probe/Invader®/MgCl2 mixture was prepared by combining 3 μL of primary probe/Invader® mix and 5 μL of 22.5 mM MgCl2 per reaction. The primary probes/Invader® mixture contained 3.5 μmol/L wild primary probe, 3.5 μmol/L mutant primary probe, 0.35 μmol/L Invader® oligonucleotide, and 10 mmol/L MOPS. Eight microliters of primary probe/Invader®/MgCl2 mixture was added per well of a 96-well plate. Seven microliters of 5 fmol/L synthetic target oligonucleotides, 10 μg/mL yeast tRNA (no target blank), and genomic DNA (15 ng/μL) were added, and denatured by incubation at 95℃ for 10 min. After 15 μL of mineral oil (Sigma, St. Louis, MO) was overlayed into all reaction wells, the plate was incubated isothermally at 63℃ for 4 h in a DNA thermalcycler (PTC-200; MJ Research, Watertown, MA) and then kept at 4℃ until fluorescence were measured. The intensity of the fluorescence was measured with a fluorescence microtiter plate reader (Cytoflour 4000; Applied Biosystems) with excitation at 485 nm/20 nm (Wavelength/Bandwidth) and emission at 530 nm/25 nm for FAM; and excitation at 560 nm/20 nm and emission at 620 nm/40 nm for RED. The genotyping was analyzed by calculating the ratio of net counts with wild primary probe to net counts with mutant primary probe. The probes used in this study were designed and synthesized by Third Wave Technologies, Inc (Madison, WI).

Data Analyses

Differences in means were evaluated with an analysis of variance. The analysis was performed with the statistical Package for Social Sciences (SPSS Japan Inc. ver. 11.5, Tokyo, Japan).

Results

We investigated the frequency and phenotypic association of *APOE* gene polymorphisms of 2,267 subjects. We found that the SNPs were in Hardy-Weinberg equilibrium. As previously described, the mean age, total cholesterol, TG, HDL-cholesterol, and LDL-cholesterol levels in this population were similar to the levels for all 12,839 patients in Serum Lipid Survey 2000[14]. We also found that the medians of total, LDL-, and HDL-cholesterol levels did not differ appreciably from the means, thereby excluding gross right-hand tailing of the distribution (data not shown). These data indicate that the participants in the gene analysis are representative of the general Japanese population.

The genotype and allelic frequency of *APOE* polymorphisms are presented in **Table 1**. The frequency of the ε2, ε3, and ε4 alleles was 4.2, 85.3, and 10.5%, respectively. As in other studies, the genotypes ε2ε2, ε2ε4, and ε4ε4 were quite rare. High frequencies of the ε3 allele are also found in Chinese, but the frequency is lower in Caucasians[15].

Table 1. Genotype and allele frequency of *APOE* gene in Japanese.

genotype	*n*	%	alleles	*n*	%
ε2/ε2	9	0.4	ε2	192	4.2
ε2/ε3	155	6.8	ε3	3,868	85.3
ε2/ε4	19	0.8	ε4	474	10.5
ε3/ε3	1,653	72.9			
ε3/ε4	407	18.0			
ε4/ε4	24	1.1			

We next examined the association of the *APOE* genotype and lipid profiles in these participants. As shown in **Table 2**, all the lipid parameters and blood glucose differed significantly among these genotypes by ANOVA. Total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, and RLP-cholesterol levels were different among the groups. The *p* values are shown in the right column. According to the post-hoc analysis, the total cholesterol level was significantly lower for genotype ε2ε2 than ε4ε4 and genotype ε2ε3 than ε3ε3, ε2ε4, or ε4ε4. The HDL-cholesterol level was significantly higher for ε2ε3 than ε2ε4. The LDL-cholesterol level was significantly lower for genotypes ε2ε2 and ε2ε3 than for ε3ε3, ε3ε4, and ε4ε4. The RLP-cholesterol level was significantly higher for ε2ε2 than ε2ε3, ε3ε3, ε3ε4, or ε4ε4 and for genotype ε2ε4 than ε2ε3, ε3ε3, or ε3ε4, although there was no significant difference in triglyceride levels according to the post-hoc analysis. Blood glucose or age did not differ significantly among the groups.

We next examined the association of the *MTHFR* C667T polymorphism with serum homocysteine levels in 505 samples randomly selected from 2,267 samples. As shown in **Table 3**, the incidence of the CC, CT, and TT genotypes was 33.9, 46.1, and 20.0%, respectively. The TT genotype was significantly associated with higher homocysteine levels in men and women, and statistical significance was found between CC and TT and between CT and TT by a post-hoc analysis. However, the difference was more prominent in men.

Discussion

There, we have shown in a large-scale study, the

Table 2. Mean of serum lipid levels and blood glucose in each genotype of *APOE* in Japanese.

	ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	total	*p* value
	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	
T-cho	165.0 ± 23.8	189.7 ± 3.00	202.9 ± 12.6	201.8 ± 0.92	206.8 ± 1.95	223.3 ± 9.18	202.1 ± 0.81	<0.0001
TG	171.4 ± 52.8	118.8 ± 8.55	189.0 ± 53.3	117.0 ± 2.41	128.0 ± 5.16	127.9 ± 18.9	119.8 ± 2.13	0.023
HDL-c	51.2 ± 9.51	63.6 ± 1.92	53.0 ± 3.07	59.8 ± 0.41	58.0 ± 0.82	61.9 ± 3.48	59.7 ± 0.36	0.007
LDL-c	70.5 ± 5.63	101.9 ± 2.75	117.2 ± 8.07	118.5 ± 0.91	120.5 ± 1.93	131.5 ± 7.97	117.7 ± 0.79	<0.0001
RLP-c	22.9 ± 1.15	4.4 ± 0.37	12.5 ± 7.59	4.7 ± 0.17	5.2 ± 0.33	4.1 ± 0.58	4.8 ± 0.15	<0.0001
FBS	121.3 ± 19.5	104.7 ± 3.27	110.6 ± 9.37	103.9 ± 0.94	103.3 ± 2.17	88.6 ± 2.54	103.9 ± 0.83	0.461
age	52.8 ± 10.1	49.5 ± 2.11	50.8 ± 53.2	46.7 ± 0.69	47.4 ± 1.30	43.2 ± 4.61	47.1 ± 0.58	0.659

T-cho: total cholesterol (mg/dL), TG: triglyceride (mg/dL), HDL-c: HDL-cholesterol (mg/dL), LDL-c: LDL-cholesterol (mg/dL), RLP-c: remnant-like particles cholesterol (mg/dL), FBS: fasting blood sugar (mg/dL), SEM: standard error of the mean

Table 3. Genotype frequency of the *MTHFR* gene and its association with serum homocysteine levels in Japanese.

total					
genotype	*n*	%	mean	SEM	
CC	171	33.9	10.9	0.3	$p<0.001$
CT	233	46.1	11.6	0.24	
TT	101	20.0	15.7	1.23	
total	505	100	12.2	0.29	
male					
genotype	*n*	%	mean	SEM	
CC	92	33.6	10.7	0.36	$p<0.001$
CT	132	48.2	12.9	0.35	
TT	50	18.2	19.8	2.41	
total	274	100	13.4	0.52	
female					
genotype	*n*	%	mean	SEM	
CC	79	34.2	10.2	0.43	$p=0.005$
CT	101	43.7	10.1	0.27	
TT	51	22.1	11.9	0.57	
total	231	100	10.5	0.23	

SEM: standard error of the mean

frequency of the *APOE* genotype in the Japanese and its association with serum lipid levels. Frequencies of *APOE* genotypes are highly heterogenous among various populations. Epidemiological data indicate that the frequency of the $\varepsilon 3$ allele is higher in Japanese and Chinese than in Caucasians, while the frequency of the $\varepsilon 4$ allele is lower in Asians than Caucasians[3, 16]. Our data indicate that the frequency of the $\varepsilon 3$ allele is quite consistent with previous reports in Japanese[8, 11, 16, 17], and is slightly higher than that of Icelandic and Hungarian populations and much higher than that in the Finnish population[15].

Our study confirmed that the $\varepsilon 4$ allele is associated with higher, and the $\varepsilon 2$ allele is associated with lower, LDL cholesterol levels. Although there was a trend for individuals with the genotypes $\varepsilon 2/\varepsilon 4$ and $\varepsilon 2/\varepsilon 2$ to have higher triglyceride levels, it was not statistically significant by a post-hoc analysis, probably because triglyceride levels are highly variable. However, individuals with $\varepsilon 2/\varepsilon 4$ and $\varepsilon 2/\varepsilon 2$ had significantly higher RLP-cholesterol levels than did those with the other genotypes, indicating that RLP-cholesterol might be better correlated with *APOE* genotype. Although in this study we could not compare the body mass index of $\varepsilon 2/\varepsilon 2$ homozygotes, it would be intriguing to know whether individuals with the $\varepsilon 2/\varepsilon 4$ and $\varepsilon 2/\varepsilon 2$ genotypes have metabolic abnormalities, such as abdominal obesity and insulin resistance, because they have higher triglyceride, RLP-cholesterol, and blood glucose levels.

Elevated levels of homocysteine have been considered a risk for cardiovascular disease. Our study is consistent with other studies that show higher homocysteine levels in people with the TT genotype. However, the relationship between the C677T *MTHFR* polymorphism and cardiovascular disease is still controversial. Because our study population is made up of healthy volunteers, a prospective study is necessary to determine which genotype is associated with cardiovascular risk.

In summary, we have provided the largest database of gene polymorphisms related to lipid metabolism and homocysteine in the general Japanese population. A prospective study is necessary to determine the contribution of these gene polymorphisms to cardiovascular risk in Japanese.

Acknowledgements

We thank Shizuya Yamashita (Osaka University) and Hideaki Bujo (Chiba University) for critical reading of the manuscript. This study was supported by research grants for health sciences from the Japanese Ministry of Health and a grant from the Japan Atherosclerosis Society. We also thank Osaka Pharmaceutical Manufactures Association for supporting our work.

References

1) Curtiss LK and Boisvert WA: Apolipoprotein E and atherosclerosis. Curr Opin Lipidol, 2000; 11:243-251
2) Davignon J, Gregg RE, and Sing CF: Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis, 1988; 8:1-21
3) Wang XL, McCredie RM, and Wilcken DE: Polymorphisms of the apolipoprotein E gene and severity of coronary artery disease defined by angiography. Arterioscler Thromb Vasc Biol, 1995; 15:1030-1034
4) Katzman R: Apolipoprotein E and Alzheimer's disease. Curr Opin Neurobiol, 1994; 4:703-707
5) Rall SC Jr. and Mahley RW: The role of apolipoprotein E genetic variants in lipoprotein disorders. J Intern Med, 1992; 231:653-659
6) Boushey CJ, Beresford SA, Omenn GS, and Motulsky AG: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA, 1995; 274:1049-1057
7) Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM, Palma-Reis RJ, Boers GH, Sheah-

an RG, Israelsson B, Uiterwaal CS, Meleady R, McMaster D, Verhoef P, Witteman J, Rubba P, Bellet H, Wautrecht JC, de Valk HW, Sales Luis AC, Parrot-Rouland FM, Tan KS, Higgins I, Garcon D, and Andria G: Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. Jama, 1997; 277:1775-1781

8) Ou T, Yamakawa-Kobayashi K, Arinami T, Amemiya H, Fujiwara H, Kawata K, Saito M, Kikuchi S, Noguchi Y, Sugishita Y, and Hamaguchi H: Methylenetetrahydrofolate reductase and apolipoprotein E polymorphisms are independent risk factors for coronary heart disease in Japanese: a case-control study. Atherosclerosis, 1998; 137:23-28
9) Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, and Ueland PM: Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol, 1998; 55:1449-1455
10) Brattstrom L, Wilcken DE, Ohrvik J, and Brudin L: Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation, 1998; 98:2520-2526
11) Shinomiya S, Sasaki J, Kiyohara C, Tsuji E, Inoue H, Marugame T, Handa K, Hayabuchi H, Hamada H, Eguchi H, Fukushima Y, and Kono S: Apolipoprotein E genotype, serum lipids, and colorectal adenomas in Japanese men. Cancer Lett, 2001; 164:33-40
12) Johnson CL, Rifkind BM, Sempos CT, Carroll MD, Bachorik PS, Briefel RR, Gordon DJ, Burt VL, Brown CD, Lippel K, and Cleeman JI: Declining serum total cholesterol levels among US adults. The National Health and Nutrition Examination Surveys. Jama, 1993; 269:3002-3008
13) Ubbink JB, Hayward Vermaak WJ, and Bissbort S: Rapid high-performance liquid chromatographic assay for total homocysteine levels in human serum. J Chromatogr, 1991; 565:441-446
14) Arai H, Yamamoto A, Matsuzawa Y, Saito Y, Yamada N, Oikawa S, Mabuchi H, Teramoto T, Sasaki J, Nakaya N, Itakura H, Ishikawa Y, Ouchi Y, Horibe H, Egashira T, Hattori H, Shirahashi N, and Kita T: Polymorphisms in four genes related to triglyceride and HDL-cholesterol levels in the general Japanese population in 2000. J Atheroscler Thromb, 2005; 12:240-250
15) Hallman DM, Boerwinkle E, Saha N, Sandholzer C, Menzel HJ, Csazar A, and Utermann G: The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am J Hum Genet, 1991; 49:338-349
16) Eto M, Watanabe K, and Ishii K: Reciprocal effects of apolipoprotein E alleles (epsilon 2 and epsilon 4) on plasma lipid levels in normolipidemic subjects. Clin Genet, 1986; 29:477-484
17) Kobori S, Nakamura N, Uzawa H, and Shichiri M: Influence of apolipoprotein E polymorphism on plasma lipid and apolipoprotein levels, and clinical characteristics of type Ⅲ hyperlipoproteinemia due to apolipoprotein E phenotype E2/2 in Japan. Atherosclerosis, 1988; 69:81-88

Circ J 2007; **71**: 1697–1702

Increased Incidence of Coronary In-Stent Restenosis in Type 2 Diabetic Patients is Related to Elevated Serum Malondialdehyde-Modified Low-Density Lipoprotein

Sakuji Shigematsu, MD; Naohiko Takahashi, MD*; Masahide Hara, MD*; Hironobu Yoshimatsu, MD*; Tetsunori Saikawa, MD**

Background Type 2 diabetes mellitus (T2DM) has been reported as a major risk factor for in-stent restenosis (ISR) after intracoronary stenting, although the details of the mechanisms remain undefined. The aim of present study was to investigate the diabetes-related risk factor for ISR.

Methods and Results A total of 131 patients who were implanted with bare metal stent(s) were enrolled in this study. Based on follow-up coronary angiography at 6 months after stenting, the patients were classified according to the presence or absence of ISR. Various coronary risk factors, including serum malondialdehyde-modified low-density lipoprotein (MDA-LDL) levels, were investigated at follow-up angiogram to relate to ISR in patients with or without T2DM. The increased incidence of ISR was observed in diabetic patients, which was significantly related to the increased serum MDA-LDL concentrations. The serum MDA-LDL concentration was positively correlated to glycohemoglobin levels in diabetic patients. In addition, MDA-LDL concentration was not altered after the treatment of ISR.

Conclusions The elevated serum MDA-LDL level is considered to be a potent risk factor for ISR in diabetic patients. MDA-LDL, which might be a consequence of metabolic abnormalities caused by diabetes, may act as a growth factor for neointimal tissues inside the implanted stent. (*Circ J* 2007; **71**: 1697–1702)

Key Words: Angioplasty; Diabetes mellitus; Lipids; Restenosis; Stents

In-stent restenosis (ISR) remains the major limitation to long-term positive outcomes after percutaneous coronary intervention (PCI)[1,2] Pathological analyses indicate that the formation of neointimal tissues in the implanted stent is a major cause of ISR, which is characterized by an inflammatory reaction at the site of balloon-induced vascular injury, the migration and proliferation of vascular smooth muscle cells, and the synthesis of excess matrix. Despite the well-defined nature of the lesion, the pathogenic mechanisms leading to accelerated neointimal hyperplasia remain largely undefined. Multiple studies have examined potential risk factors for ISR and have reported that conventional coronary risk factors such as hypertension, hyperlipidemia, smoking habits, and a family history of coronary artery disease (CAD) are not associated with an increased risk of ISR[2,3] This may be attributable to differences in the pathology of post-stenting restenosis and atheromatous plaque in CAD[4]

In contrast, type 2 diabetes mellitus (T2DM) has been reported as a strong risk factor for ISR, possibly because patients with diabetes display a tendency toward exaggerated intimal hyperplasia inside the stent[3,5,6] Studies in animal models have suggested that the inflammatory and proliferative responses to balloon-induced vascular injury are enhanced in diabetes[7,8] The metabolic alterations that occur as a result of hyperglycemia or hyperinsulinemia can accelerate many of the pathophysiologic processes that lead to restenosis. Diabetes results in endothelial dysfunction and accelerated platelet deposition, both of which increase the propensity to thrombosis. Several growth factors known to promote the restenosis process are overexpressed in the presence of hyperglycemia. Advanced glycosylation promotes inflammatory cell recruitment and smooth muscle cell proliferation[9] Although the accumulating evidence from in vitro and in vivo studies demonstrate the increased risk of ISR in diabetes, the precise mechanism underlying the clinical observations remains uncertain.

Oxidative modification of low-density lipoprotein (LDL) has been demonstrated to play a central role in the initiation and acceleration of atherosclerosis[10] Oxidized LDL exerts several proatherogenic effects, including direct cytotoxicity to endothelial cells, the promotion of increased synthesis and secretion of adhesion molecules, increased monocyte chemotaxis and adhesion, and enhanced foam cell formation in atherosclerotic lesions[11,12] It has been reported that oxidized LDL promotes vascular smooth muscle cell proliferation[13,14] Recently, immunoassays using monoclonal antibodies prepared against oxidized LDL demonstrated that human blood contains oxidized LDL and that higher levels were found in patients with CAD[15] Serum level of

(Received February 8, 2007; revised manuscript received June 16, 2007; accepted July 30, 2007)

*Department of Cardiology, National Hospital Organization Beppu Medical Center, Beppu, Departments of *Internal Medicine and **Laboratory Medicine, Faculty of Medicine, Oita University, Oita, Japan*

Mailing address: Sakuji Shigematsu, MD, Department of Cardiology, National Hospital Organization Beppu Medical Center, 1473 Uchikamado, Beppu 874-0011, Japan. E-mail: sshige@med.oita-u.ac.jp

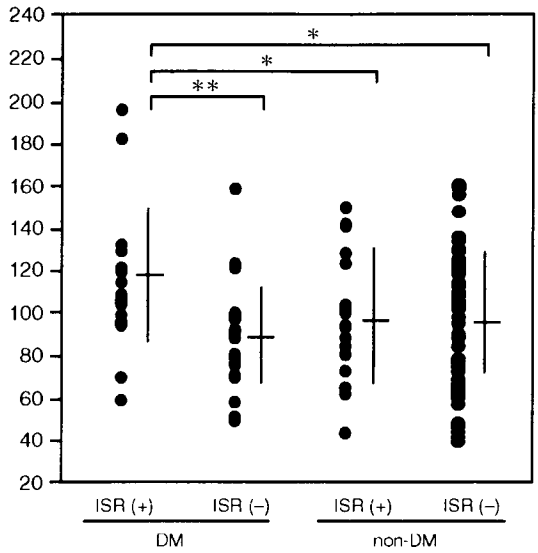

Fig 1. Serum malondialdehyde-modified low-density lipoprotein (MDA-LDL) concentrations in diabetic (DM) and non-diabetic (non-DM) patients with or without in-stent restenosis (ISR). Serum MDA-LDL concentrations were significantly higher in diabetic patients who developed ISR than in patients of the other groups. Data are means ± SD. Statistical analysis was performed with 1-way analysis of variance followed by Fisher's post-hoc test. Significant differences between the groups are indicated. *p<0.05, **p<0.01.

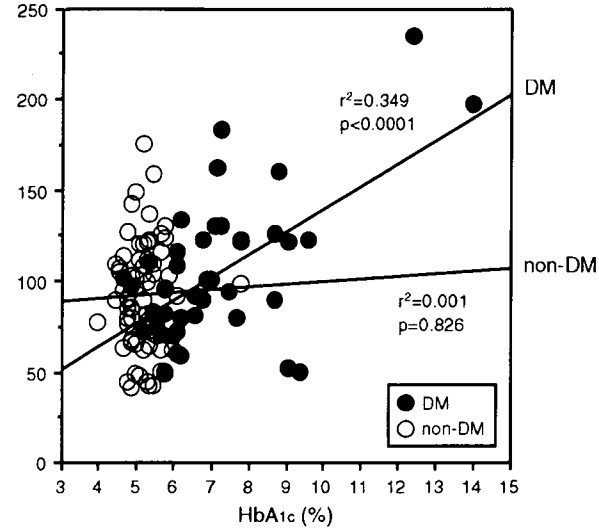

Fig 2. Linear regression analysis and Pearson correlation coefficients for comparisons between the glycohemoglobin (HbA_{1c}) and serum malondialdehyde-modified low-density lipoprotein (MDA-LDL) concentrations in diabetic (DM) and non-diabetic (non-DM) patients. A positive correlation was seen only for diabetic patients ($p<0.0001$, $r^2=0.349$).

oxidized LDL was also increased in diabetic patients who developed vascular complications![16] A high concentration of circulating oxidized LDL is considered as an independent and significant predictor for future cardiac events in T2DM patients with CAD![17] Although the nature of the oxidized LDL in the serum of diabetic patients has not been defined, adverse effects of circulating oxidized LDL in diabetes have been strongly suggested. In the present study, we investigated the potential risk factors for ISR, especially in T2DM patients, and we disclose for the first time that an elevated serum level of malondialdehyde-modified LDL (MDA-LDL), a major oxidized LDL, relates to the increase in the incidence of ISR observed in diabetic patients.

Methods

Patient Population

A total of 131 patients (old myocardial infarction, 65; stable effort angina pectoris, 66) who underwent coronary stenting were enrolled in this study. Patients with acute myocardial infarction, unstable angina pectoris, chronic total occluded lesion, renal failure (serum creatinine >2 mg/dl), liver disorder, or malignancy were excluded. All patients were treated with 100 mg/day aspirin and 200 mg/day ticlopidine after stenting. Ticlopidine was given for 1 month after stenting, but aspirin was administered indefinitely. Subacute stent thrombosis did not occur in the enrolled patients. None of the patients in this study received drug-eluting stents, intracoronary brachytherapy, or any other type of investigational local drug therapy or investigational stent implantation.

Coronary Angiographic Evaluation

All patients who received intracoronary stent(s), underwent an angiographic follow-up examination 6 months after stenting. Coronary angiograms were obtained in multiple projections in identical views at baseline, immediately after stent placement, and at follow-up after 6 months, and were stored as digital images. Quantitative angiographic analysis was made by operators unaware of the clinical characteristics of the patients, using the automated edge detection system (CAAS II for Toshiba Infinix; Pie Medical imaging, The Netherlands). The length of the stenosis was estimated with electronic calipers and the contrast-filled catheter tips were used for calibration. The diameters of the proximal and distal reference segments were averaged by the system to yield the reference luminal diameter (RD). The percent diameter stenosis was calculated as the difference between the minimal luminal diameter (MLD) and RD. Binary ISR was defined as >50% diameter stenosis at follow-up.

Measurement of MDA-LDL

Venous blood sampling, including MDA-LDL, was performed in the fasting state upon admission for follow-up angiography. In some diabetic patients, MDA-LDL was measured just before and 30 min after treatment of ISR (ie, re-ballooning of restenosis site). We used an enzyme-linked immunosorbent assay for the detection of MDA-LDL, based on the principles previously reported by Kotani et al![18] Venous blood samples were collected and the sera were separated within 4 h. Prior to storage at −20°C, the serum samples were mixed with a stabilizing reagent according to the procedure described by Kitano et al![19] The samples were diluted 2,000-fold in a dilution buffer containing SDS. Duplicate 100-μl aliquots of the diluted sample were then added to the wells of plates coated with monoclonal antibody against MDA-LDL (ML25; Daiich Pure Chemicals, Tokyo, Japan). ML25 has previously been shown to recognize MDA residues. After incubation for 2 h at room temperature, the plates were washed, and β-galactosidase-conjugated monoclonal antibody against apoprotein B (AB16; Daiich Pure Chemicals) was added. It has been shown that the combination of positive immunoreactions with both ML25 and

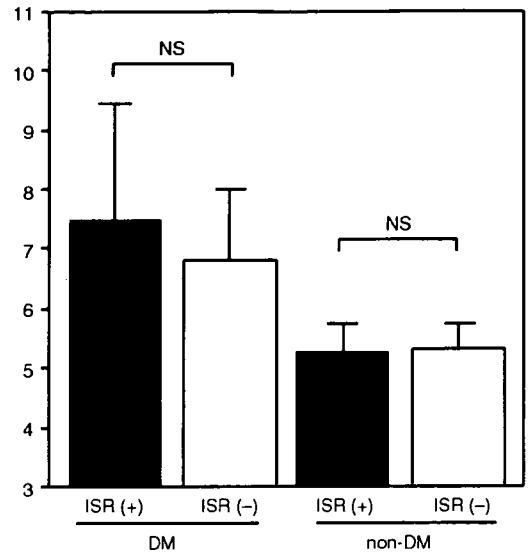

Fig 3. Glycohemoglobin (HbA_{1c}) levels in diabetic (DM) and non-diabetic (non-DM) patients with or without in-stent restenosis (ISR). HbA_{1c} levels were not significant difference between ISR (+) and ISR (−) in both DM and non-DM patients. Data are means ± SD. Statistical analysis was performed with 1-way analysis of variance followed by Fisher's post-hoc test. NS, no significance.

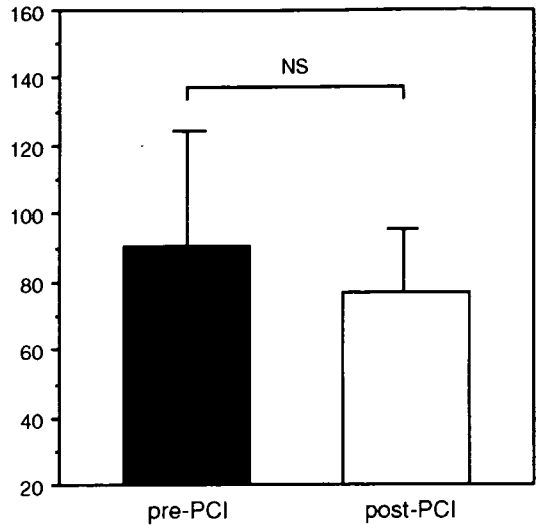

Fig 4. Change in the serum malondialdehyde-modified low-density lipoprotein (MDA-LDL) level before (pre-percutaneous coronary intervention (pre-PCI)) and after (post-PCI) treatment of in-stent restenosis. Serum MDA-LDL was not statistically different after the mechanical crushing of the neointima with a PCI balloon. Data are means ± SD. Statistical analysis was performed with Student's unpaired t-test. NS: no significance.

Table 1 Clinical and Biochemical Data Relating to In-Stent Restenosis in the Enrolled Patients

Characteristic	*Restenosis (+) (n=38)*	*Restenosis (−) (n=93)*	*Univariate p value*	*Multivariate p value*
Age (years)	*69 (10)*	*69 (11)*	*0.981*	*NS*
Sex (M/F)	*32/6*	*60/33*	*0.023**	*NS*
OMI/SAP	*17/21*	*47/46*	*0.547*	*NS*
Body mass index (kg/m²)	*23.3 (2.9)*	*24.5 (4.2)*	*0.119*	*NS*
T2DM	*50%*	*26%*	*0.009***	*0.009***
Hypertension	*47%*	*51%*	*0.694*	*NS*
Smoking	*20%*	*22%*	*0.786*	*NS*
Medication of statin	*32%*	*57%*	*0.014**	*NS*
Fasting plasma glucose (mg/dl)	*109 (30)*	*104 (24)*	*0.344*	*NS*
Glycohemoglobin (%)	*6.3 (1.8)*	*5.7 (1.1)*	*0.037**	*NS*
LDL-cholesterol (mg/dl)	*109 (24)*	*105 (22)*	*0.408*	*NS*
Triglyceride (mg/dl)	*121 (56)*	*117 (55)*	*0.761*	*NS*
HDL-cholesterol (mg/dl)	*43.1 (12.2)*	*45.9 (11.2)*	*0.208*	*NS*
Remnant-like particle (mg/dl)	*4.0 (1.5)*	*4.1 (1.9)*	*0.772*	*NS*
Lipoprotein(a)	*19.2 (14.4)*	*24.6 (21.7)*	*0.200*	*NS*
Uric acid (mg/dl)	*6.1 (1.6)*	*5.8 (1.3)*	*0.199*	*NS*
hs-CRP	*0.179 (0.220)*	*0.138 (0.175)*	*0.298*	*NS*
MDA-LDL (U/L)	*107.0 (34.2)*	*95.4 (29.6)*	*0.058*	*NS*
Stent diameter (mm)	*3.0 (0.4)*	*3.0 (0.4)*	*0.766*	*NS*
Stent length (mm)	*16.6 (4.5)*	*15.5 (3.5)*	*0.109*	*NS*
MSA (mm²)	*6.38 (1.58)*	*6.45 (1.55)*	*0.782*	*NS*

*Data are means (SD) or percentages. Statistical analysis was conducted using a stepwise multiple regression analysis. Values of p<0.05 indicate significant differences. *p<0.01; **p<0.05.*
NS, no significance; OMI, old myocardial infarction; SAP, stable angina pectoris; T2DM, type 2 diabetes mellitus; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; MDA-LDL, malondialdehyde-modified LDL; MSA, minimum stent area immediately after percutaneous coronary intervention.

AB16 recognizes MDA-LDL. After an additional incubation for 1 h at room temperature, the plates were washed, and 100-μl of 10 mmol/L *o*-nitrophenyl-galactopyranoside was added. The reaction was stopped after 2 h by adding 100-μl of 0.2 mol/L sodium carbonate (pH 12). The absorbance at 405 nm and the reference absorbance at 650 nm were determined using a microplate reader (M-Vmax, Molecular Devices, CA). A prepared solution of MDA-LDL was used as a primary standard, in which 15% of the total amino groups were modified. We tentatively defined 1 unit/L MDA-LDL as the absorbance obtained with the primary standard at a concentration of 1 mg/L. A calibration curve was prepared using 500- to 8,000-fold dilutions of a reference serum, the secondary standard, and the amount of MDA-LDL in the samples was calculated from the curve. The reference sera were prepared from pooled sera from

Table 2 Clinical and Biochemical Data Relating to In-Stent Restenosis in T2DM Patients

Characteristic	Restenosis (+) (n=19)	Restenosis (−) (n=24)	Univariate p value	Multivariate p value
Age (years)	66 (11)	69 (11)	0.442	NS
Sex (M/F)	17/2	12/13	0.012*	NS
OMI/SAP	8/11	12/13	0.607	NS
Body mass index (kg/m^2)	23.7 (3.1)	26.0 (4.8)	0.085	NS
Hypertension	41%	58%	0.282	NS
Smoking	35%	17%	0.179	NS
Insulin treatment	35%	33%	0.903	NS
Medication of statin	21%	54%	0.032*	NS
Fasting plasma glucose (mg/dl)	121 (19)	131 (35)	0.351	NS
Glycohemoglobin (%)	7.2 (2.0)	6.9 (1.3)	0.576	NS
LDL-cholesterol (mg/dl)	119 (21)	104 (19)	0.180	NS
Triglyceride (mg/dl)	140 (65)	116 (41)	0.165	NS
HDL-cholesterol (mg/dl)	40.5 (8.7)	49.4 (12.5)	0.024*	NS
Remnant-like particle (mg/dl)	4.2 (1.7)	4.4 (1.8)	0.790	NS
Lipoprotein(a)	25.6 (17.1)	25.4 (21.7)	0.978	NS
Uric acid (mg/dl)	6.4 (1.7)	5.8 (1.2)	0.199	NS
hs-CRP	0.104 (0.096)	0.158 (0.167)	0.251	NS
MDA-LDL (U/L)	117.2 (32.2)	88.2 (24.1)	0.008**	0.008**
Stent diameter (mm)	3.0 (0.2)	2.9 (0.3)	0.207	NS
Stent length (mm)	16.0 (4.2)	15.8 (3.6)	0.446	NS
MSA (mm^2)	6.35 (1.07)	5.98 (1.25)	0.252	NS

*Data are means (SD) or percentages. Statistical analysis was conducted using a stepwise multiple regression analysis. Values of p<0.05 indicate significant differences. *p<0.01; **p<0.05.*
Abbreviations see in Table 1.

healthy volunteers. In the preliminary study, serum MDA-LDL level in the healthy volunteers was 58.8±17.9 U/L (n=86) (unpublished data).

Statistical Analysis

A simultaneous and a stepwise multiple regression analysis were used to evaluate the independent association of various risk factors with ISR. A comparison of the variables among 4 groups (Figs 1,3) was performed using 1-way analysis of variance with a post-hoc test (Fisher's PLSD). Linear regression analysis and Pearson correlation coefficients was used for comparisons between the glycohemoglobin and MDA-LDL concentrations (Fig 2). Student's unpaired t-test was used for comparison of MDA-LDL level before and after PCI (Fig 4). Statistical significance was defined as $p<0.05$.

Results

Clinical and laboratory characteristics of the enrolled patients are shown in Table 1. Of 131 patients, 38 developed ISR within 6 months after stenting and 93 patients did not. A stepwise multiple regression analysis was used to evaluate the independent association of various risk factors with ISR. Sex difference (male gender), T2DM, and elevation of glycohemoglobin (HbA_{1c}) were positive univariate factors, and medication of HMG-CoA reductase inhibitor (statin) was a negative univariate factor for ISR. However, T2DM was the only independent risk factor for ISR (Table 1).

The analysis shown in Table 1 clearly indicates that T2DM is a significant risk factor for ISR. In fact, the percentage ISR rate was 44.2% in diabetic patients (19/43 patients) compared with 21.6% in non-diabetic patients (19/88 patients). Thus, we investigated the diabetes-related risk factors that promote ISR. A stepwise multiple regression analysis was also used to evaluate the independent association of these variables with ISR in diabetic patients (Table 2). Male gender and elevated serum MDA-LDL concentration were positive univariate factors, and medication with HMG-CoA reductase inhibitor (statin) and the HDL-cholesterol concentration were negative univariate factors for ISR. However, only elevation of the serum MDA-LDL concentration was an independent risk factor for ISR (Table 2).

Fig 1 compares the average concentration of serum MDA-LDL in diabetic and non-diabetic patients with or without ISR. A significant elevation of serum MDA-LDL concentration was observed only in the diabetic patients who developed ISR. We examined whether the serum MDA-LDL level relates to the control of diabetes and it was positively correlated to HbA_{1c} in diabetic patients (Fig 2). Interestingly, this trend was not observed in non-diabetic patients. In contrast, the HbA_{1c} level was not statistically different between the ISR and non-ISR groups of diabetic patients (Fig 3). These results suggest the possibility that an elevated plasma glucose level does not directly promote ISR, but rather the metabolic abnormality caused by a high plasma glucose level increases serum MDA-LDL, which results in the increased rate of ISR. To exclude the possibility that an elevated serum MDA-LDL is simply derived from the restenosis site (ie, neointima in the stent), we investigated whether the serum MDA-LDL level is altered after treatment of ISR, but as shown in Fig 4, it was not significantly decreased after treatment (ie, mechanical crushing of the neointima with a PCI balloon). This result strongly suggests that an elevated level of serum MDA-LDL in diabetic patients is not merely a consequence of restenosis.

Discussion

T2DM is a serious risk factor for poor outcome after percutaneous transluminal coronary angioplasty.[21] Although coronary stents improve the acute results and decrease the rate of restenosis compared with balloon angioplasty, diabetes remains a key independent predictor of ISR.[5,21,22] After stenting of native coronary arteries, diabetic patients