high output heart failure showed decrease in both pulmonary and systemic vascular resistance[6]. The removal of plasmacytoma decreased the serum VEGF level and improved clinical symptoms. Therefore, elevated serum VEGF can explain many of the clinical features containing cardiac manifestations in this syndrome. Although the hemodynamic disorder in our case was similar to theirs, we could not measure VEGF at the first admission. The time interval from the first admission till the measurement of VEGF was long. Therefore, elevated serum VEGF could not be proved as the cause of the high output heart failure in our case. However, low grade fever under 38 ℃, mild anemia and slightly decreased serum thiamine level could not explain the marked increase in cardiac output and decrease in vascular resistance. Furthermore, we used neither steroid hormone nor alkylating agents that influence serum VEGF level. Therefore, the serum VEGF level may have been high from the beginning of heart failure and caused the clinical features in our case.

Steroid treatment improved the pulmonary hypertension and high VEGF level in another case[8]. The pulmonary hypertension may have been caused by the increase in vascular resistance. Presumably a vicious cycle was established in which interstitial/perivascular edema due to hyperpermeability caused by VEGF reduced gas diffusion and up-regulated the expression of VEGF. However, hypoxia was not documented and pulmonary vascular resistance fell in our case. VEGF stimulates vasodilation and endothelial nitric oxide production except for vascular hyperpermeability and angiogenesis[9]. Therefore, the main cause of pulmonary hypertension may be the high output secondary to vasodilation with VEGF elevation rather than the increase in vascular resistance.

In contrast, a young female presented with ischemic cardiomyopathy with severe coronary stenoses and reduced ejection fraction[5]. An abnormal immune reaction containing VEGF elevation may have caused coronary arteritis. Although the treatment improved the ejection fraction as VEGF decreased, the hemodynamic data containing cardiac output and vascular resistance were not reported. There was no coronary artery stenosis and ejection fraction was normal in our case.

Other types of cardiac manifestations with this syndrome have also been reported: A case of left ventricular hypertrophy similar to hypertrophic cardiomyopathy with normal coronary artery[10] (cardiac output was unknown), a case of moderate left ventricular hypertrophy and pulmonary hypertension[11] with marked elevation of both pulmonary and systemic vascular resistance, a case with normal systemic vascular resistance and markedly elevated pulmonary vascular resistance[12], and a case resembling dilated cardiomyopathy with normal coronary artery[13] (cardiac output and vascular ressistance were unknown).

These cardiac manifestations were different to those of our case. Therefore, cardiac involvement of this syndrome can vary widely. It is not clear whether only VEGF can explain all these various patterns of cardiac complications systematically because serum VEGF was not measured in those four cases. Further examination is required to determine the uniform mechanism including any other bioactive products in addition to VEGF which may be important in the wide spectrum of cardiac involvement of this syndrome.


## 要　約

### 血管内皮増殖因子の上昇を伴い高心拍出量性心不全を呈した Crow-Fukase症候群の1例

白木　照夫　佐藤　慎二　杉山　洋樹　小林　誠
高村　俊行　梶山　晃雄　斎藤　大治

Crow-Fukase症候群は，形質細胞の傷害に基づく疾患で，うっ血性心不全はその生命予後を左右する最大の合併症である．57歳の男性が，浮腫と発熱を主訴に当院へ入院した．神経所見では手袋と靴下型の末梢神経障害が認められ，血液検査で副腎皮質刺激ホルモン，甲状腺刺激ホルモンおよび免疫グロブリンA型のM成分が上昇していた．全身の皮膚にはポリープ様病変がみられ，心臓を含む内臓の腫大があった．これらの臨床所見から，本症例はCrow-Fukase症候群と診断された．

心臓カテーテル検査では，高心拍出性心不全と肺高血圧が認められた．利尿薬が水分過剰状態の改善に有効で，Ca拮抗薬とアンジオテンシン変換酵素阻害薬の併用が体血圧の低下に有効であった．血清中の血管内皮増殖因子は著明に上昇しており，本症候群の心症状への関与が疑われた．

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# Targeted Deletion of Class A Macrophage Scavenger Receptor Increases the Risk of Cardiac Rupture After Experimental Myocardial Infarction

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***Background***—Class A macrophage scavenger receptor (SR-A) is a macrophage-restricted multifunctional molecule that optimizes the inflammatory response by modulation of the activity of inflammatory cytokines. This study was conducted with SR-A–deficient ($SR\text{-}A^{-/-}$) mice to evaluate the relationship between SR-A and cardiac remodeling after myocardial infarction.

***Methods and Results***—Experimental myocardial infarction (MI) was produced by ligation of the left coronary artery in $SR\text{-}A^{-/-}$ and wild-type (WT) male mice. The number of mice that died within 4 weeks after MI was significantly greater in $SR\text{-}A^{-/-}$ mice than in WT mice ($P=0.03$). Importantly, death caused by cardiac rupture within 1 week after MI was 31% (17 of 54 mice) in $SR\text{-}A^{-/-}$ mice and 12% (6 of 51 mice) in WT mice ($P=0.01$). In situ zymography demonstrated augmented gelatinolytic activity in the infarcted myocardium in $SR\text{-}A^{-/-}$ mice compared with WT mice. Real-time reverse transcription–polymerase chain reaction at day 3 after MI showed that the expression of matrix metalloproteinase-9 mRNA increased significantly in the infarcted myocardium in $SR\text{-}A^{-/-}$ mice compared with WT mice. Furthermore, $SR\text{-}A^{-/-}$ mice showed augmented expression of tumor necrosis factor-$\alpha$ and reduction of interleukin-10 in the infarcted myocardium at day 3 after MI. In vitro experiments also demonstrated increased tumor necrosis factor-$\alpha$ and decreased interleukin-10 expression in activated $SR\text{-}A^{-/-}$ macrophages.

***Conclusions***—The present findings suggest that SR-A deficiency might cause impairment of infarct remodeling that results in cardiac rupture via insufficient production of interleukin-10 and enhanced expression of tumor necrosis factor-$\alpha$ and of matrix metalloproteinase-9. SR-A might contribute to the prevention of cardiac rupture after MI. (***Circulation.*** **2007; 115:&NA;-.)**

**Key Words:** cytokines ■ macrophages ■ myocardial infarction ■ receptors ■ remodeling

Myocardial infarction (MI) causes complex structural alterations that involve both the infarcted and noninfarcted left ventricular (LV) myocardium.[1] The dynamic synthesis and breakdown of extracellular matrix (ECM) proteins play an important role in the post-MI LV remodeling,[2] which is a compensatory mechanism against LV dysfunction. However, the excessive degradation of ECM components in the infarcted regions appears to lead to pathological cardiac remodeling, which results in LV rupture.[3]

LV rupture is a lethal complication that accounts for 5% to 30% of in-hospital mortality in patients with acute MI and is most likely to occur during the first week after the onset of symptoms.[4] In addition, it is difficult to predict LV rupture in spite of the management of several risk factors such as transmural MI, a first MI, and hypertension. Recent studies have demonstrated that members of the matrix metalloproteinase (MMP) gene family play a central role in the degradation of ECM after MI.[5–7] Therefore, MMPs appear to play a major role in post-MI LV rupture. Furthermore, it has been shown that a key component of LV healing and remodeling after MI is the inflammatory response triggered by a wide variety of chemoattractants and inflammatory cytokines, which can modulate post-MI LV tissue repair.[8,9] We and others have reported that macrophage infiltration into the infarcted myocardium accelerated LV remodeling via increased activity of MMPs, which indicates macrophages that infiltrate into the infarcted regions are important contributors to the modulation of LV remodeling after MI.[10–12]

**Clinical Perspective p 0000**

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Class A macrophage scavenger receptor (SR-A) is the prototypic member of an expanding family of membrane receptors collectively termed scavenger receptors. SR-A is also a macrophage-restricted multifunctional molecule.[13-15] SR-A can bind with high affinity to an unusually broad range of polyanionic ligands, which includes modified lipoproteins, lipopolysaccharide of Gram-negative bacteria, lipoteichoic acid of Gram-positive species, β-amyloid, and advanced glycation end products. On the basis of this broad ligand specificity, SR-A may play a role in a wide range of macrophage-associated physiological and pathophysiological processes.[16] Previous studies reported that SR-A was associated with the modification of atherosclerosis, macrophage adhesion, host defense, clearance of dying cells, and nervous system disorders. Moreover, Cotena et al recently showed that SR-A could ensure an inflammatory response of the appropriate magnitude via modulation of the activities of proinflammatory receptors and the production of several chemokines.[17] This suggests that SR-A might regulate inflammation itself and consequent tissue remodeling via macrophage function in the pathological conditions such as MI. However, there is no evidence that shows the involvement of SR-A in LV remodeling after MI. In the current paper, we report that a deficiency in the SR-A gene (SR-A$^{-/-}$) contributes to the risk of cardiac rupture after experimental MI via the modification of inflammatory cytokines and MMP expression.

## Methods

### Animals

Mice with targeted disruption of the SR-A gene (SR-A$^{-/-}$),[18] which is essential for the formation of SR-A, were used after at least the 8th backcross into the control C57BL/6J strain was reached.[19] Genotyping of animals was performed by use of polymerase chain reaction (PCR) of DNA obtained from tail biopsies. Recipient C57BL/6J mice and wild-type (WT) mice of the same genetic background were originally purchased from the Jackson Laboratory (Bar Harbor, Me). SR-A$^{-/-}$ and WT male mice were bred at the Animal Resource Facility at the Kumamoto University under specific pathogen-free conditions. All animal procedures were approved by the Animal Research Committee at Kumamoto University, and all procedures conformed to the *Guide for the Care and Use of Laboratory Animals* by the Institute of Laboratory Animal Resources. The SR-A$^{-/-}$ and WT mice were fed a regular chow diet and were used for experiments between 8 and 12 weeks of age.

### Left Coronary Ligation

Mice were anesthetized with pentobarbital sodium (70 mg/kg) via intraperitoneal injection, and MI was induced by permanent occlusion of the left anterior descending coronary artery with an 8-0 Prolene suture under artificial ventilation, as previously described by our laboratory.[10] Significant electrocardiographic and color changes in the ischemic area were considered indicative of successful coronary occlusion. In the sham experiments, the same surgical procedure was performed, with the exception of coronary ligation.

### Survival Rate

To evaluate survival after MI, the operation and autopsy were performed by a group of investigators who were blinded to the results of the genotyping. SR-A$^{-/-}$ and WT mice underwent coronary artery ligation and were monitored rigorously for morbidity and mortality. Autopsy was immediately performed in each animal after death to determine the cause of death, particularly with reference to cardiac rupture.

### Echocardiography and Organ Weight Measurement for Assessment of LV Function

Echocardiographic measurements at baseline and on days 7 and 28 after surgery were performed with a Sonos 4500 with a high-frequency transducer (12 MHz; Philips Co., Tokyo, Japan) as previously described.[10,20] Good 2-dimensional views of the LV were obtained for guided M-mode measurements of interventricular septal wall thickness (IVS), posterior wall thickness (PW), LV end-diastolic diameter (LVDd), and end-systolic diameter (LVDs) as surrogate markers of LV dilatation caused by LV remodeling. M-mode percent fractional shortening (%FS) and LV mass were calculated by the following formulas:

$$(1)\qquad \%FS=\frac{LVDd-LVDs}{LVDd}\times 100$$

and

$$(2)\qquad LV\ mass=1.055\times[(IVS+PW+LVDd)^3-LVDd^3]\times 10^3,$$

respectively.[21] After in vivo echocardiographic studies at day 7 post-MI, the heart and lung were excised and their weights were determined. Moreover, the lungs were used to determine water content as a marker of lung congestion by calculation of the wet-to-dry ratio after desiccation for 24 hours at 50°C.[22]

### Light Microscopy and Morphometric Analysis

At days 1, 3, 5 and 7 after coronary ligation, mice were euthanized for gross and microscopic cardiac analysis, with 6 to 10 animals studied at each time point. Heart tissues were fixed in 4% paraformaldehyde solution at 4°C for 4 hours, embedded in OCT compound (Sakura Finetechnical Co., Tokyo, Japan), frozen in liquid nitrogen, and cut by a cryostat into sections 6 μm thick. Sections were routinely stained with hematoxylin and eosin for light microscopy and with Masson's trichrome for evaluation of myocardial fibrosis. Infarct size and infarct area were determined by a previously reported method.[10] In brief, infarct size (%) was calculated as the total infarct circumference divided by the total LV circumference times 100; infarct area was calculated as the percentage of MI area relative to the entire LV tissue area.

### Immunohistochemistry

Immunohistochemistry was performed according to an indirect immunoperoxidase method with the following antibodies: anti-CD204 for SR-A (2F8; Serotec, Oxford, UK); anti-CD68 for macrophages (FA-11; Serotec); anti-Ly-6G for granulocytes (Gr-1; Southern Biotechnology, Birmingham, Ala); anti-CD31 for endothelial cells (MEC13.3; Pharmingen, San Diego, Calif); anti–smooth muscle α-actin for myofibroblasts (1A4; Dako, Glostrup, Denmark). After inhibition of endogenous peroxidase activity by the method of Isobe et al,[23] the sections were incubated with the monoclonal antibodies described above at 4°C overnight. Goat anti-rat Ig-conjugated peroxidase-labeled polymer amino acid (Nichirei, Tokyo, Japan) was used as the secondary antibody. After visualization with 3,3′-diaminobenzidine, sections were stained with hematoxylin for nuclear staining and were mounted with resin. As negative controls, the same procedures were performed but without the primary antibodies. For cell enumeration, the number of positive cells in the infarcted region for each antibody was counted and expressed as the number per mm$^2$.

### Detection of Gelatinolytic Activity by In Situ Zymography

To detect gelatinolytic activity in the infarcted heart tissues, we performed in situ zymography by use of a previously reported approach.[10] Gelatin films with sections were incubated for 6 hours at 37°C in a moisture chamber and stained with Biebrich Scarlet solution (Wako, Osaka, Japan). The gelatin in contact with the proteolytic areas of the sections was digested, which demonstrated zones of enzymatic activity indicated by negative staining.

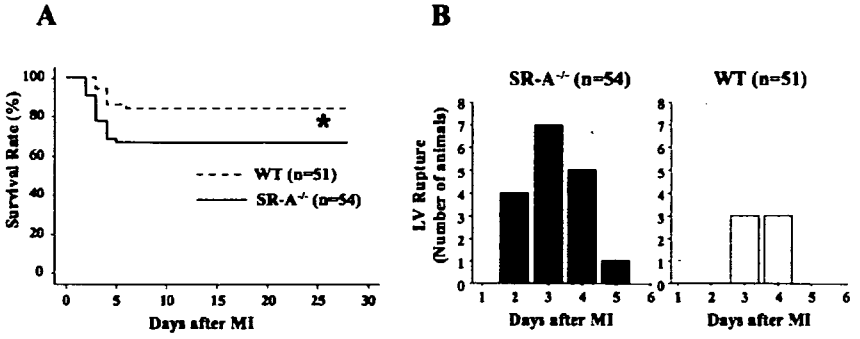


**Figure 1.** A, Survival of SR-A$^{-/-}$ and WT mice after MI. Survival curves are plotted for both SR-A$^{-/-}$ mice (n=54) and WT mice (n=51) as determined with the Kaplan-Meier method. *Log-rank $P$=0.027. B, Number of mice with cardiac rupture in SR-A$^{-/-}$ (17 of 54 mice) and WT (6 of 51 mice) mice within 1 week post-MI.

### Real-Time Reverse Transcriptase-PCR Assay

Total RNA from heart tissues at day 3 after MI was extracted by the RNAzol B method (Tel-Test Inc., Friendswood, Tex). Total RNA was reverse-transcribed into cDNA using random primers (Life Technologies Inc., Rockville, Md). For detection of MMP-2, MMP-9, tissue inhibitor of metalloproteinase-1 (TIMP-1), TIMP-4, tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin-1$\beta$ (IL-1$\beta$), IL-10 and transforming growth factor-$\beta$ (TGF-$\beta$) mRNA levels in heart tissue, real-time reverse transcriptase PCR was performed with an Applied Biosystems 7300 Real-time PCR System with TaqMan Universal PCR Master Mix and TaqMan Gene Expression Assays (Applied Biosystems, Foster City, Calif). Ribosomal eukaryotic 18S RNA (Applied Biosystems) was used as an endogenous control gene. A standard curve for the serial dilution of murine heart cDNA was generated. The amplification cycle consisted of 2 minutes at 50°C, 10 minutes at 95°C, 15 seconds at 95°C, and 1 minute at 60°C. The mRNA levels were normalized to the endogenous 18S ribosomal RNA gene expression.

### In Vitro Assay of Cell Culture of Peritoneal Macrophages

To clarify the role of SR-A in the alteration of cytokine production in macrophages, we employed an in vitro assay system of peritoneal macrophages. Peritoneal macrophages were collected and suspended in RPMI 1640 medium (Sigma, St. Louis, Mo) that was supplemented with 10% fetal calf serum, 0.1 mg/mL streptomycin, and 100 U/mL penicillin, and then sowed at the density of $1.0\times10^6$/well on a plastic 24-well plate (Corning, Inc, Corning, NY).[24] The multiwell plate was incubated for 2 hours at 37°C in a humidified incubator with 5% $CO_2$. Each well was washed 10 times with 1 mL of PBS to remove nonadherent cells and was assigned either to receive acetylated low-density lipoprotein as a ligand for SR-A or not. Adherent macrophages were additionally cultured for 6 hours or 12 hours, and the supernatant was collected to determine the levels of TNF-$\alpha$ and IL-10 with sandwich ELISA. The assay was performed with commercially available mouse TNF-$\alpha$ ELISA kit (BioSource International, Camarillo, Calif) and mouse IL-10 Quantikine kit (R&D Systems, Minneapolis, Minn). Furthermore, to determine the inhibitory effects of recombinant mouse IL-10 (rmIL-10; R&D Systems) on TNF-$\alpha$ production of activated macrophages, we incubated peritoneal macrophages from both SR-A$^{-/-}$ and WT mice with 50 ng/mL and 200 ng/mL of rmIL-10 simultaneously with acetyl-low-density lipoprotein, a ligand for SR-A.

### Statistical Analysis

Data are expressed as mean±2 SEM. Analyses of survival after MI were carried out by the Kaplan-Meier method with the log-rank test to compare survival curves between groups. Group comparisons were made with $\chi^2$ tests for nominal data, and unpaired $t$ tests or Mann-Whitney $U$ tests were used for continuous data. Results with $P<0.05$ were considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

## Results

### Survival and Cardiac Rupture after MI

The post-MI survival rates of SR-A$^{-/-}$ and WT mice were compared. There were no deaths in the sham-operated groups. As shown in Figure 1A, WT mice (84%, 43 of 51 mice) had significantly better survival after MI compared with SR-A$^{-/-}$ mice (67%, 36 of 54 mice). Interestingly, the most frequent cause of death in both SR-A$^{-/-}$ (17 of 18 mice) and WT (6 of 8 mice) mice was LV rupture, which was confirmed by blood coagulation around the pericardial sac and small slits commonly observed in the LV wall. As shown in Figure 1B, the number of the mice that died of LV rupture, which occurred within 5 days, was significantly greater in SR-A$^{-/-}$ mice (31%, 17 of 54 mice) than in WT mice (12%, 6 of 51 mice; $P$=0.01). During the experiments, no mice in either group were observed to have died from infectious diseases.

### Physiological and Echocardiographic Measurement

As shown in Table 1, there were no significant differences in body weight, LVDd, M-mode %FS, LV mass at baseline or at days 7 and 28 post-MI between the 2 groups. Body weight, LVDd, and LV mass increased, and M-mode %FS decreased in both SR-A$^{-/-}$ and WT mice at days 7 and 28 post-MI. However, there were no significant differences between the 2 groups. Interestingly, relative heart weight (heart weight/body weight), relative lung weight (lung weight/body weight) and lung wet-to-dry ratio increased significantly in SR-A$^{-/-}$ mice compared with WT mice at day 7 post-MI.

### Histomorphometric and Immunohistochemical Analysis

To evaluate the role of SR-A in the extent of ischemic damage in the infarcted myocardium, infarct size and infarct area were measured at day 7 post-MI. Histological analysis demonstrated that sham-operated myocardium had no signs of myocardial degeneration such as necrosis, fibrosis, hypertrophic change, or inflammatory response. As shown in Table 1, infarct size and area were nearly identical between the 2 groups (54.6±4.9% and 41.8±5.5%, respectively, for SR-A$^{-/-}$ versus 53.5±4.0% and 43.1±3.9%, respectively, for WT). Immunohistochemical analysis revealed the gradual infiltration of FA-11–positive macrophages into the infarcted region, which peaked at day 5 post-MI (Figure 2B). The accumulation of Gr-1–positive granulocytes into the infarcted region increased earlier, peaked at day 3 after MI, and gradually decreased in both groups (Figure 2C). The

**General Characteristics of SR-A$^{-/-}$ and WT Mice**

| Parameter | Phase | SR-A$^{-/-}$ | WT |
|---|---|---|---|
| BW, g | Baseline | 22.5±1.2 | 22.0±0.2 |
| | Day 7 after MI | 23.6±1.1 | 23.8±1.0 |
| | Day 28 after MI | 26.2±1.2 | 26.8±0.7 |
| Echocardiographic measurement | | | |
| LVDd, mm | Baseline | 2.70±0.08 | 2.66±0.17 |
| | Day 7 after MI | 3.26±0.22 | 3.27±0.23 |
| | Day 28 after MI | 4.92±0.32 | 5.08±0.27 |
| M-mode, %FS | Baseline | 38.4±3.8 | 38.0±3.3 |
| | Day 7 after MI | 29.9±2.0 | 29.5±2.2 |
| | Day 28 after MI | 27.1±1.8 | 24.7±2.3 |
| LV mass/BW | Baseline | 3.55±0.29 | 3.15±0.37 |
| | Day 7 after MI | 6.69±0.70 | 6.15±0.67 |
| | Day 28 after MI | 6.38±0.69 | 6.02±0.72 |
| Heart and lung weights | | | |
| Heart weight/BW, mg/g | Day 7 after MI | 7.92±0.42* | 6.27±0.56 |
| Lung weight/BW, mg/g | Day 7 after MI | 6.38±0.64* | 5.34±0.30 |
| Lung W/D weight ratio | Day 7 after MI | 4.42±0.29* | 4.04±0.11 |
| Histomorphometric measurement | | | |
| Infarct size, % | Day 7 after MI | 54.6±4.9 | 53.5±4.0 |
| Infarct area, % | Day 7 after MI | 41.8±5.5 | 43.1±3.9 |

BW indicates body weight; LVDd, left ventricular end-diastolic diameter; %FS, percent fractional shortening; and W/D, wet-to-dry.
*$P<0.05$ vs WT mice.

temporal change in infiltration of these inflammatory cells was identical between the 2 groups. In addition, immunostaining for CD31 and anti–smooth muscle α-actin revealed a similar presence of new blood vessels and myofibroblasts in the infarcted region of both groups (Figure 2A). SR-A–positive cells gradually appeared in the infarcted region of WT mice, although these cells were not observed in the preinfarcted heart tissues from both groups. The spatial and temporal SR-A expression corresponded to that of FA-11–positive macrophages. SR-A$^{-/-}$ mice were not immunopositive for the SR-A antibody at all, in contrast to WT mice (Figure 2A).

Masson's trichrome staining showed impaired healing of the infarcted myocardial tissue in SR-A$^{-/-}$ mice. That is, as shown in Figure 3A and 3C, there were loosely distributed collagen fibers and the retention of unprocessed necrotic myocardium in the infarcted region in SR-A$^{-/-}$ mice at day 7 post-MI. Conversely, as shown in Figure 3B and 3D, there was dense fibrosis in the entire infarcted region in WT mice.

## MMP Expression and Activation in Infarcted Region

To evaluate the expression of MMPs and TIMPs, which play an important role in cardiac rupture after MI, we quantified cardiac MMP-2, MMP-9, TIMP-1, and TIMP-4 mRNA levels in SR-A$^{-/-}$ and WT mice. As shown in Figure 4A, the expression of MMP-9 mRNA increased significantly in the infarcted region compared with the noninfarcted or sham-operated myocardial tissues at day 3 post-MI in both SR-A$^{-/-}$ and WT mice, and was greater in the infarcted region of SR-A$^{-/-}$ mice than in that of WT mice. TIMP-1 mRNA expression also increased significantly in the infarcted region compared with the noninfarcted or sham-operated myocardial tissues at day 3 post-MI in both groups, and was inhibited more strongly in the infarcted region of SR-A$^{-/-}$ mice than WT mice. On the other hand, there was no significant difference in MMP-2 and TIMP-4 mRNA expression in the infarcted region between the SR-A$^{-/-}$ and WT mice, although MMP-2 mRNA was upregulated, and TIMP-4 mRNA

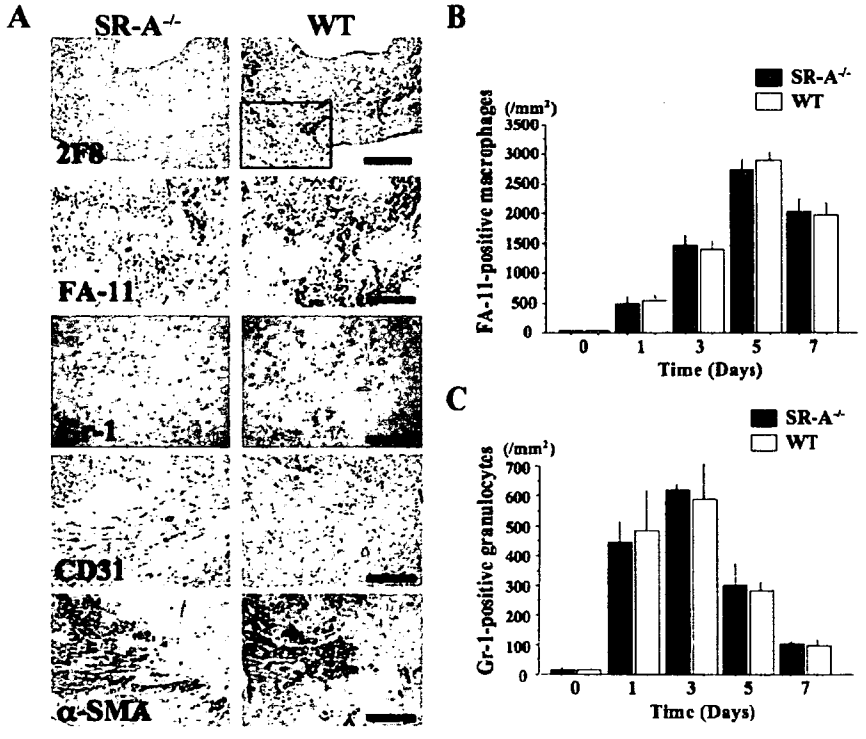


**Figure 2.** A, Immunohistochemical analysis of SR-A expression (2F8) and appearance of macrophages (FA-11), granulocytes (Gr-1), endothelial cells (CD31), and myofibroblasts (α-SMA) in infarcted regions of SR-A$^{-/-}$ (left) and WT (right) mice at day 3 post-MI. Scale bars represent 300 μm in each row. Cell enumeration of FA-11–positive macrophages (B) and Gr-1–positive granulocytes (C) in infarcted regions of SR-A$^{-/-}$ and WT mice at day 0, 1, 3, 5, and 7 post-MI. Data points represent the number of positive cells per mm$^2$. Bars represent mean±2 SEM.

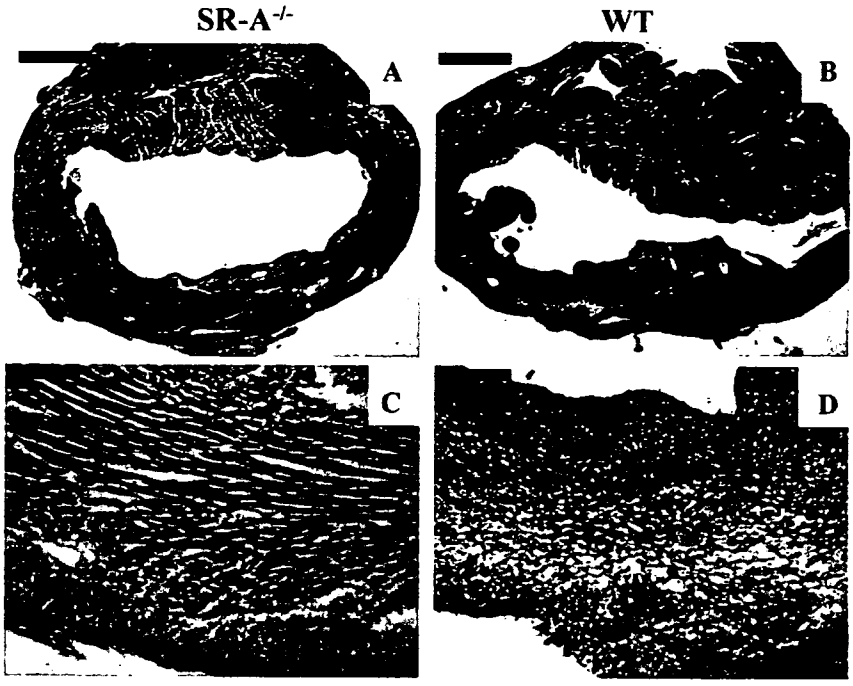


**Figure 3.** Representative low-power (A and B) and high-power (C and D) microphotographs of LV cross-sections stained with Masson's trichrome from SR-A$^{-/-}$ (A and C) and WT (B and D) mice at day 7 post-MI. Scale bars represent 1 mm in A and B and 200 μm in C and D.

was downregulated in the infarcted region compared with the noninfarcted or sham-operated myocardial tissues at day 3 post-MI in both SR-A$^{-/-}$ and WT mice.

We performed in situ zymography with gelatin films to detect gelatinolytic activity derived from the augmented MMP-9 mRNA expression in infarcted myocardial tissue. No gelatinolytic activity was detected at 1 day post-MI in either group (data not shown). In situ zymography demonstrated greater gelatinolytic activity in the infarcted region of SR-A$^{-/-}$ mice than WT mice at day 5 post-MI (Figure 4B). Moreover, morphometrically quantitative analysis of the gelatinolytic area/infarct area at day 5 post-MI confirmed the increased gelatinolytic activity in SR-A$^{-/-}$ mice (Figure 4C).

## Expression of Inflammatory Cytokines

To examine the molecular mechanism that underlies the increased MMP activity in SR-A$^{-/-}$ mice, we determined cardiac mRNA expression of several cytokines and growth factors by real-time reverse transcriptase PCR. Interestingly, the proinflammatory cytokine TNF-α mRNA was upregulated in the infarcted region compared with the noninfarcted or sham-operated myocardial tissues at day 3 post-MI in both SR-A$^{-/-}$ and WT mice, and was greater in the infarcted region of SR-A$^{-/-}$ mice than WT mice (Figure 5A). The quantification of protein levels by ELISA also demonstrated upregulated TNF-α protein expression in the infarcted region of SR-A$^{-/-}$ mice (Figure 5E). In contrast, the antiinflammatory cytokine IL-10 mRNA was also upregulated in

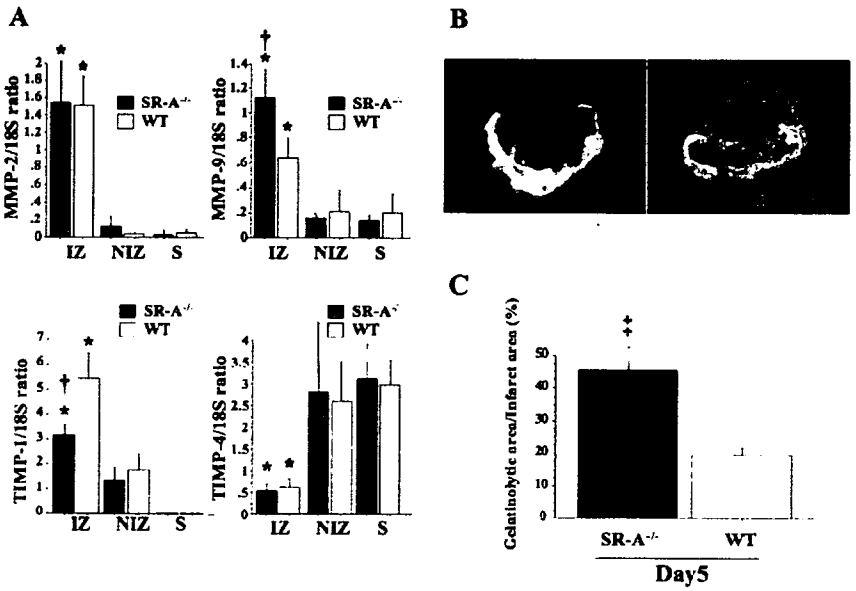


**Figure 4.** A, Real-time reverse transcriptase PCR results for MMP-2, MMP-9, TIMP-1, and TIMP-4 mRNA levels in infarcted zone (IZ), noninfarcted zone (NIZ), and sham-operated myocardium (S) from SR-A$^{-/-}$ and WT mice at day 3 post-MI. These mRNA levels were standardized by the levels of endogenous control 18S ribosomal RNA gene. Bars represent mean°2 SEM. *$P$<0.05 versus NIZ and S in each strain. †$P$<0.05 versus IZ in WT mice. B, In situ zymography in sections of infarcted myocardium from SR-A$^{-/-}$ (left) and WT (right) mice at day 5 post-MI. Lysis of gelatin in contact with the proteolytic areas of the sections is indicated by negative staining on the slides. C, Morphometric analysis of the gelatinolytic area/infarct area at day 5 post-MI in SR-A$^{-/-}$ and WT mice. Bars represent mean±2 SEM. ‡$P$<0.01 versus WT mice.

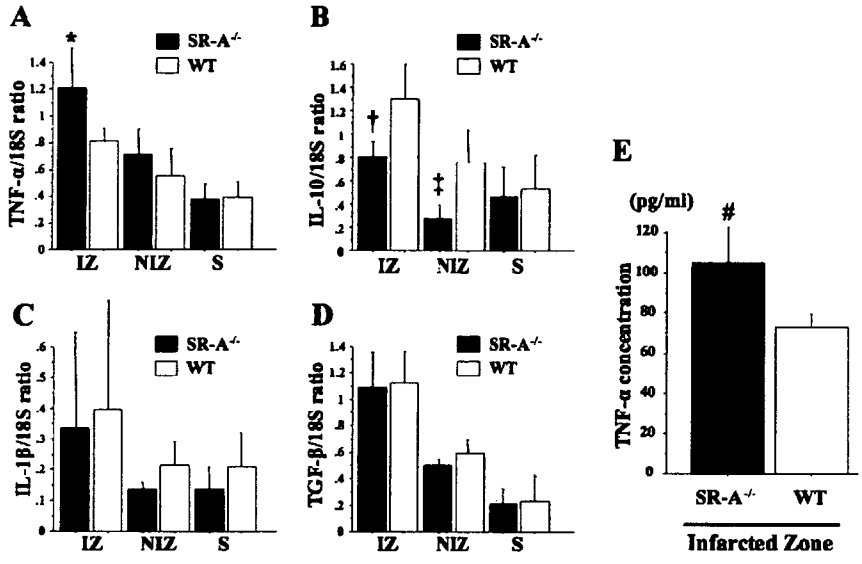


**Figure 5.** Real-time reverse transcriptase PCR for TNF-α (A), IL-10 (B), IL-1β (C), and TGF-β (D) mRNA levels in infarcted zone (IZ), noninfarcted zone (NIZ), and sham-operated myocardium (S) from SR-A$^{-/-}$ and WT mice at day 3 after MI. These mRNA levels were standardized by the levels of endogenous control 18S ribosomal RNA gene. Bars represent mean±2 SEM. *$P$<0.05 versus IZ in WT mice. †$P$<0.005 versus IZ in WT mice. ‡$P$<0.005 versus NIZ in WT mice. E, Quantification of TNF-α protein level by ELISA in infarcted region obtained from SR-A$^{-/-}$ and WT mice at day 3 after MI. Bars represent mean±2 SEM. #$P$<0.05 versus WT mice.

the infarcted region compared with the noninfarcted or sham-operated myocardial tissues at day 3 post-MI in both SR-A$^{-/-}$ and WT mice, and was significantly lower in the infarcted and noninfarcted region of SR-A$^{-/-}$ mice than WT mice (Figure 5B). Although other mRNAs of inflammatory cytokines and growth factors such as TGF-β, which appeared to regulate the synthesis and breakdown of ECM components, were also induced in the infarcted region compared with the noninfarcted region or sham-operated tissues, there were no significant differences between SR-A$^{-/-}$ and WT mice (Figure 5C and 5D).

### In Vitro Analysis With Peritoneal Macrophages

We conducted in vitro experiments with peritoneal macrophages to confirm whether the imbalance in production of inflammatory cytokines detected in in vivo experiments could be reproduced in vitro. As shown in Figure 6A, IL-10 production increased significantly in both groups after 6 hours of incubation, but not after 12 hours. The levels of IL-10 production were significantly lower in SR-A$^{-/-}$ macrophages than WT macrophages when cultured with SR-A ligand for 6 hours, but not for 12 hours. As shown in Figure 6B, TNF-α production was significantly greater after 12 hours of incubation compared with production after 6 hours in both groups. The levels of TNF-α production were greater in SR-A$^{-/-}$ macrophages than WT macrophages when cultured with SR-A ligand for 12 hours, but not for 6 hours. With respect to the effects of rmIL-10 addition, the levels of TNF-α production by activated macrophages were significantly down-regulated in both SR-A$^{-/-}$ and WT macrophages after administration of 50 ng/mL rmIL-10, and the difference between both macrophages was diminished (Figure 6B). In contrast, in the group that received 200 ng/mL rmIL-10, the inhibitory effects on TNF-α production were absent (data not shown).

## Discussion

Our study provided the first evidence that SR-A was essential for normal healing of infarcted myocardium. Specifically, we showed that SR-A$^{-/-}$ mice had a higher mortality mainly as a result of LV rupture after MI. The fragility of the LV wall in SR-A$^{-/-}$ mice might be attributable to markedly enhanced MMP activity in the infarcted myocardium. In the upstream mechanism, SR-A might be involved in the regulation of cytokine production and the ischemia-derived inflammatory response.

In the present study, we found that SR-A$^{-/-}$ mice had loosely distributed collagen fibers and retention of unprocessed necrotic myocardium in the infarcted region stained with Masson's trichrome. It has been demonstrated that MMPs might be the

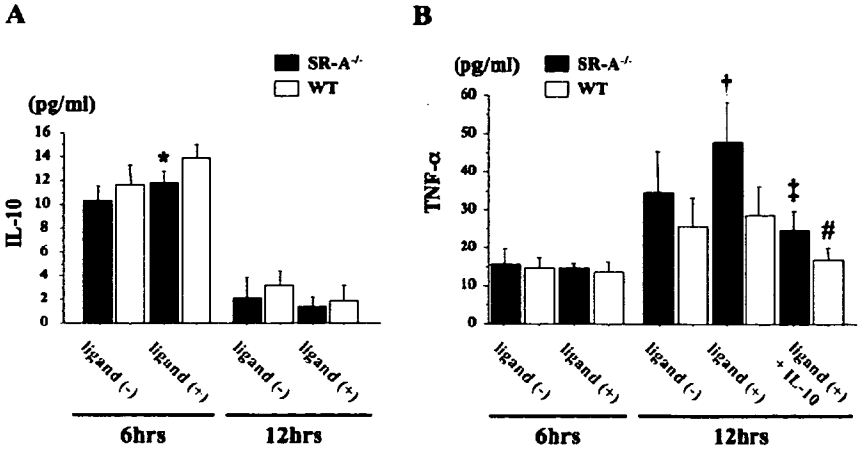


**Figure 6.** Quantification of IL-10 (A) and TNF-α (B) concentrations in culture medium of SR-A$^{-/-}$ and WT peritoneal macrophages in the presence or absence of SR-A ligand (acetyl-low-density lipoprotein). Bars represent mean±2 SEM. *$P$<0.05 versus WT macrophages incubated with SR-A ligand for 6 hours. †$P$<0.05 versus WT macrophages incubated with SR-A ligand for 12 hours. ‡$P$<0.01 versus SR-A$^{-/-}$ macrophages incubated with SR-A ligand for 12 hours without rmIL-10. #$P$<0.05 versus WT macrophages incubated with SR-A ligand for 12 hours without rmIL-10.

major pathophysiological regulators of ECM degradation and might be implicated in the pathogenesis of LV remodeling after MI.[5] Indeed, several studies in genetically-manipulated mice documented that gelatinases (ie, MMP-2 and MMP-9) play a crucial role in the LV remodeling process and may contribute to LV rupture.[6,7,25] Heymans et al demonstrated that MMP-9 deficiency prevented cardiac rupture after MI.[7] The significance of MMP-9 activity in early infarct healing and rupture was emphasized by the observation that MMP-9 was predominantly found in leukocytes and macrophages, and that its activity peaked around day 2, the period in which most ruptures occur. In the present study, quantitative reverse transcriptase PCR revealed increased MMP-9 mRNA expression and decreased TIMP-1 mRNA expression in the infarcted region of SR-A$^{-/-}$ mice at day 3 post-MI. In addition, in situ zymography indicated excessive gelatinolytic activity around the infarcted region of SR-A$^{-/-}$ mice. Taking these findings into consideration, enhanced gelatinolytic activity may directly contribute to the increased risk of post-MI LV rupture in SR-A$^{-/-}$ mice.

With regard to the upstream mechanism under the augmented MMP activity, Jugdutt reported that the net proteolytic activity of MMPs depended on transcription, activation, and inhibition of these molecules.[26] Transcription from MMP genes to pro-MMPs is stimulated by several factors such as inflammatory cytokines and growth factors.[27] Indeed, Sun et al recently showed that elevated local TNF-$\alpha$ in infarcted myocardium of TNF-$\alpha$–deficient mice contributes to acute cardiac rupture via augmented MMP-9 expression.[28] TNF-$\alpha$ is a master proinflammatory cytokine that is produced in the infarcted myocardium very soon after MI and is potentially a major contributor to post-MI LV rupture.[28,29] On the other hand, antiinflammatory cytokines such as IL-10 are thought to have a protective role after MI through the suppression of the acute inflammatory process.[30–32] Very recently, Fulton et al demonstrated that SR-A$^{-/-}$ mice displayed reduced levels of lipopolysaccharide-induced IL-10 production, which regulated the inflammatory process in endotoxemia and sepsis.[33] Consistent with these findings, our data revealed that the expression of IL-10 mRNA was attenuated in the infarcted region of SR-A$^{-/-}$ mice compared with WT mice at day 3 post-MI. On the other hand, the expression of TNF-$\alpha$ mRNA increased significantly more in the infarcted region of SR-A$^{-/-}$ mice than in that of WT mice at day 3 post-MI. Moreover, we subsequently performed in vitro experiments with peritoneal macrophages to verify the contribution of SR-A to the imbalanced production of these proinflammatory and antiinflammatory cytokines. The present findings suggested that SR-A deficiency might enhance TNF-$\alpha$ secretion as a result of the suppression of IL-10 secretion in SR-A$^{-/-}$ macrophages. The imbalance of the production of inflammatory cytokines could be responsible for the markedly augmented MMP activity and increased risk of post-MI LV rupture. Furthermore, we showed the inhibitory effects of rmIL-10 administration on TNF-$\alpha$ secretion in the cell culture experiments. The more intense effect was observed in SR-A$^{-/-}$ macrophages. Therefore, SR-A–mediated IL-10 production may be a key step involved in the regulation of TNF-$\alpha$ production in activated macrophages.

In addition, organ weight analysis indicated that heart weight, lung weight, and lung wet-to-dry ratio increased significantly in SR-A$^{-/-}$ mice compared with WT mice at day 7 post-MI. These results suggested the exacerbation of post-MI myocardial hypertrophy or lung injury in SR-A$^{-/-}$ mice. The putative mechanisms for these findings include a direct effect of upregulated TNF-$\alpha$ on myocardial hypertrophy or lung congestion based on the adverse influence of TNF-$\alpha$ on post-MI heart and lung tissues.[28,34,35] In the present study, however, there was no significant difference in the echocardiographic measurements of LV cavity dimensions, M-mode %FS, and LV mass between the 2 groups. We must emphasize that echocardiographic measurements and histomorphological and biological analyses could only be performed in the surviving mice. It is possible that the degree of LV expansion, LV wall thinning, and LV dysfunction might have been greater in the mice that died as a result of early cardiac rupture and this may have biased the results. In addition, LV dimensions and function could have been greatly influenced by the stage of the anesthesia and hemodynamic parameters such as heart rate.[20] The reason why there were no significant differences in echocardiographic measurements between the 2 groups might have been caused by the exclusion of nonsurviving mice or hemodynamic changes as a result of anesthesia.

In summary, the present findings provide the first evidence of a pathophysiological role for SR-A in LV remodeling after MI and add further support for the importance of macrophages in the healing process after MI. Further research about the SR-A–related signaling pathway might provide innovative therapeutic approaches to prevent cardiac rupture after MI.

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## Disclosures

None.

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## CLINICAL PERSPECTIVE

Despite improved treatments for acute myocardial infarction (MI), post-MI cardiac rupture remains a serious acute complication. Unfortunately, cardiac rupture mainly develops in younger patients with a transmural MI and is also unpredictable and fatal because of the absence of treatment. Accumulated experimental and clinical studies have indicated that members of the matrix metalloproteinase gene family play a central role in pathogenesis of cardiac rupture. Furthermore, we believe that understanding inflammatory response is critical for the prevention of post-MI cardiac rupture because the matrix metalloproteinase activation may be regulated mainly by various inflammatory mediators. In the present study, we evaluated the role of class A macrophage scavenger receptor (SR-A), which was a macrophage-restricted multifunctional molecule that optimized the inflammatory response in post-MI tissue repair. Our data revealed that a deficiency in the SR-A gene increased the risk of cardiac rupture after experimental MI via enhanced matrix metalloproteinase expression in infarcted myocardium. Moreover, we observed that SR-A deficiency enhanced tumor necrosis factor-α secretion as a result of the suppression of interleukin-10 secretion in SR-A–deficient macrophages. These findings suggest that SR-A might regulate macrophage-associated inflammatory responses in infarcted regions and might modulate consequent tissue remodeling in the healing process after MI. Further research into the SR-A–related signaling pathway might provide innovative therapeutic approaches to prevent cardiac rupture.

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# Future adverse cardiac events can be predicted by persistently low plasma adiponectin concentrations in men and marked reductions of adiponectin in women after acute myocardial infarction

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**Abstract**

There is conflicting information about whether mortality after AMI is higher in women than men. We investigated the significance of plasma adiponectin concentrations on major adverse cardiac events (MACE) after acute myocardial infarction (AMI) to delineate any differences between men and women. The study patients consisted of 114 men and 42 women with AMI. The incidence of MACE was significantly higher in women than men during the entire follow-up period ($p < 0.05$). Compared with men for post-AMI MACE, the hazard ratio for women was 5.6 after adjustment for prognostic factors. Killip class ($p < 0.001$) and sex differences ($p < 0.05$) were independent predictors of MACE at 1 year post-AMI. Plasma adiponectin levels in women were significantly higher than men on admission (8.66 μg/mL [range: 6.6–14.08] versus 4.71 μg/mL [range: 3.47–7.27], $p < 0.0001$) and during the post-AMI course (all $p < 0.0001$). Multivariate analysis identified plasma adiponectin level on admission as an independent predictor of MACE in men ($p < 0.001$) and the difference between plasma adiponectin levels at discharge and on admission in women ($p < 0.05$). Patterns of serial changes in plasma adiponectin concentrations are different between men and women and plasma adiponectin concentrations can be used to predict future adverse cardiac events in AMI patients.


*Keywords:* Myocardial infarction; Adiponectin; Prognosis; Sex

## 1. Introduction

The issue of whether women have more unfavorable prognosis than men after acute myocardial infarction (AMI) has provoked much controversy [1–4]. Women are reported to have higher relative risk than men in the early phase, especially within 1 year after AMI, and it may be accounted for the older age and more unfavorable risk characteristics of women [1,2]. However, other studies reported no significant differences related to mortality between men and women even after adjusting for age, coronary risk and other prognostic factors [3,4].

Low levels of plasma adiponectin, a representative new member of adipocyte-derived proteins, have been observed in patients with coronary artery disease [5,6]. We previously reported that coronary plaque rupture resulting in the onset

*Abbreviations:* AMI, acute myocardial infarction; CK, creatinine phosphokinase; ELISA, enzyme-linked immunosorbent assay; HDL, high-density lipoprotein; LAD, left artery descending; LCx, left circumflex artery; MACE, major adverse cardiac events; POBA, plain old balloon angioplasty; RCA, right coronary artery; ROC, receiver operating characteristic; TIMI, thrombolysis in myocardial infarction; VD, vessel disease

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of AMI might reduce plasma concentrations of adiponectin [7]. Adiponectin accumulates in the vascular subendothelial space after damage of the endothelial barrier *in vivo* [8], suggesting that the protein has vessel repair properties. Plasma adiponectin levels may also predict adverse cardiac events and adiponectin may act as a protective factor for the cardiovascular system [9]. High concentrations of adiponectin are associated with future lower risk of AMI [10]. On the other hand, plasma adiponectin levels are lower in men than in women probably due to a selective reduction by testosterone through inhibition of adiponectin secretion from adipocytes [11,12]. However, sex-based differences in clinical outcomes after AMI with regard to adiponectin have not been well defined.

The purpose of the present study was to investigate the serial changes in plasma adiponectin concentrations after AMI in men and women, to delineate any differences between the two sexes with regard to the incidence of adverse cardiac events after AMI, and the role of plasma adiponectin level in such difference.

## 2. Methods

### 2.1. Patients

The study patients consisted of 114 men and 42 women who were admitted to our hospital with AMI from October 2000 to March 2004. AMI was defined as elevated myocardial enzyme concentrations, with either typical chest pain persisting longer than 30 min or electrocardiographic changes (including ischemic ST-segment depression, ST-segment elevation, or pathologic Q waves). Elevated enzyme concentrations were defined as peak creatine phosphokinase (CK) concentrations of more than twice the normal upper limit. All patients also met the following criteria: (1) they were admitted to the hospital within 24 h after the onset of AMI, (2) plasma adiponectin concentrations were measured repeatedly up to discharge (mean period of hospitalization of $25 \pm 9$ days, range: 12–46 days) after the onset of AMI, (3) provided consent to the study, and (4) no post-AMI major adverse cardiac events (MACE) during hospitalization. MACE was defined as the development of the following complications: cardiac-related death, recurrent myocardial infarction, unstable angina, and heart failure requiring emergency rehospitalization. Heart failure defined as dyspnea and/or edema was accompanied by pulmonary congestion on the chest roentgenogram and left ventricular dysfunction on echocardiogram. Patients who were treated with antihypertensive drugs or those whose baseline blood pressure was $\geq$140/90 mm Hg were considered hypertensive. Diabetes mellitus was diagnosed according to the criteria of the World Health Organization [13], however none of the patients was taking any type of thiazolidinedione. Cigarette smoking was defined as active smoking. Killip classes on hospital admission, depending on the clinical manifestations of cardiac failure, were also assessed (Killip 1, no heart failure; Killip 2, $S_3$ and/or basal lung crepitations; Killip 3, acute pulmonary edema; Killip 4, cardiac shock) [14]. The study protocol was approved by the Human Ethics Review Committee of Kumamoto University and a signed consent form was obtained from each subject.

### 2.2. Blood sampling, quantification of plasma adiponectin and emergency coronary angiography

In patients with confirmed AMI, blood samples were obtained immediately after hospitalization for measurement of plasma adiponectin concentrations. Furthermore, blood samples were taken every 4 h over the first 24 h for determination of peak CK levels. Venous blood samples were also taken at 24, 72 h, and 7 days after admission and at discharge to measure plasma adiponectin levels. Blood samples for biochemical assessments, such as total cholesterol, triglyceride and high-density lipoprotein (HDL) cholesterol, were obtained after a 12 h fast after admission. Plasma adiponectin levels were determined by enzyme-linked immunosorbent assay (ELISA) as described previously [15].

Emergency coronary angiography was performed in all patients and the allocation of reperfusion therapy was determined by the attending physician and interventional cardiologists independent of this study. The perfusion grade of the infarct-related artery was assessed in accordance with the thrombolysis in myocardial infarction (TIMI) study classification [16]. The final TIMI flow grade was assessed on the final shot of the emergency coronary angiography.

### 2.3. Follow-up study

After hospital discharge, 156 patients were prospectively followed-up every month with a clinic visit or until occurrence of one MACE. Only the first cardiac event after the enrollment into the study was considered the endpoint in the follow-up analysis. Follow-up data were available for 100% of patients at the entire follow-up period.

### 2.4. Statistical analysis

Group data of normally distributed continuous variables were expressed as mean $\pm$ S.D., and continuous variables that did not show normal distribution were expressed as the median value (25–75th percentile range). Comparisons of continuous variables were performed using the unpaired *t*-test and the Mann–Whitney *U*-test, as appropriate. Categorical variables were presented by frequency counts, and intergroup comparisons were analyzed by the $\chi^2$-test. We plotted cumulative event curves using the Kaplan–Meier survival method and tested differences between the curves of the two groups for statistical significance by the log-rank analysis. The Spearman two-way test was used to assess the relation between two quantitative variables with non-normal distribution. Variables with non-normal distribution

Table 1
Clinical characteristic of the two study groups

| | Men ($n=114$) | Women ($n=42$) | $p$ |
|---|---|---|---|
| Age (years) | 61 ± 12 | 72 ± 10 | <0.0001 |
| Time to admission to hospital (h)[a] | 2.5 (1.5–4.0) | 5.5 (2.0–12.0) | 0.0031 |
| Hypertension, $n$ (%) | 57 (50) | 27 (64) | 0.1124 |
| Diabetes mellitus, $n$ (%) | 35 (31) | 13 (31) | 0.9760 |
| Total cholesterol (mg/dL) | 206 ± 61 | 218 ± 47 | 0.2584 |
| Triglyceride (mg/dL)[a] | 131 (91–178) | 120 (88–149) | 0.4403 |
| HDL cholesterol (mg/dL) | 46 ± 14 | 55 ± 17 | 0.0011 |
| Smoking, $n$ (%) | 81 (71) | 7 (17) | <0.0001 |
| Body mass index (kg/m$^2$) | 24 ± 3 | 22 ± 3 | 0.0018 |
| Killip class 1/2/3/4, $n$ (%) | 96 (84)/15 (13)/2 (2)/1 (1) | 32 (78)/6 (15)/1 (2)/2 (5) | 0.4311 |
| Culprit artery RCA/LAD/LCx, $n$ (%) | 41 (36)/54 (47)/19 (17) | 17 (40)/21 (50)/4 (10) | 0.5288 |
| Vessel involvement 1VD/2VD/3VD, $n$ (%) | 64 (56)/37 (32)/13 (11) | 22 (53)/14 (33)/6 (14) | 0.8628 |
| Reperfusion therapy, $n$ (%) | | | 0.8248 |
| None | 5 (4) | 3 (7) | |
| Thrombolysis | 9 (8) | 2 (5) | |
| POBA | 20 (18) | 7 (17) | |
| Stent | 80 (70) | 30 (71) | |
| TIMI 3, $n$ (%) | 112 (98) | 40 (95) | 0.2935 |
| Peak creatine kinase (IU/L)[a] | 2257 (1179–4300) | 1315 (759–2800) | 0.0643 |
| Peak creatine kinase-MB (IU/L)[a] | 170 (84–305) | 166 (63–364) | 0.4253 |

HDL, high-density lipoprotein; LAD, left artery descending; LCx, left circumflex artery; POBA, plain old balloon angioplasty; RCA, right coronary artery; TIMI, thrombolysis in myocardial infarction; VD, vessel disease.
[a] Median (25–75th percentiles).

were transformed logarithmically before multivariate analysis to fulfill the conditions required for this type of analysis. Cox proportional-hazards analysis was performed to assess the short-term prognosis (1-year MACE post-AMI). We also assessed the independent predictors of MACE during the entire follow-up period using multivariate logistic regression analysis including variables that were significantly associated with MACE in univariate analysis in men and in women, respectively. These analyses were performed using SPSS (SPSS Inc., Chicago, Illinois). Statistical significance was defined as $p<0.05$.

The best cutoff for predicting MACE after AMI was defined as that which yielded the highest product of sensitivity and specificity [17]. To determine the best cutoff level for plasma adiponectin, a receiver operating characteristic (ROC) curve was generated by using the computer program LABROC5 provided by Metz et al. [18].

## 3. Results

### 3.1. Clinical background, outcomes and classical predictors of MACE

Table 1 shows the clinical characteristics of men and women with AMI. Women were significantly older and less likely to be smokers, and had significantly higher HDL cholesterol levels and lower body mass index (BMI) than men. Furthermore, the time between the onset of AMI and hospitalization was significantly longer in women. Patients were followed for a mean period of 663 ± 384 days (range: 28–1623 days). During the entire follow-up period, MACE was observed in 13 men (cardiac-related death [$n=1$], recurrent myocardial infarction [$n=2$], unstable angina [$n=7$], heart failure [$n=3$]) and in 10 women (cardiac-related death [$n=2$], recurrent myocardial infarction [$n=1$], unstable angina [$n=5$], heart failure [$n=2$]). Fig. 1 shows event-free survival curves of patients after AMI. Compared with men for post-AMI MACE, the hazard ratio for women was 2.4 after non-adjustment (95% CI: 1.018–5.608, $p=0.0454$) and 5.6 after adjustment for prevalent variables such as age, time to hospitalization, hypertension, diabetes mellitus, total cholesterol, triglyceride, HDL cholesterol, smoking, body mass index, Killip class, vessel involvement, and peak CK (95% CI: 1.029–30.984, $p=0.0463$). In all women, MACE

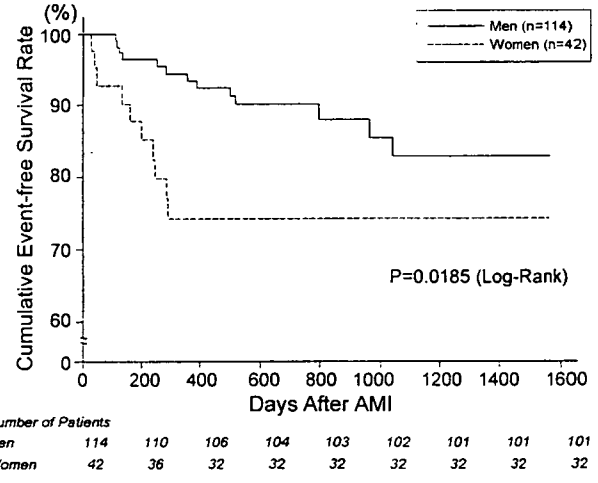


Fig. 1. Event-free survival after acute myocardial infarction (AMI) according to sex.

Table 2
Prognostic variables for 1 year major adverse cardiac events after acute myocardial infarction

| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | $p$ | OR | 95% CI | $p$ |
| Age (year$^{-1}$) | 1.026 | 0.982–1.071 | 0.2511 | 1.009 | 0.912–1.098 | 0.9921 |
| Women | 4.292 | 1.484–12.500 | 0.0072 | 24.390 | 1.499–500.000 | 0.0248 |
| Log-time to admission to hospital (h$^{-1}$) | 10.543 | 2.678–41.506 | 0.0008 | 4.301 | 0.345–53.560 | 0.2569 |
| Hypertension | 2.792 | 0.858–9.081 | 0.0880 | 4.368 | 0.634–30.118 | 0.1344 |
| Diabetes mellitus | 1.015 | 0.332–3.100 | 0.9794 | 0.771 | 0.125–4.760 | 0.7797 |
| Total cholesterol (per mg/dL) | 0.993 | 0.981–1.006 | 0.2751 | 0.983 | 0.962–1.005 | 0.1288 |
| Log-triglyceride (per mg/dL) | 0.395 | 0.037–4.234 | 0.4430 | 2.170 | 0.015–310.011 | 0.7596 |
| HDL cholesterol (per mg/dL) | 1.004 | 0.968–1.040 | 0.8458 | 1.010 | 0.950–1.075 | 0.7408 |
| Smoking | 0.976 | 0.344–2.772 | 0.9644 | 8.148 | 0.657–101.032 | 0.1024 |
| Body mass index (per kg/m$^2$) | 0.927 | 0.795–1.080 | 0.3296 | 1.093 | 0.856–1.396 | 0.4760 |
| Killip class >1 | 2.899 | 1.495–5.623 | 0.0016 | 26.316 | 3.846–166.667 | 0.0008 |
| Multi-vessel involvement | 1.645 | 0.826–3.276 | 0.1564 | 4.132 | 0.6219–27.027 | 0.1420 |
| Log-peak creatine kinase (per IU/L) | 2.253 | 0.553–9.178 | 0.2571 | 1.463 | 0.193–11.070 | 0.7127 |

HDL, high-density lipoprotein.

occurred within the first year after AMI (10 women with 1-year MACE), while it was observed uniformly across the follow-up period in men (seven men with 1-year MACE). Therefore, we investigated the prevalent prognostic variables on 1-year MACE for all patients using multivariate analysis. The results showed that Killip class and female sex were independent and significant predictors (Table 2). However, during the entire follow-up period, MACE could be predicted only by Killip class (95% CI: 2.762–37.037, $p=0.0005$), but sex difference was not identified as an independent predictor of MACE in multivariate analysis (95% CI: 0.512–19.608, $p=0.2147$).

### 3.2. Serial changes in plasma adiponectin concentrations and clinical determinants of adiponectin on admission

We examined the serial changes in plasma adiponectin during hospitalization. Plasma adiponectin concentrations in women were significantly higher than men on admission (8.66 μg/mL [range: 6.6–14.08] versus 4.71 μg/mL [range: 3.47–7.27], $p<0.0001$), at 24 h (8.44 μg/mL [range: 5.38–10.96] versus 4.31 μg/mL [range: 3.15–6.67], $p<0.0001$), at 72 h (8.07 μg/mL [range: 5.33–10.62] versus 4.07 μg/mL [range: 3.02–6.54], $p<0.0001$), at 7 days (9.00 μg/mL [range: 6.28–11.96] versus 4.60 μg/mL [range: 2.98–7.82], $p<0.0001$), and at discharge (9.57 μg/mL [range: 6.05–11.99] versus 4.70 μg/mL [range: 3.23–7.81], $p<0.0001$) (Fig. 2).

To assess the determinants of plasma adiponectin concentrations on admission, multiple regression analysis was performed after a stepwise regression that included clinical variables such as age, sex, time to admission to hospital, hypertension, diabetes mellitus, total cholesterol, triglyceride, HDL cholesterol, smoking, BMI, Killip class, culprit coronary artery, and multivessel involvement. The results of this model ($R^2=0.428$, $p<0.0001$) revealed that plasma adiponectin concentrations were significantly associated with sex ($\beta=0.308$, $p<0.0001$), BMI ($\beta=-0.253$, $p=0.0006$), serum triglyceride ($\beta=-0.221$, $p=0.0015$) and age ($\beta=0.181$, $p=0.0171$).

### 3.3. Adiponectin as prognostic biomarker in men and women

The ROC area under the curves (mean ± S.E.M.) were 0.794 ± 0.068 (95% CI: 0.637–0.901) in men and 0.388 ± 0.107 (95% CI: 0.203–0.605) in women during the follow-up period. The sensitivity and specificity for prediction of MACE using the best cutoff level for plasma adiponectin on admission (3.8 μg/mL) were 73% and 70% in men, respectively. In case of women, the sensitivity and specificity were 58% and 60%, respectively, for the best cutoff level on admission of 8.5 μg/mL. The event-free survival curves using the above-mentioned cutoff levels in men and women are shown in Fig. 3. In men, patients with adiponectin ≤3.8 μg/mL on admission were more likely to develop MACE than those with adiponectin >3.8 μg/mL,

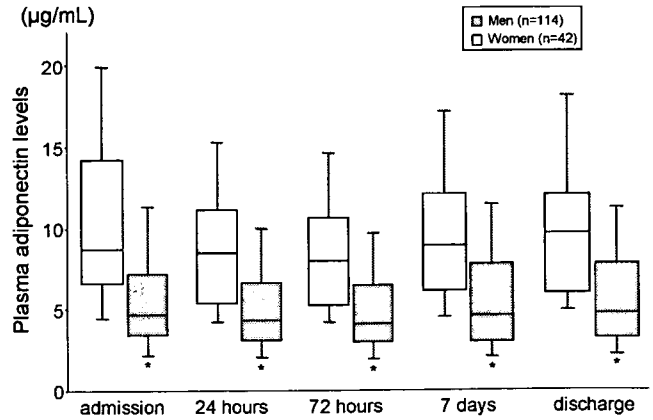


Fig. 2. Serial changes in plasma adiponectin concentrations in men and women. Values are expressed as the median value (25–75th percentile range). *$P<0.0001$ compared with women at the corresponding time point.

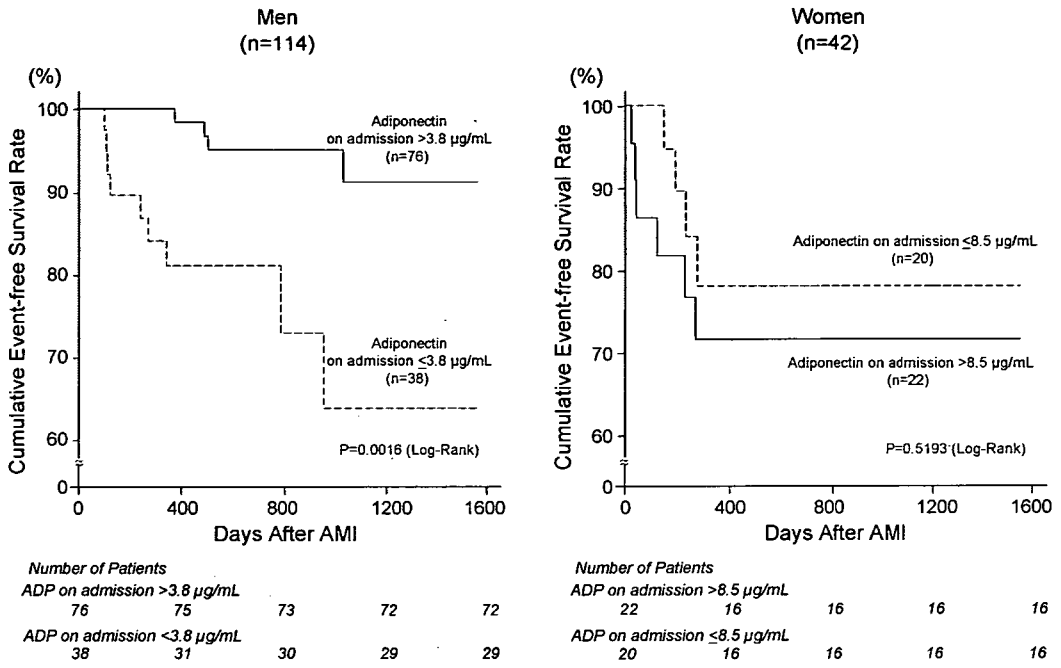


Fig. 3. Event-free survival after acute myocardial infarction (AMI) according to best cutoff value on admission in men (3.8 μg/mL) and women (8.5 μg/mL). ADP, adiponectin.

whereas no such correlation for the cutoff level of adiponectin was found in women.

Furthermore, we examined the serial changes in plasma adiponectin in relation to the development of MACE in men and in women (Fig. 4). Plasma adiponectin concentrations on admission were significantly lower in men who developed future MACE than those free of MACE (2.60 μg/mL [range: 1.74–3.87] versus 4.98 μg/mL [range: 3.70–8.06], $p = 0.0008$) and the same results were obtained at other time points (24 h: 2.20 μg/mL [range: 1.85–4.31] versus 4.40 μg/mL [range: 3.27–7.06], $p = 0.0009$; 72 h: 2.63 μg/mL [range: 1.65–3.70] versus 4.52 μg/mL [range: 3.24–6.69], $p = 0.0003$; 7 days: 2.43 μg/mL [range: 2.03–4.34] versus 4.74 μg/mL [range: 3.37–8.29], $p = 0.0023$; discharge: 2.67 μg/mL [range: 2.06–4.68] versus 4.93 μg/mL [range: 3.45–8.33], $p = 0.0033$). On the other hand, plasma

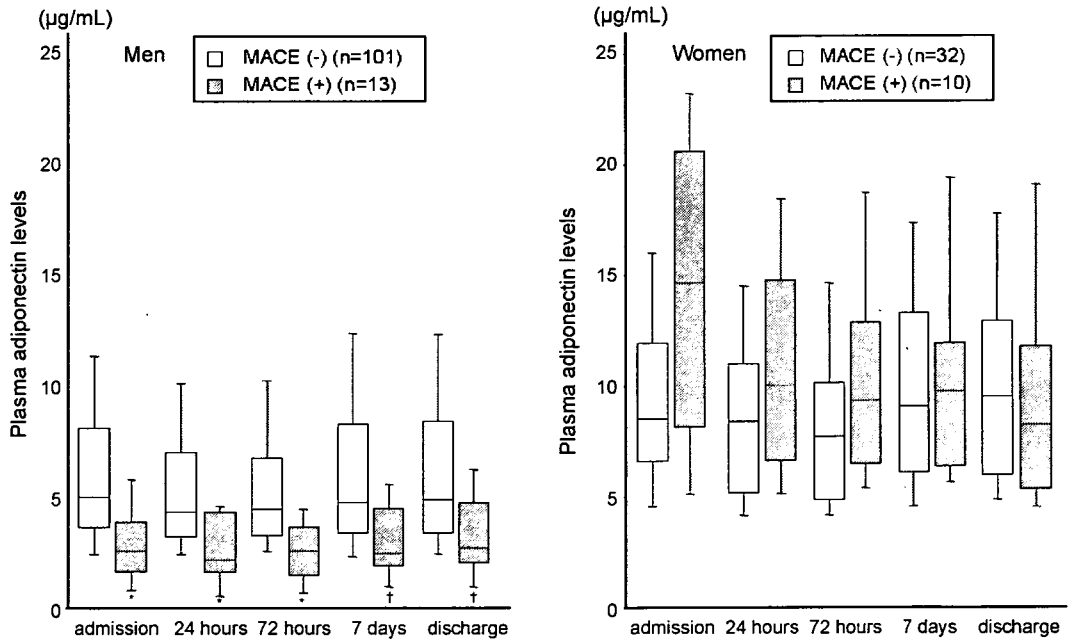


Fig. 4. Serial changes in plasma adiponectin concentrations in men and in women patients with acute myocardial infarction who subsequently developed major adverse cardiac events [MACE(+)] or did not develop major adverse cardiac events [MACE(−)] during follow-up. Values are expressed as the median value (25–75th percentile range). $^{*}p < 0.001$ and $^{\dagger}p < 0.005$, compared with MACE(−) patients at the corresponding time point.

Table 3
Predictors of post-AMI major adverse cardiac events in men

| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | p | OR | 95% CI | p |
| Age (year$^{-1}$) | 0.987 | 0.941–1.037 | 0.6080 | | | |
| Log-time to admission to hospital (h$^{-1}$) | 2.425 | 0.614–9.588 | 0.2063 | | | |
| Hypertension | 0.841 | 0.282–2.506 | 0.7559 | | | |
| Diabetes mellitus | 2.155 | 0.724–4.639 | 0.1679 | | | |
| Total cholesterol (per mg/dL) | 0.995 | 0.983–1.007 | 0.3941 | | | |
| Triglyceride (per mg/dL) | 1.000 | 0.996–1.003 | 0.8208 | | | |
| HDL cholesterol (per mg/dL) | 0.959 | 0.912–1.009 | 0.1059 | | | |
| Smoking | 1.786 | 0.393–8.130 | 0.4528 | | | |
| Body mass index (per kg/m$^2$) | 1.072 | 0.918–1.252 | 0.3796 | | | |
| Killip class >1 | 8.300 | 2.773–24.846 | 0.0002 | 5.944 | 1.859–19.009 | 0.0027 |
| Multi-vessel involvement | 1.302 | 0.435–3.902 | 0.6369 | | | |
| Log-peak creatine kinase (per IU/L) | 4.233 | 0.852–21.022 | 0.0776 | | | |
| Log-adiponectin (per μg/mL) | | | | | | |
| Admission | 0.023 | 0.004–0.136 | <0.0001 | 0.053 | 0.010–0.288 | 0.0007 |
| 24 h | 0.023 | 0.004–0.118 | <0.0001 | | | |
| 72 h | 0.012 | 0.002–0.072 | <0.0001 | | | |
| 7 days | 0.026 | 0.004–0.182 | 0.0002 | | | |
| Discharge | 0.052 | 0.008–0.302 | 0.0010 | | | |
| Log-Δadiponectin (per μg/mL)[a] | | | | | | |
| 24 h | 0.331 | 0.003–41.585 | 0.6537 | | | |
| 72 h | 0.108 | 0.001–7.988 | 0.3105 | | | |
| 7 days | 2.493 | 0.091–68.082 | 0.5883 | | | |
| Discharge | 15.277 | 0.316–738.730 | 0.1683 | | | |

HDL, high-density lipoprotein.
[a] Log-difference between plasma adiponectin concentrations at each subsequent blood sampling point and on admission (μg/mL).

adiponectin concentrations on admission tended to be higher in women who developed future MACE than those who did not, albeit insignificantly at all time points (admission: 14.67 μg/mL [range: 8.18–20.93] versus 8.60 μg/mL [range: 6.60–12.62], $p=0.1839$; 24 h: 10.02 μg/mL [range: 6.96–14.50] versus 8.44 μg/mL [range: 5.21–10.96], $p=0.2877$; 72 h: 9.35 μg/mL [range: 6.74–12.69] versus 8.22 μg/mL [range: 5.04–10.39], $p=0.2148$; 7 days: 9.72 μg/mL [range: 6.61–11.74] versus 9.05 μg/mL [range: 6.28–13.46], $p=0.6053$; discharge: 8.47 μg/mL [range: 5.82–11.47] versus 9.75 μg/mL [range: 6.07–13.00], $p=0.7010$).

The delta change in plasma adiponectin concentration during follow-up (i.e., difference between plasma adiponectin concentration at each subsequent blood sampling point and on admission) in women with future MACE was greater than that of women who did not develop MACE (24 h: −2.20 μg/mL [range: −5.04 to −0.58] versus −0.55 μg/mL [range: −1.87 to 0.03], $p=0.0479$; 72 h: −3.57 μg/mL [range: −6.50 to −1.10] versus −1.48 μg/mL [range: −2.02 to −0.29], $p=0.0628$; 7 days: −3.49 μg/mL [range: −7.51 to 0.02] versus −0.37 μg/mL [range: −1.56 to 2.12], $p=0.0549$; discharge: −2.07 μg/mL [range: −7.98 to −0.71] versus 0.52 μg/mL [range: −1.56 to 2.00], $p=0.0106$). These findings suggest that the development of MACE after AMI may be associated with plasma adiponectin levels in men, but with marked reduction of plasma adiponectin during follow-up after AMI in women.

In women, the ROC area under the curves (mean ± S.E.M.) for the delta change in plasma adiponectin concentration at 24, 72 h, 7 days and at discharge were 0.735 ± 0.098 (95% CI: 0.516–0.886), 0.725 ± 0.104 (95% CI: 0.500–0.886), 0.732 ± 0.102 (95% CI: 0.505–0.889), and 0.785 ± 0.090 (95% CI: 0.572–0.919), respectively. The ROC area under the curve for the delta change in plasma adiponectin concentration at discharge was the highest in women and was therefore applied for MACE as a prognostic variable in logistic regression analysis of this group. On the other hand, in the same analysis for men we used plasma adiponectin concentrations on admission because fluctuations in these concentrations were small. The results of logistic regression analysis showed that plasma adiponectin concentration on admission was an independent and significant predictor of MACE in men (Table 3). In women, the delta change in plasma adiponectin concentration at discharge was an independent and significant predictor of MACE (Table 4).

The sensitivity and specificity of ROC curve for prediction of MACE in women using the best cutoff value for the delta change in plasma adiponectin concentration at discharge (−1.7 μg/mL) were 75% and 70%, respectively. Therefore, we divided women subjects into two subgroups using the delta change in adiponectin concentration to assess the prognosis of women with AMI. Female patients with delta change in plasma adiponectin levels at discharge ≤ −1.7 μg/mL were significantly more likely to develop cardiac events than those

Table 4
Predictors of post-AMI major adverse cardiac events in women

| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *p* | OR | 95% CI | *p* |
| Age (year$^{-1}$) | 0.999 | 0.937–1.064 | 0.9705 | | | |
| Log-time to admission to hospital (h$^{-1}$) | 5.022 | 1.256–30.083 | 0.0250 | 4.468 | 0.858–23.273 | 0.0754 |
| Hypertension | 5.747 | 0.729–45.455 | 0.0968 | | | |
| Diabetes mellitus | 0.864 | 0.223–3.345 | 0.8331 | | | |
| Total cholesterol (per mg/dL) | 0.987 | 0.969–1.005 | 0.1537 | | | |
| Triglyceride (per mg/dL) | 0.990 | 0.975–1.004 | 0.1592 | | | |
| HDL cholesterol (per mg/dL) | 1.004 | 0.962–1.074 | 0.8519 | | | |
| Smoking | 1.524 | 0.323–7.194 | 0.5946 | | | |
| Body mass index (per kg/m$^2$) | 0.905 | 0.745–1.099 | 0.3142 | | | |
| Killip class >1 | 4.649 | 1.330–16.247 | 0.0161 | 1.860 | 0.451–7.674 | 0.3908 |
| Multi-vessel involvement | 1.676 | 0.473–5.940 | 0.4240 | | | |
| Log-peak creatine kinase (per IU/L) | 1.331 | 0.264–6.708 | 0.7291 | | | |
| Log-adiponectin (per μg/mL) | | | | | | |
| Admission | 10.315 | 0.795–133.772 | 0.0743 | | | |
| 24 h | 6.056 | 0.356–103.142 | 0.2130 | | | |
| 72 h | 6.669 | 0.442–105.390 | 0.1778 | | | |
| Discharge | 0.671 | 0.037–12.036 | 0.7865 | | | |
| Log-Δadiponectin (per μg/mL)[a] | | | | | | |
| 24 h | 0.0000908 | $0.160 \times 10^{-7}$ to 0.515 | 0.0348 | | | |
| 72 h | 0.001 | $0.176 \times 10^{-6}$ to 4.662 | 0.1080 | | | |
| 7 days | 0.001 | $0.384 \times 10^{-6}$ to 0.247 | 0.0141 | | | |
| Discharge | 0.000126 | $0.608 \times 10^{-6}$ to 0.026 | 0.0010 | 0.00028 | $0.937 \times 10^{-7}$ to 0.088 | 0.0053 |

HDL, high-density lipoprotein.
[a] Log-difference between plasma adiponectin concentrations at each subsequent blood sampling point and on admission (μg/mL).

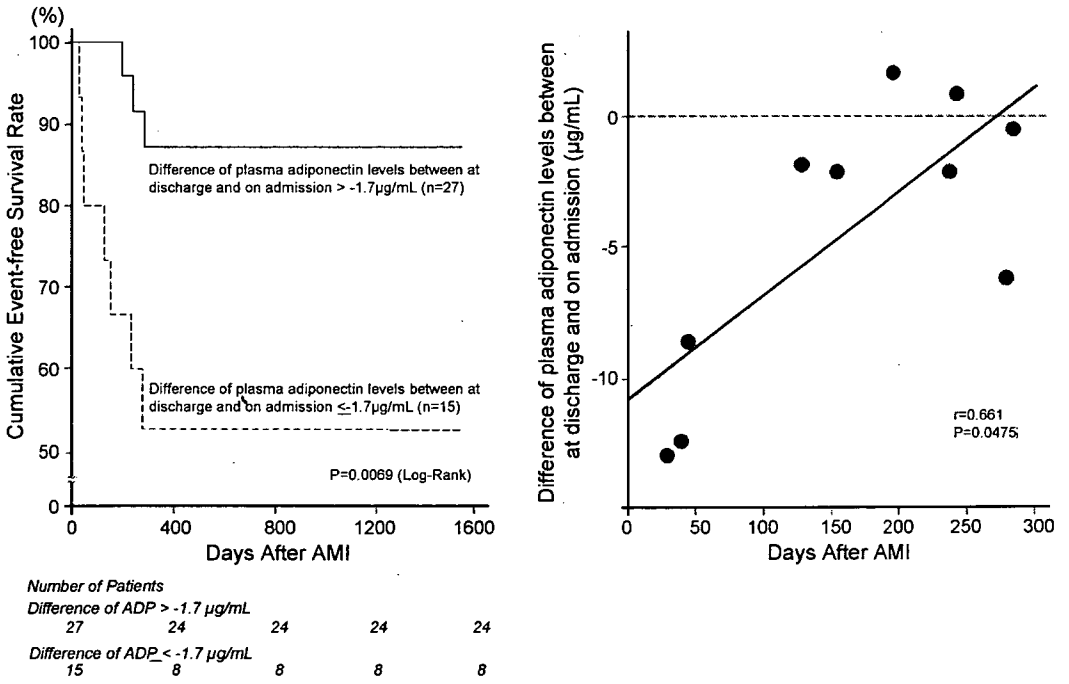


Fig. 5. *Left*: event-free survival after acute myocardial infarction (AMI) according to best cutoff value of the difference of plasma adiponectin concentrations between at discharge and on admission in women (−1.7 μg/mL). *Right*: correlation between the difference of plasma adiponectin levels between at discharge and on admission and the number of days after AMI in women. ADP, adiponectin.

with a delta change >−1.7 μg/mL (Fig. 5, left). Furthermore, the delta change in plasma adiponectin concentrations at discharge correlated with the number of days from the onset of AMI to the occurrence of MACE (Fig. 5, right).

## 4. Discussion

In the present study, we found that plasma adiponectin concentrations during the post-AMI course were lower in men than those in women. Furthermore, plasma adiponectin concentrations on admission were significantly influenced by sex. Low plasma adiponectin concentrations were associated with poor prognosis in men, while a reduction in adiponectin concentration during hospitalization correlated with future cardiac events in women with AMI. Especially in women, a decrease in plasma adiponectin concentration correlated with the number of days from AMI onset to the occurrence of MACE.

Previous studies reported sex differences in adiponectin concentrations in healthy subjects [11]. Our results showed lower plasma adiponectin concentrations in men than in women even after the onset of AMI. While sex hormones are considered to affect adiponectin levels, all women in the present study were postmenopausal. Recent reports indicated that testosterone reduced plasma adiponectin concentrations by inhibiting its secretion from adipocytes [11,12] and that testosterone replacement therapy caused a decrease of adiponectin level in hypogonadal patients [12]. However, other studies showed that plasma adiponectin concentrations were comparable between pre- and postmenopausal women and they were not affected by hormone replacement therapy in postmenopausal women [11,19]. A remarkable decline in adiponectin levels occurs during the progression of puberty in boys, however, such change is not observed in girls [20]. The testosterone levels in boys correlate negatively with adiponectin levels, whereas estradiol concentrations in girls are not associated with adiponectin [20]. The sex difference in adiponectin may be observed under the influence of testosterone-regulated adiponectin. In fact, in men, testosterone levels decrease gradually with age, however; the levels are relatively maintained at a range that cannot be ignored [21]. Therefore, sex differences should be taken into account in studies of adiponectin.

Our results showed that low adiponectin concentrations during the post-AMI follow-up were associated with poor prognosis in men. Our results are in agreement with those reported by Pischon et al. [10] who showed that high plasma adiponectin concentrations were associated with future lower risk of AMI in men. Based on the results of several experimental studies, adiponectin is considered to be involved in the initiation and progression of atherosclerosis through its anti-atherosclerotic effects [22]. Furthermore, adiponectin was reported to increase the expression of tissue inhibitor of metalloproteinases in human monocyte-derived macrophages, which is known to control the rupture of atherogenic plaque lesions [23]. Therefore, in men, persistently low adiponectin levels may indirectly promote the progression of coronary artery disease, by canceling its protective anti-atherosclerotic actions, leading to future cardiac events.

This study demonstrated that MACE in women after AMI was greater than that in men. The increase in 1-year MACE can be explained by Killip class and sex, whereas the difference in overall mortality is explained only by Killip class. As for this reason, 1-year MACE seems to increase in women, but is later followed by an increase in MACE in men. This excess 1-year MACE in women cannot be explained by the frequency of prevalent risk factors. There is conflicting information on whether short-term mortality after AMI is higher in women than in men after adjustment for prognostic factors (1–4). The data reported by Vaccarino et al. [24,25] indicated that younger women who survive hospitalization for myocardial infarction had a higher mortality rate than men. However, they did not identify the factors that could explain the sex differences. Therefore, a novel marker to unravel a mystery of sex-related differences is required and we paid much attention to plasma adiponectin levels and those changing pattern in AMI. In our study, we examined post-AMI MACE, rather than mortality, as the primary endpoint and all women who developed MACE did so within the first year after AMI. Furthermore, our results indicated that marked reduction of plasma adiponectin levels was closely associated with the occurrence of MACE in women. Plasma adiponectin concentrations in women who developed future MACE were comparable to those without MACE at discharge, however, adiponectin concentrations at the time of development of MACE could be further decreased relative to their levels at discharge. Therefore, especially in women, marked reduction from relatively high plasma adiponectin concentrations may trigger the progression of coronary atherosclerosis, which may be aggravated by low plasma concentrations of adiponectin. There is some possibility of occurrence of MACE provided that plasma adiponectin levels at discharge are still lower than those on admission in women.

The precise mechanism of the reduction of plasma adiponectin concentrations immediately after the onset of AMI remains unclear. Adiponectin accumulates in the vascular subendothelial space when the endothelial barrier is damaged [8]. We reported previously that adiponectin might target the ruptured plaques resulting in their consumption in the circulating plasma [7]. In addition, adiponectin may play a role in the scaffold of formed collagen in myocardial remodeling after ischemic injury by its uptake into the interstitium and around the infarcted lesion, which may decrease plasma adiponectin levels [26]. Therefore, this protein is considered to have vessel repair and tissue healing properties and reduced concentrations during the post-AMI period may be accounted for by the consequence of coronary plaque rupture followed by infarcted myocardium. Plasma adiponectin has been recently recognized to exist in three isoforms: (1) trimer, basic unit of the multimeric adiponectin, referred to

as low molecular weight (LMW) adiponectin, (2) hexamer, linked two subunits of trimer, known as middle molecular weight (MMW) adiponectin, and (3) a high molecular weight (HMW) adiponectin comprising 12–18 subunits [27,28]. The HMW adiponectin levels in women are higher than those in age- and BMI-matched men because testosterone regulates the secretion of HMW adiponectin from adipocytes, whereas the MMW and LMW adiponectin levels are comparable between the two sexes [12]. Therefore, there may be sex-related differences in total adiponectin levels. It has been proposed recently that patients with coronary heart disease have a selective reduction in HMW adiponectin, suggesting that the oligomeric complex distribution of adiponectin is critical for anti-atherogenic activity [29]. It is also possible that the absolutely small amount of circulating total adiponectin, including the HMW component, in men and the decrease of total adiponectin caused by selective consumption of the HMV component in women, contributes to the development of coronary artery disease in men and women, respectively. Recent studies reported the involvement of total adiponectin levels in future coronary events in men but not in women [10,30]. HMW adiponectin levels or the ratio of HMW to total adiponectin may be of prognostic significance especially in women. In short, persistently low adiponectin concentrations in men and marked reductions of adiponectin in women after AMI may be suggestive of future cardiac events. Adiponectin may not only act as a marker of cardiovascular risk but also a causal risk factor.

The patient population was relatively small in the present study and the study was limited to the Japanese population. However, we merely tested a plasma marker for predicting later onset of cardiac events. The present study demonstrated that plasma adiponectin concentrations could be potentially used as a marker for prediction of post-AMI MACE. We measured consecutive plasma adiponectin concentrations up to discharge after the onset of AMI but we did not measure them at the occurrence of MACE. However, the pattern of changes in plasma adiponectin concentrations was quite different between men and women, which might provide a clue to solve the problem of whether women have more unfavorable short-term post-AMI outcome than men. Further studies of larger and other ethnic populations are needed to confirm our findings.

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