Table 2
Follow-up data of IGT patients in control group and pravastatin-group

	Control	Pravastatin	*p*-Value
BMI (kg/m^2)			
Baseline	23.9 ± 2.4	23.0 ± 2.3	0.23
Follow-up	24.0 ± 2.2	22.9 ± 1.9	0.09
Change (%)	0.4 ± 4.1	−0.1 ± 3.7	0.68
Body weight (kg)			
Baseline	59.8 ± 8.7	59.1 ± 10.2	0.81
Follow-up	60.2 ± 8.4	58.9 ± 9.6	0.66
Change (%)	0.4 ± 1.9	−0.2 ± 1.8	0.33
Total cholesterol (mg/dL)			
Baseline	183 ± 21	188 ± 16*	0.31
Follow-up	181 ± 29	159 ± 21*	<0.01
Change (%)	−1.1 ± 10.7	−15.6 ± 8.4	<0.01
LDL-cholesterol (mg/dL)			
Baseline	118 ± 20	122 ± 15*	0.43
Follow-up	118 ± 24	94 ± 16*	<0.01
Change (%)	0.6 ± 17.0	−23.1 ± 8.9	<0.01
HDL-cholesterol (mg/dL)			
Baseline	49 ± 10	46 ± 10	0.29
Follow-up	50 ± 10	49 ± 9	0.92
Change (%)	1.2 ± 16.3	9.8 ± 25.5	0.27
Triglycerides (mg/dL)			
Baseline	101 (85–129)	122 (92–162)	0.15
Follow-up	107 (82–132)	125 (89–153)	0.26
Change (%)	0.9 ± 24.1	−0.7 ± 22.0	0.65
hsCRP (mg/L)			
Baseline	1.1 (0.5–2.0)	1.4 (0.8–2.3)*	0.36
Follow-up	0.7 (0.5–2.8)	0.6 (0.4–1.2)*	0.24
Change (%)	19.6 ± 75.3	−36.7 ± 41.3	<0.01
HbA1c (%)			
Baseline	5.3 ± 0.4*	5.3 ± 0.4	0.91
Follow-up	5.6 ± 0.5*	5.2 ± 0.3	0.02
Change (%)	4.1 ± 5.4	−1.4 ± 5.6	<0.01
HOMA-IR			
Baseline	1.5 ± 0.9	1.7 ± 1.1	0.54
Follow-up	1.8 ± 1.1	1.5 ± 0.9	0.29
Change (%)	37.6 ± 72.4	8.8 ± 84.5	0.03
HOMA-Iβ			
Baseline	86.6 ± 48.7	100.8 ± 70.2	0.46
Follow-up	95.4 ± 72.6	89.4 ± 60.4	0.78
Change (%)	12.8 ± 43.8	16.8 ± 85.2	0.48
Adiponectin (μg/mL)			
Baseline	5.8 (4.1–6.7)	5.2 (4.0–6.5)*	0.73
Follow-up	5.4 (4.0–6.8)	6.1 (5.1–9.3)*	0.32
Change (%)	0.1 ± 31.4	35.4 ± 40.6	<0.01
Fasting glucose (mg/dL)			
Baseline	91 ± 8	92 ± 11	0.63
Follow-up	96 ± 12	90 ± 7	0.09
Change (%)	5.4 ± 13.9	−1.7 ± 8.9	0.10
Fasting insulin (μU/mL)			
Baseline	6.5 ± 3.7	7.2 ± 4.5	0.57
Follow-up	7.7 ± 4.8	6.6 ± 4.1	0.42
Change (%)	27.1 ± 48.1	10.3 ± 82.9	0.06
2 h glucose (mg/dL)			
Baseline	157 ± 17	165 ± 24*	0.23
Follow-up	165 ± 38	142 ± 26*	0.04
Change (%)	5.6 ± 23.8	−13.5 ± 11.3	<0.01
2 h insulin (μU/mL)			
Baseline	94.3 ± 41.0	94.9 ± 44.8*	0.97
Follow-up	98.0 ± 55.7	74.0 ± 42.6*	0.13
Change (%)	2.3 ± 36.8	−23.5 ± 23.4	0.01
Peak glucose (mg/dL)			
Baseline	202 ± 33	199 ± 31†	0.80
Follow-up	204 ± 34	180 ± 26†	0.02
Change (%)	1.5 ± 11.6	−8.1 ± 16.2	0.04
$AUC_{glucose}$ (mg/dL per 2 h)			
Baseline	19847 ± 2462	20094 ± 2517*	0.76
Follow-up	20179 ± 3728	18053 ± 2154*	0.04
Change (%)	1.7 ± 15.3	−9.5 ± 11.0	0.01
$AUC_{insulin}$ (μU/mL per 2 h)			
Baseline	7906 ± 3050	8123 ± 4112*	0.85
Follow-up	8049 ± 3728	6446 ± 3333*	0.16
Change (%)	3.3 ± 30.9	−18.3 ± 23.1	0.03
Insulinogenic index			
Baseline	0.596 ± 0.442	0.628 ± 0.409	0.81
Follow-up	0.659 ± 0.396	0.651 ± 0.396	0.95
Change (%)	44.3 ± 121.0	90.6 ± 269.8	0.73
Insulin sensitivity index			
Baseline	5.5 ± 2.9	5.3 ± 2.7†	0.78
Follow-up	5.2 ± 3.6	6.9 ± 3.9†	0.16
Change (%)	−4.5 ± 38.9	35.6 ± 49.2	<0.01

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-Iβ, homeostasis model assessment of pancreatic β-cell function; HbA1c, glycosylated hemoglobin A1c; AUC, area under the concentration–time curve. Triglycerides, hsCRP and adiponectin were expressed as medians with interquartile range.

* $p < 0.01$ vs. baseline values.

† $p < 0.05$ vs. baseline values.

In the pravastatin group, levels of total cholesterol (16%), LDL-cholesterol (23%) and hsCRP (37%) were significantly decreased. Pravastatin therapy was associated with a significant elevation of adiponectin levels among patients with IGT (35%; Table 2 and Fig. 2). At the end of follow-up, total cholesterol, LDL-cholesterol and HbA1c levels were significantly lower in the pravastatin group than in the control group. The magnitude of the decrease in total cholesterol, LDL-cholesterol, HbA1c, hsCRP levels and HOMA-IR and the magnitude of the increase in adiponectin levels were significantly greater in the pravastatin group than the control group. Pancreatic β-cell function assessed by HOMA-Iβ was not significantly modulated from baseline to follow-up in each group (Table 2).

3.3. Oral glucose tolerance test

At baseline, 2 h glucose values in OGTT were not significantly different between the pravastatin and control groups (Table 1 and Fig. 1). At 6 months follow-up, the glucose levels at the 2 h in OGTT were significantly lower in the pravastatin group than in the control group ($p = 0.04$; Table 2 and Fig. 1).

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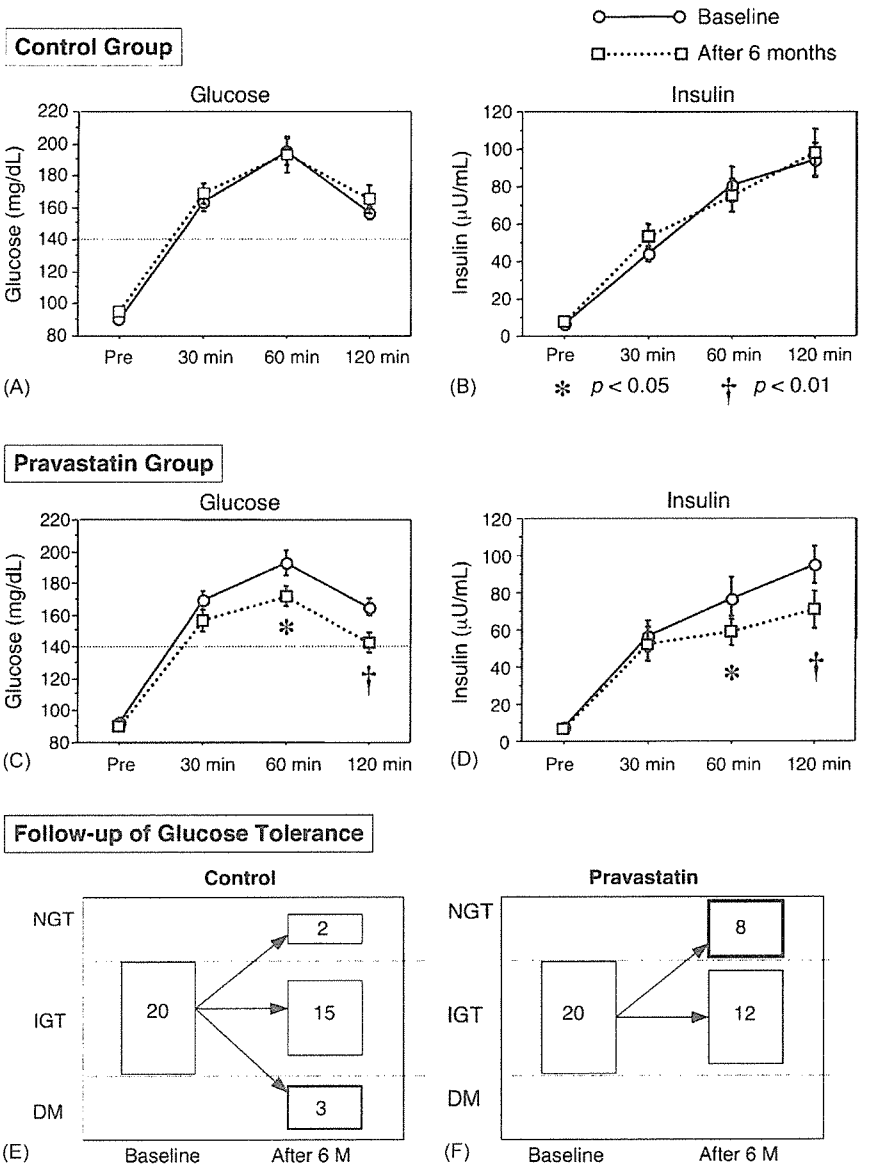

Fig. 1. Glucose concentrations during 75 g OGTT in control group (A) and pravastatin group (C) at baseline (solid lines) and after 6 months follow-up (dotted lines). Insulin concentrations during 75 g OGTT in control group (B) and pravastatin group (D) at baseline (solid lines) and after 6 months follow-up (dotted lines) $^{*}p<0.05$; $^{\dagger}p<0.01$, baseline vs. 6 months follow-up. Results of evaluation of glucose tolerance by 75 g OGTT at baseline and 6 months follow-up in control group (E) and pravastatin group (F). NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus.

The peak glucose levels were also significantly lower in the pravastatin group than in the control group at follow-up. A significantly greater reduction from baseline in $AUC_{glucose}$ and $AUC_{insulin}$ during OGTT was observed in the pravastatin group compared with the control group (Table 2 and Fig. 1). Pravastatin treatment significantly increased the insulin sensitivity index and decreased the 2 h insulin values at 6 months follow-up (Table 2). The improvement in insulin sensitivity index was significantly greater in the pravastatin group than the control group (Table 2). The early phase insulin response after OGTT assessed by insulinogenic index was not significantly different at baseline or at 6 months in both groups (Table 2).

3.4. Contributing factors to the improvement of post-load hyperglycemia

Changes in the 2 h glucose values in OGTT were significantly correlated with changes in LDL-cholesterol levels ($r=0.329$; $p=0.038$), hsCRP levels ($r=0.363$; $p=0.021$) and adiponectin levels ($r=-0.462$; $p=0.003$; Table 3 and Fig. 2) from baseline to follow-up. As expected, changes in the fasting glucose ($r=0.450$; $p=0.004$), HbA1c ($r=0.470$; $p=0.002$) and HOMA-IR ($r=0.363$; $p=0.021$) were significantly correlated with decrease of the 2 h glucose levels. Stepwise regression analysis indicated that the change in adiponectin, fasting glucose and HbA1c levels were

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Table 3
Correlation between change in BMI and blood parameters and decrease of the 2 h glucose values in OGTT

Parameter	Univariate regression		Stepwise regression	Multivariate regression ($R^2 = 0.344$)		
	r-Value	p-Value	F-value	B (S.E.M.)	β-Value	p-Value
BMI	0.236	0.142	0.027	–	–	–
Total cholesterol	0.213	0.187	0.150	–	–	–
HDL-cholesterol	−0.221	0.171	1.876	–	–	–
LDL-cholesterol	0.329	0.038	0.047	–	–	–
Triglycerides	0.156	0.338	0.821	–	–	–
hsCRP	0.363	0.021	0.763	–	–	–
Adiponectin	**−0.462**	**0.003**	**4.832***	**−6.40 (2.91)**	**−0.304**	**0.035**
Fasting glucose	0.450	0.004	3.435*	0.80 (0.43)	0.262	0.072
Fasting insulin	0.296	0.064	0.106	–	–	–
HbA1c	**0.470**	**0.002**	**4.136***	**29.62 (14.57)**	**0.298**	**0.049**
HOMA-IR	0.363	0.021	0.098	–	–	–
HOMA-Iβ	0.087	0.593	0.054	–	–	–

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, glycosylated hemoglobin A1c; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-Iβ, homeostasis model assessment of pancreatic β-cell function; hs-CRP, high-sensitivity C-reactive protein.
* Significant variables by stepwise regression analysis ($F > 3.0$).

significantly associated with reduction in the 2 h post-loaded glucose values ($F = 7.82$; $p < 0.001$). Multiple regression analysis revealed that change in adiponectin levels was significantly and independently associated with reduction in the 2 h glucose values ($\beta = -0.304$; $p = 0.035$).

3.5. Clinical outcome during follow-up

Three patients in the control group developed new-onset diabetes after 6 months follow-up, compared with no patients in the pravastatin group (Fig. 1). More than 10 mg/dL reduction at the 2 h glucose values in OGTT was observed in 85% of the pravastatin group and 35% of the control group ($p = 0.001$). Logistic regression analysis with the covariates listed in Table 1 identified only pravastatin therapy as a significant predictive factor for the improvement of post-loaded hyperglycemia (more than 10 mg/dL reduction at the 2 h glucose values) in patients with IGT (odds ratio 5.7; 95% confidence interval 1.7–19.3; $p = 0.003$). Finally, eight patients (40%) in the pravastatin group and two patients (10%) in the control group achieved the normal glucose tolerance by 6 months ($p = 0.03$; Fig. 1). There was no significant difference in changes in adiponection, hsCRP and BMI values between 8 patients converted to normal glucose tolerance from IGT and 12 patients presented IGT again in the pravastatin group (data not shown).

4. Discussion

Treatment with pravastatin in CAD patients with IGT significantly improved glucose tolerance associated with increasing plasma levels of adiponectin. Postprandial hyperglycemia is a significant risk factor for developing diabetes, which itself poses a significant risk for cardiovascular events [6]. The results of the present study suggest that pravastatin could contribute to an improvement in postprandial, rather than fasting, glucose metabolism. Patients with CAD have decreased adiponectin levels [9] which is associated with an increased risk of myocardial infarction [10]. Adiponectin

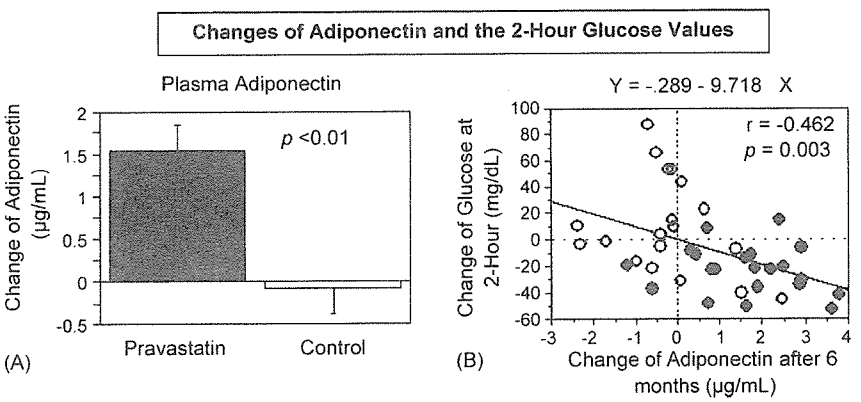

Fig. 2. A bar graph showing changes of plasma adiponectin levels from baseline to 6 months follow-up in control group (white bars) and pravastatin group (black bars) (A). A line graph and a dot-plot graph demonstrating the significant inverse correlation between the change of plasma adiponectin levels and change of glucose levels at 2 h following OGTT in IGT patients with CAD. Open circles, control group; closed circles, pravastatin group ($r = -0.462$; $p = 0.003$) (B).

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may also have anti-inflammatory and anti-atherogenic activity [15]. Based on our present findings, the increase in adiponectin levels associated with pravastatin therapy may have clinical benefit in preventing cardiovascular events in patients with CAD, in conjunction with the benefits conferred by the cholesterol-lowering and glucose metabolism-improving effects of this drug.

Data from the WOSCOP trial showed that pravastatin therapy was associated with a significant reduction in the risk of new-onset diabetes [4]. Although the mechanism by which pravastatin conferred this benefit was unclear, it was suggested that it may be due to its effect on reducing triglyceride levels [1], its anti-inflammatory activity [16] or its effect on endothelial functions [17]. The WOSCOP investigators suggested that all these mechanisms might well be active, along with other as yet unidentified direct or indirect effects on glucose metabolism, to reduce the propensity for the development of diabetes. In our study, we found that pravastatin improved glucose tolerance and was associated with elevation of adiponectin in patients with IGT, which may further explain the beneficial effects of pravastatin as a potent "metabolic modifier".

We showed, for the first time, that pravastatin therapy results in an increase in plasma adiponectin levels in humans. Adiponectin is an adipocyte-derived secreted protein that has several important metabolic and endocrinologic functions that are of particular relevance to glucose metabolism and the development of type-2 diabetes. In humans, increased serum concentrations of adiponectin are associated with increased insulin sensitivity and glucose tolerance [11]. Patients with type-2 diabetes have reduced levels of adiponectin, and, conversely, high levels of adiponectin are independently associated with a reduced risk of type-2 diabetes in apparently healthy subjects [18]. In our study, the pravastatin-induced elevation in adiponectin levels may have improved the patients' systemic insulin sensitivity and lowered their blood glucose levels at the 2 h following OGTT. Interestingly, other statins have not been found to have any significant effect on plasma adiponectin levels [19,20]. The effects of pravastatin on adipose tissue and adipocytes are very interesting and we have not yet examined the direct effects of pravastatin on adipocytes in vitro. As pravastatin is a relatively hydrophilic drug and may not accumulate in adipose tissue [21], it is unclear whether the effect on adiponectin levels is a direct result of pravastatin uptake into adipocytes. Furthermore, it would be interesting to determine whether there was any redistribution of fat tissue, especially the changes in visceral fat depots assessed by abdominal computed tomography scan may be involved in the increase in insulin sensitivity and adiponectin levels by pravastatin. Further studies will be needed to clarify the mechanism of the pravastatin-induced increase in plasma adiponectin levels.

In our study, early insulin secretion levels remain unchanged, while total insulin secretion ($AUC_{insulin}$) and the 2 h insulin levels in OGTT were significantly decreased in the pravastatin group. At the same time, insulin sensitivity was increased in the pravastatin group. Insulin resistance and hyperinsulinemia are known to be involved in the pathogenesis of metabolic syndrome [22], and plasma levels of adiponectin are also associated with obesity, insulin resistance and the metabolic syndrome [8]. Pravastatin has recently been shown to improve insulin sensitivity in patients with metabolic syndrome [23] and in animal models of glucose intolerance [24]. Pravastatin can be considered a potentially effective treatment modality for patients with metabolic syndrome and elevated cholesterol levels [25]. In the present study, 12 of 40 patients (30%) were obese (BMI $\geq$ 25) and we did not measure abdominal circumference to diagnose metabolic syndrome. It will be interesting to determine the clinical value of pravastatin use in patients with metabolic syndrome with abdominal obesity in the future study.

Pravastatin is a hydrophilic molecule and is selectively taken up by hepatocytes via Na^+-independent multispecific anion transporters and energy-dependent transporters [26] thereby having direct activity in the liver but not in peripheral tissue and other organs. It has been reported that pravastatin, but not other hydrophobic statins, can exert beneficial effects on phenotypes of hepatocytes [27,28]. Pravastatin may potentially improve insulin sensitivity in the liver through modulation of hepatocyte functions and acceleration of lipid metabolism, leading to a subsequent lowering blood glucose levels at the postprandial state.

Results from the Diabetes Epidemiology: COllaborative Analysis of Diagnostic Criteria in Europe Study suggest that postprandial high blood glucose levels can be an important risk factor for cardiovascular events [6]. As IGT and diabetes mellitus are successive diseases, early management of blood glucose levels could prevent the progression to diabetes and, subsequently, serious cardiovascular events. IGT has been recognized as a practical target to prevent diabetes and cardiovascular complications, however, effective clinical strategies to treat IGT have not yet been established [29]. Many patients with IGT frequently have abnormal lipid metabolism, which together are key components of the metabolic syndrome [23], leading to development of CAD. Because treatment of ischemic heart disease with consideration to the patient's glucose tolerance and cholesterol levels is a clinically valuable approach, pravastastain may be useful as the first line statin for patients at earlier stages in the atherogenic and diabetogenic processes.

Hypoadiponectinemia has been recognized as the risk factor for cardiovascular events [10], thus it would be beneficial for patients with hypoadiponectinemia to increase plasma adiponectin. Recently, Gannage-Yared et al. demonstrated that pravastatin did not significantly alter plasma levels of adiponectin and insulin sensitivity in healthy subjects with normal plasma levels of adiponectin [30]. Pravastatin might potentially increase the plasma levels of adiponectin in patients with insulin resistance and hypoadiponectinemia rather than the healthy subjects.

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5. Limitations

Because the present study was the open-labeled design with the small size of patients group, the double-blinded study with larger number of patients will be required to confirm the present results. We did not measure the circulating plasma levels of free fatty acid (FFA) at fast or in the postprandial state in the present study. It would be interesting to investigate whether pravastatin may alter the FFA levels in the postprandial state. OGTT, the most common method for evaluating whole body glucose tolerance in the daily practical medicine and is the physiological examination, has often been used to assess glucose tolerance and insulin sensitivity in the clinical studies. Euglycemic hyperglycemic clamp study is designed for measuring peripheral glucose utilization by exogenously infused insulin, whereas plasma glucose responses during OGTT are the results of hepatic glucose production and peripheral glucose utilization by endogenously secreted insulin. The glucose clamp is the "gold standard" and more accurate way in measurement insulin sensitivity than OGTT, then, we will be able to get more detailed information of pravastatin-induced improvement of insulin resistance by the euglycemic hyperglycemic clamp in the future investigation.

In conclusion, we have demonstrated that pravastatin significantly improved glucose tolerance and significantly elevated plasma levels of adiponectin in CAD patients with IGT. Pravastatin therapy may be an advantageous clinical option to improve hypercholesterolemia and glucose metabolism in patients with CAD.

Acknowledgements

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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 ± 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 χ^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

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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

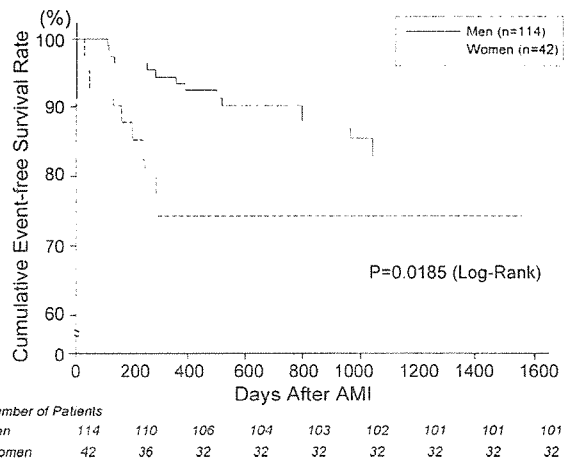

Fig. 1. Event-free survival after acute myocardial infarction (AMI) according to sex.

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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,

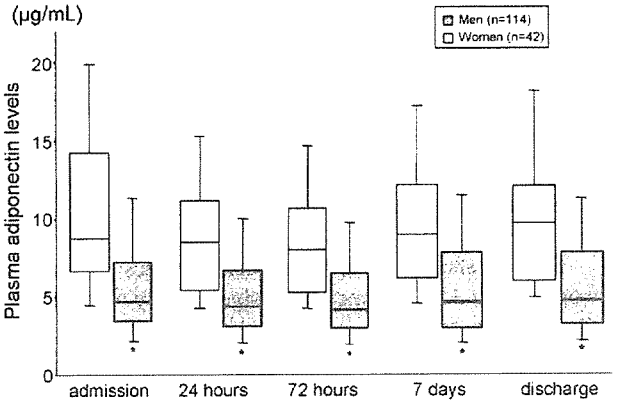

Fig. 2. Serial changes in plasma adiponectin concentrations in men and women. Values are expressed as the median value (25–75th percentile range). *$P < 0.0001$ compared with women at the corresponding time point.

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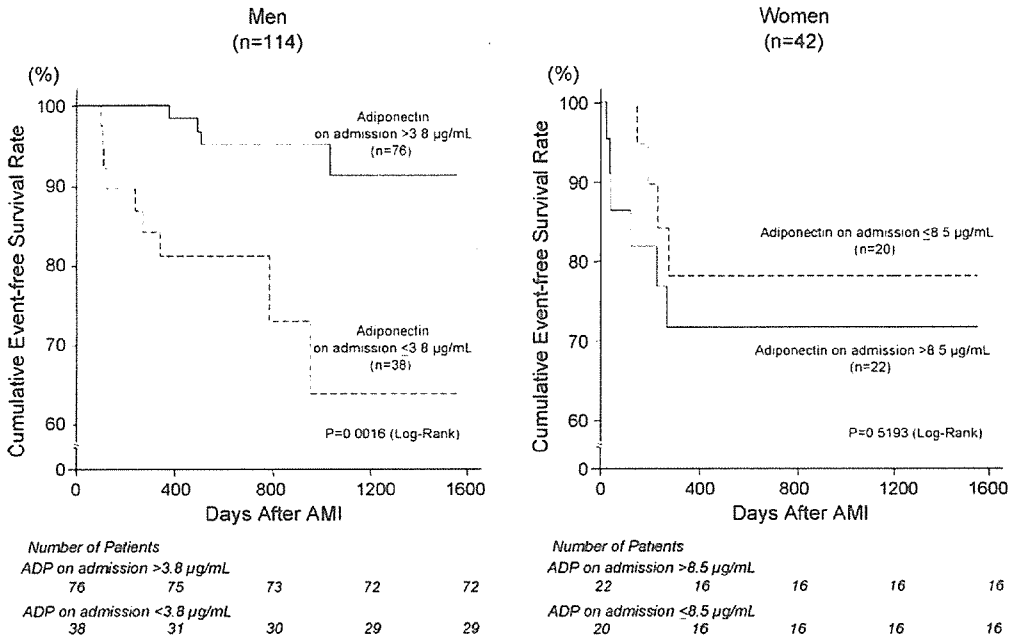

Fig. 3. Event-free survival after acute myocardial infarction (AMI) according to best cutoff value on admission in men (3.8 μg/mL) and women (8.5 μg/mL). ADP, adiponectin.

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

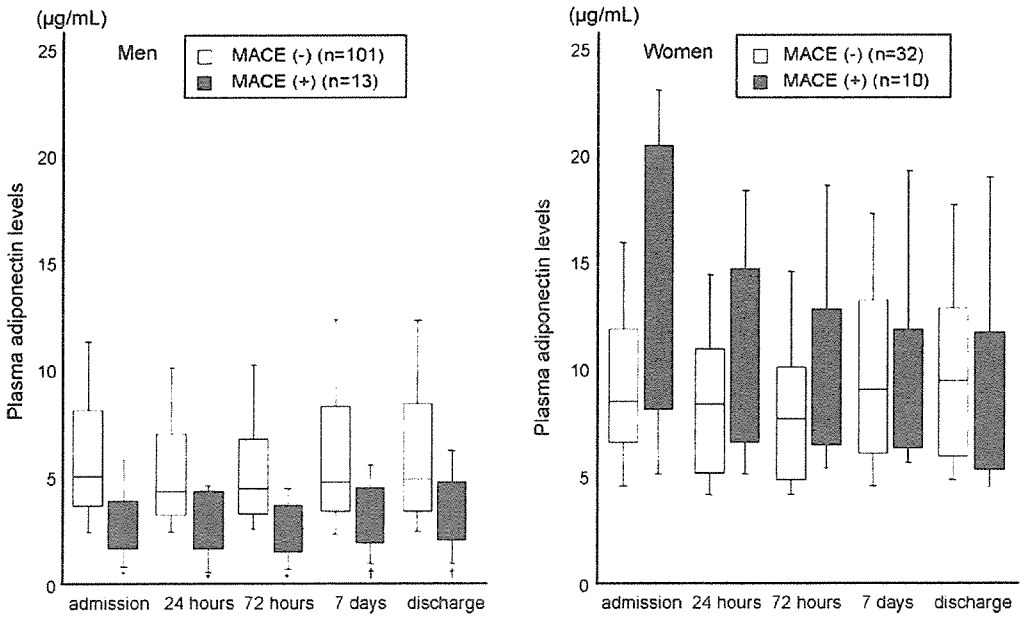

Fig. 4. Serial changes in plasma adiponectin concentrations in men and in women patients with acute myocardial infarction who subsequently developed major adverse cardiac events [MACE(+)] or did not develop major adverse cardiac events [MACE(−)] during follow-up. Values are expressed as the median value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

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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

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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	0.160×10^{-7} to 0.515	0.0348			
72 h	0.001	0.176×10^{-6} to 4.662	0.1080			
7 days	0.001	0.384×10^{-6} to 0.247	0.0141			
Discharge	0.000126	0.608×10^{-6} to 0.026	0.0010	0.00028	0.937×10^{-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).

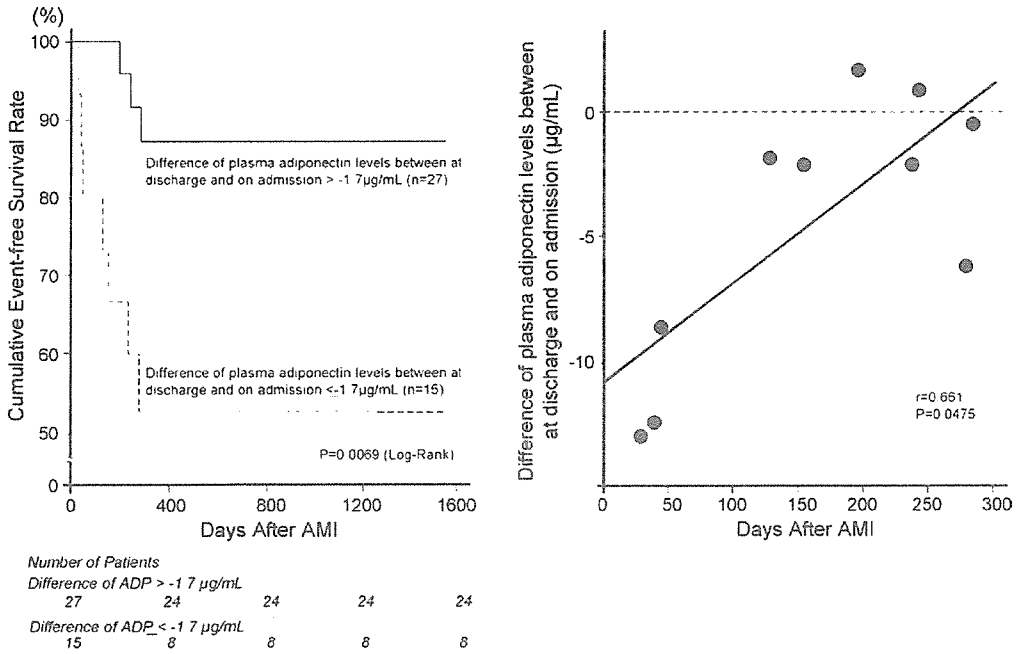

Fig. 5. *Left*: event-free survival after acute myocardial infarction (AMI) according to best cutoff value of the difference of plasma adiponectin concentrations between at discharge and on admission in women (−1.7 μg/mL). *Right*: correlation between the difference of plasma adiponectin levels between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

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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

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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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see commentary on page 1678

Blockade of Angiotensin II type-1 receptor reduces oxidative stress in adipose tissue and ameliorates adipocytokine dysregulation

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Dysregulated production of adipocytokines may be involved in the development of atherosclerotic cardiovascular disease in metabolic syndrome and chronic kidney disease (CKD) associated with metabolic syndrome. The aim of this study was to determine the effects of treatment with angiotensin II (Ang II) type-1 receptor blocker (ARB) on the regulation of adipocytokines. Olmesartan, an ARB, significantly blunted the age- and body weight-associated falls in plasma adiponectin both in genetically and diet-induced obese mice, without affecting body weight, but had no effect on plasma adiponectin levels in lean mice. Olmesartan also ameliorated dysregulation of adipocytokines in obesity, such as tumor necrosis factor-α, plasminogen activator inhibitor-1, monocyte chemotactic protein-1, and serum amyloid A3. Olmesartan significantly reduced reactive oxygen species originating from accumulated fat and attenuated the expression of nicotinamide adenine dinucleotide phospho hydrogenase oxidase subunits in adipose tissue. In cultured adipocytes, olmesartan acted as an antioxidant and improved adipocytokine dysregulation. Our results indicate that blockade of Ang II receptor ameliorates adipocytokine dysregulation and that such action is mediated, at least in part, by targeting oxidative stress in obese adipose tissue. Ang II signaling and subsequent oxidative stress in adipose tissue may be potential targets for the prevention of atherosclerotic cardiovascular disease in metabolic syndrome and also in metabolic syndrome-based CKD.

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KEYWORDS: angiotensin II type1 receptor blocker; adiponectin; adipocytokine; oxidative stress; NADPH oxidase

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The metabolic syndrome is a cluster of insulin resistance, hypertension, dyslipidemia, and visceral obesity.[1] Recent studies demonstrated that the metabolic syndrome is also strongly associated with chronic kidney disease (CKD).[2,3] In addition to the effects of elevated blood pressure and blood glucose, there is evidence to suggest that visceral obesity *per se* can cause vascular endothelial dysfunction resulting in renal damage (termed obesity-related glomerulopathy).[4,5]

Cardiovascular diseases are major causes of mortality and morbidity in the metabolic syndrome and also in CKD.[6] Several biomarkers such as microalbuminuria and plasma homocysteine, and C-reactive protein levels, have been investigated as potential risk factors for cardiovascular disease in CKD.[7–12] Adipose tissue was previously considered as an energy-storage organ, but recent research has demonstrated that this tissue produces and secretes a variety of biologically active molecules, conceptualized as adipocytokines.[13–18] Increased production of plasminogen activator inhibitor-1 (PAI-1) from accumulated visceral fat leads to thrombotic tendency, and high levels of plasma tumor necrosis factor-α (TNF-α) result in inflammation.[15,16,19] Adiponectin, which is an adipose-specific endocrine factor, exhibits anti-atherogenic, anti-diabetic, and anti-inflammatory properties,[20–23] but its plasma level is low in visceral obesity.[18] Hypoadiponectinemia is associated with coronary artery disease, type 2 diabetes, essential hypertension, and metabolic syndrome.[24–28] Interestingly, it was demonstrated that plasma adiponectin level could be a negative predictor of cardiovascular outcomes among patients with end-stage renal disease.[29,30] Considered together, dysregulation of adipocytokine may be also involved in the development of cardiovascular diseases in CKD associated with metabolic syndrome.

Regimens designed to increase plasma adiponectin levels and to ameliorate adipocytokine dysregulation might prevent the development of cardiovascular diseases. The renin–angiotensin system (RAS), a hormonal cascade that includes angiotensinogen, renin, angiotensin-converting enzyme (ACE), angiotensin (Ang), and its receptors, is involved in the maintenance of systemic blood pressure. In pathological

state, Ang II also functions as a local biologically active mediator in the progression of cardiovascular remodeling and nephropathy through Ang II type-1 (AT1) receptor.[31,32] Excessive production of local Ang II in the kidney increases intraglomerular pressure resulting in microalbuminuria, and enhances synthesis of extracellular matrix.[32] Therefore, AT1 receptor blockers (ARBs) are considered to have cardio- and reno-protective effects beyond their anti-hypertensive effects.[33–35] Moreover, several reports demonstrated that ARBs reduced the risk of new-onset diabetes in humans.[36] Recently, RAS blockers were reported to increase plasma adiponectin concentrations in patients with essential hypertension.[37] However, the effect of ARB on adipose-function, that is, adipocytokine production, has not been defined in experimental study. In the present study, we investigated the effects of olmesartan, an ARB, on the dysregulation of adipocytokines, with a special focus on adiponectin.

RESULTS

Effects of olmesartan on plasma adiponectin levels

We treated lean C57BL/6J and obese KKAy mice with olmesartan and investigated the changes in plasma adiponectin levels. We analyzed KKAy mice as an obese mouse model because KKAy mice exhibit phenotypes of the metabolic syndrome including obesity, diabetes, dyslipidemia, fatty liver, hypertension and hypoadiponectinemia.[38] Plasma adiponectin concentrations were similar in 8- and 20-week-old C57BL/6J mice (34.3 ± 1.4 and $38.1 \pm 1.2\,\mu g/ml$, respectively). In contrast, plasma adiponectin concentrations in obese KKAy mice decreased with age, in parallel with the increase in body weight (8-week-old: 25.2 ± 1.3, 20-week-old: $16.5 \pm 0.9\,\mu g/ml$). Lean C57BL/6J and obese KKAy mice (21-week-old) were treated with olmesartan for 2 weeks. Olmesartan treatment resulted in a significant fall in blood pressure level but did not affect the body weights of both C57BL/6J and KKAy mice, compared with control mice (Table 1). Olmesartan significantly prevented the decline in plasma adiponectin in obese KKAy mice (Figure 1a, Table 1). In contrast, olmesartan treatment did not significantly change plasma adiponectin concentrations in lean C57BL/6J mice (Figure 1b, Table 1). To confirm the effect of olmesartan in preventing the reduction in plasma adiponectin concentrations during the development of obesity, we repeated the experiments in genetically obese *db/db* and diet-induced obesity (DIO) mice, and investigated the changes in plasma adiponectin concentrations. Olmesartan treatment blunted the reduction of plasma adiponectin both in *db/db* and diet-induced obesity mice (Figure 1c and d). Furthermore, adiponectin messenger RNA (mRNA) levels in adipose tissue were significantly higher in olmesartan-treated mice compared with control KKAy mice (Figure 1e). Insulin tolerance tests showed improved insulin sensitivity in olmesartan-treated KKAy mice (Figure 1f), compared with control KKAy mice.

Effects of olmesartan on adipocytokine expression

To test whether olmesartan directly affects the adipose tissue to improve the obesity-related low plasma adiponectin level, we investigated the expression of AT1a receptor mRNA in various tissues of mice. The expression level of AT1a receptor in adipose tissue was abundant, compared with heart, kidney, and skeletal muscle, which are considered as the major target organs of ARB,[39,40] but there was no significant difference in AT1a receptor mRNA levels of adipose tissues between lean C57BL/6J and obese KKAy mice (Figure 2a). These results suggest that the adipose tissue is one of the major target organs of ARB *in vivo*. We also investigated the effect of ARB on the dysregulated expressions of other adipocytokines. The mRNA expression levels of TNF-α, PAI-1, monocyte chemotactic protein-1 (MCP-1), and serum amyloid A3 (SAA3), which is an acute phase reactant in adipose tissue like C-reactive protein,[41,42] were overexpressed in the obese

Table 1 | Body weight, SBP, plasma glucose, insulin, and plasma adiponectin concentrations in C57BL/6 and KKAy mice before and after 2-week treatment with olmesartan

	Control (n=4)		Olmesartan (n=4)	
	Before	After	Before	After
C57BL/6 mice				
Body weight (g)	22.0±0.7	24.2±1.3¶	22.5±0.4	24.5±0.3¶
SBP (mmHg)	100.3±2.9	105.5±1.3	106.7±3.0	86.0±4.1§,*
Glucose (mmol/l)	10.3±0.5	10.8±0.2	9.9±0.5	10.5±1.2
Insulin (nmol/l)	0.06±0.01	0.05±0.01	0.07±0.01	0.05±0.01
Adiponectin (μg/ml)	37.7±2.6	38.0±3.3	38.8±1.0	37.8±2.9
KKAy mice				
Body weight (g)	51.2±1.8	51.0±1.6	50.9±1.1	51.7±0.8
SBP (mmHg)	116.4±3.3	119.8±3.9	110.9±2.4	80.0±2.9§,*
Glucose (mmol/l)	26.3±2.7	27.4±2.8	26.6±2.2	24.1±3.0
Insulin (nmol/l)	13.9±0.7	17.6±1.0¶	13.0±1.1	14.6±2.3
Adiponectin (μg/ml)	15.6±1.1	12.6±0.4 ¶	15.1±1.7	18.1±1.6*

SBP, systolic blood pressure.
Values are mean±s.e.m. *$P<0.01$, compared with control mice.
¶$P<0.05$, §$P<0.01$, compared with the basal value (before olmesartan treatment).

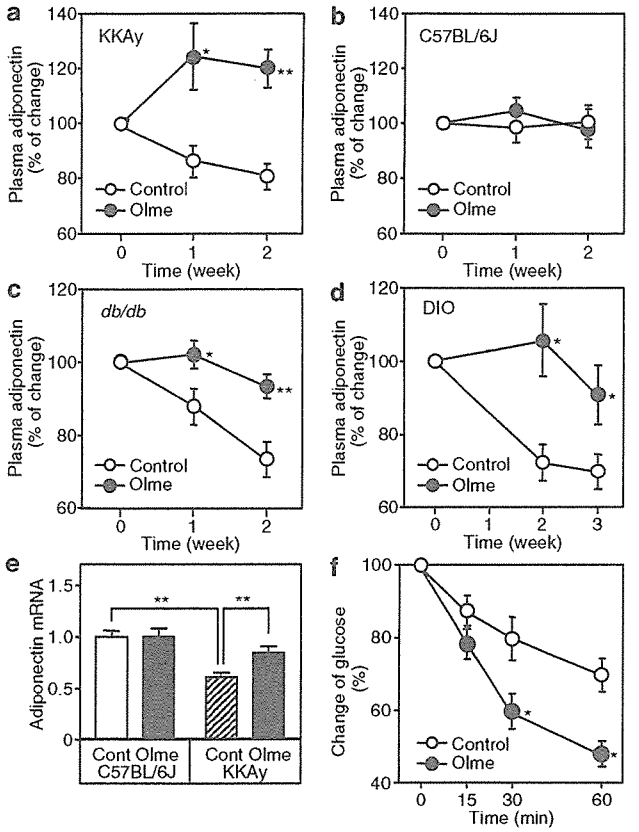

Figure 1 | Effects of olmesartan on plasma concentrations of adiponectin in mice. (**a** and **b**) Twenty-one-week-old KKAy and C57BL/6J mice were treated with olmesartan (Olme), or no drug (Control) for 2 weeks ($n = 4$, each). Plasma adiponectin concentrations were measured once a week. (**c**) Sixteen-week-old *db/db* mice were treated with olmesartan (Olme), or no drug (Control) ($n = 9$, each) for 2 weeks. (**d**) Eight-week-old C57BL/6J mice were fed high fat/high sucrose. At 21 weeks of age, they were fed the same diet mixed with either olmesartan (Olme) or no drug (Control) ($n = 5$, each) for 3 weeks. Plasma adiponectin concentrations were presented as percentage change relative to their levels before olmesartan treatment. (**e**) Adiponectin mRNA expression in adipose tissues. The values were normalized to the level of cyclophilin mRNA. (**f**) Insulin sensitivity of olmesartan-treated KKAy mice. Glucose curves under the insulin tolerance test. Plasma glucose levels were normalized to those at 0 min (100%). In sections **a–d** and **f**, open circles: control group, solid circles: olmesartan group. Values are mean ± s.e.m., $^*P < 0.05$ compared with control mice. Similar results were obtained in another independent experiment.

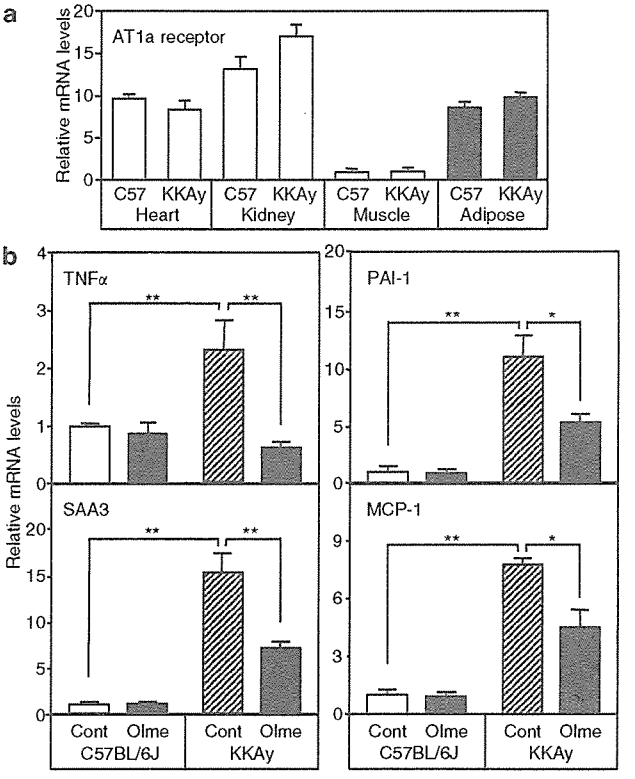

Figure 2 | Effects of olmesartan on mRNA expression of adipocytokines in mice. (**a**) Expression levels of AT1a receptor mRNA in various mouse tissues. Values are normalized to the level of 36B4 mRNA. (**b**) The mRNA expression levels of TNF-α, PAI-1, SAA3, and MCP-1 in adipose tissue of C57BL/6J and KKAy mice treated with olmesartan (Olme), or no drug (Cont) ($n = 4$, each). The values were normalized to the level of cyclophilin mRNA. Data displayed in A and B are expressed as mean ± s.e.m. $^*P < 0.05$, $^{**}P < 0.01$.

KKAy mice compared with lean C57BL/6J mice. Figure 2b clearly shows that treatment with olmesartan attenuated the enhanced mRNA expressions of TNF-α, PAI-1, SAA3, and MCP-1 in obese KKAy mice, but not in lean C57BL/6J mice. These findings indicate that olmesartan does not affect the expression of adipocytokines in the lean mouse, but improves the dysregulation of adipocytokines in the obese mouse. In the next step, we examined the molecular mechanism of the action of ARB on adipocytokines in obese mice.

Effect of olmesartan on reactive oxygen species in adipose tissue

It has been reported that ARBs improve oxidative stress in blood vessels and skeletal muscles.[40,43] Recently, Furukawa *et al.*[38] reported that reactive oxygen species (ROS) are increased in obesity before the rise in plasma glucose concentrations. They demonstrated that actively produced ROS originated from accumulated fat, which subsequently caused dysregulation of various adipocytokines, such as adiponectin, PAI-1, and TNF-α. ARBs might ameliorate adipocytokine dysregulation through a reduction of oxidative stress in adipose tissue. To test this hypothesis, we measured the concentration of adipose thiobarbituric acid reactive substance (TBARS), a marker of lipid peroxidation (LPO), in C57BL/6J and KKAy mice. Adipose TBARS concentrations were higher in KKAy mice than in C57BL/6J mice, in agreement with previous data.[38] Treatment with olmesartan markedly attenuated adipose TBARS concentrations in obese KKAy mice, but no such effect was observed in lean C57BL/6J mice (Figure 3a). Moreover, plasma levels of H_2O_2, a hazardous ROS against tissues and cells, were also attenuated

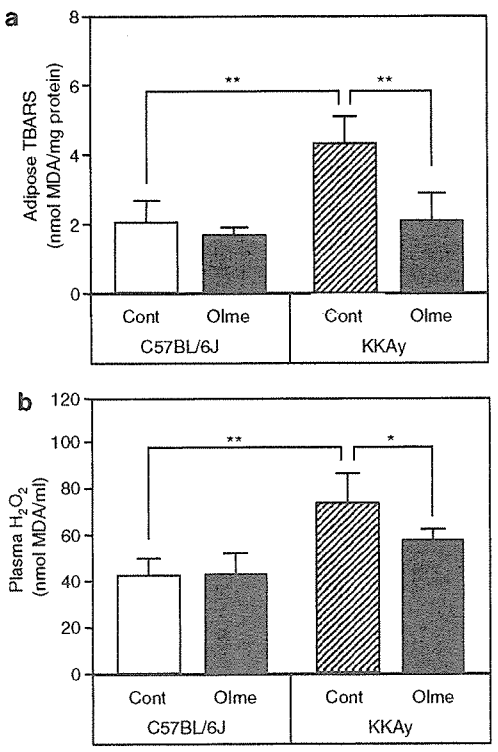

Figure 3 | (a) Effects of olmesartan on LPO levels in adipose tissues of C57BL/6J and KKAy mice treated for 2 weeks with olmesartan (Olme), or no drug (Cont) ($n = 4$, each). The level of LPO in adipose tissue homogenates was measured as TBARS. (**b**) Effects of olmesartan on plasma H_2O_2 concentrations in mice. Data are mean ± s.e.m. $^*P < 0.05$, $^{**}P < 0.01$. MDA, malondialdehyde.

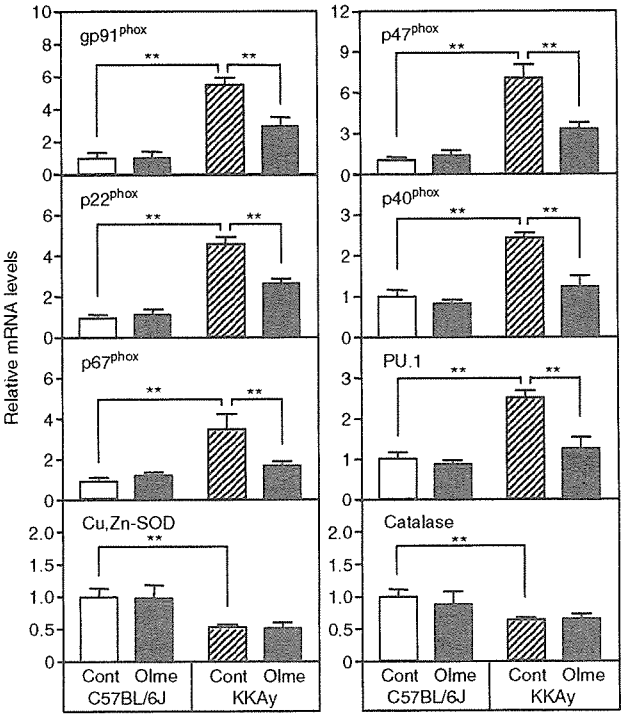

Figure 4 | Effects of olmesartan on mRNA expression levels of NADPH oxidase subunits, PU.1, Cu, Zn-superoxide dismutases and catalase in adipose tissues of C57BL/6J and KKAy mice treated for 2 weeks with olmesartan (Olme), or no drug (Cont) ($n = 4$, each). Values are normalized to the level of cyclophilin mRNA and are expressed as mean ± s.e.m. $^*P < 0.05$, $^{**}P < 0.01$.

by olmesartan treatment in obese KKAy mice but not in lean C57BL/6J mice (Figure 3b).

Effects of olmesartan on production and removal system of fat ROS

Next, we investigated the expression of genes related to the production and removal of ROS in adipose tissue and the effects of olmesartan on their expression levels. Nicotinamide adenine dinucleotide phospho hydrogenase (NADPH) oxidase complex is a major source of ROS in various organs especially in accumulated fat.[38,44] The NADPH oxidase complex consists of membrane-associated flavocytochrome b_{558} family of proteins, which includes $gp91^{phox}$ and $p22^{phox}$ as well as cytosolic components $p47^{phox}$ $p67^{phox}$, and $p40^{phox}$ The expression levels of all subunits were markedly augmented in adipose tissue of obese KKAy mice (Figure 4), as described previously.[38] In obese KKAy mice, we also found increased expression of transcriptional factor PU.1, which is known to upregulate the transcription of NADPH oxidase subunits.[45] Olmesartan clearly reduced the expression of NADPH oxidase subunits and PU.1 in the adipose tissue of obese KKAy mice, but had no effect on the expression of these genes in lean C57BL/6 mice. The expression levels of CU, Zn-superoxide dismutases, and catalase, the ROS elimination system, were decreased in obese KKAy mice, but olmesartan did not affect the expression of these genes.

Effects of olmesartan on adipocytokines in adipocytes

In the above experiments, we demonstrated that ARB reduced ROS production in obese adipose tissue. Recently, RAS inhibitors were reported to prevent oxidative stress-induced apoptosis of vascular endothelial cells.[46] Therefore, to investigate whether ARB directly affects downstream ROS-mediated adipocytokine dysregulation, we conducted *in vitro* experiments using primary adipocytes. We used differentiated adipocytes derived from stromal vascular cells (SVCs) of mouse adipose tissues. The secretion of adiponectin from adipocytes was diminished by incubation with H_2O_2 in a dose-dependent manner (Figure 5a). Antioxidant *N*-acetyl cysteine canceled the H_2O_2-induced inhibition of adiponectin secretion, as described previously.[38] These results validated the experimental procedure and H_2O_2 treatment in this study. In subsequent experiments, olmesartan resulted in a significant amelioration in the inhibitory effect of H_2O_2 on adiponectin secretion, whereas it had no effect on basal adiponectin secretion (Figure 5b). ACE inhibitor, captpril, significantly but slightly ameliorated oxidative stress-induced impairment of adiponectin production (Figure 5c). The