Acute-Phase Glucose Fluctuation Is Negatively Correlated With Myocardial Salvage After Acute Myocardial Infarction – Involvement of Monocyte Subsets –

Ikuko Teraguchi, MD; Toshio Imanishi, MD, PhD; Yuichi Ozaki, MD; Takashi Tanimoto, MD, PhD; Minoru Ueyama; Makoto Orii, MD; Yasutsugu Shiono, MD; Kunihiro Shimamura, MD; Kohei Ishibashi, MD; Takashi Yamano, MD; Yasushi Ino, MD, PhD; Tomoyuki Yamaguchi, MD; Kumiko Hirata, MD, PhD; Takashi Kubo, MD, PhD; Tokio Sanke, MD, PhD; Takashi Akasaka, MD, PhD

Background: It remains unclear whether glycemic fluctuation immediately after acute myocardial infarction (AMI) can affect myocardial damage. This study investigated the impact of glucose fluctuation on myocardial salvage following successful recanalization of primary AMI.

Methods and Results: A total of 36 consecutive patients with AMI were studied. Glycemic variability, as indicated by the mean amplitude of glycemic excursion (MAGE), was measured on a continuous glucose monitoring system. Three subsets (CD14+CD16−, CD14++CD16+ and CD14+−CD16+) were measured on flow cytometry 1, 2, 3, 4 and 5 days after AMI onset. A 2-h oral glucose test was performed in 23 patients who had no previous diagnosis of diabetes and/or glycated hemoglobin <6.5%, after the onset of AMI at 2 weeks. Plasma active glucagon-like peptide (GLP)-1 level was measured in each sample. The extent of myocardial salvage 7 days after AMI was evaluated on cardiovascular magnetic resonance imaging. MAGE and the peak CD14+CD16− monocyte level were significantly negatively correlated with myocardial salvage index (MSI). MAGE was significantly correlated with peak CD14+CD16− monocyte level. Of interest, plasma GLP-1 level was significantly positively correlated with MSI and significantly negatively correlated with MAGE.

Conclusions: Glycemic fluctuations during the acute phase of AMI affect MSI, indicating that manipulation of glucose variability from peak to nadir might be a potential therapeutic target for salvaging ischemic damage. (Circ J 2014; 78: 170–179)

Key Words: Acute myocardial infarction; Glucagon-like peptide-1; Glucose fluctuation; Monocyte; Myocardial salvage

Numerous studies have shown an association between plasma glucose level on admission and an increased risk of mortality and poor prognosis after acute myocardial infarction (AMI), regardless of diabetes status.1–4 The Japan Acute Coronary Syndrome Study (JACSS)5 found that in patients without a history of diabetes, there was a linear relation between blood glucose level at admission and in-hospital mortality, while there was a U-shaped relationship between blood glucose level and mortality in patients with a history of diabetes. In line with these findings, we found a significantly positive correlation between impaired myocardial salvage index (MSI) and stress hyperglycemia on admission in non-diabetic, but not diabetic, patients with AMI.6 Glycemic disorders during the early phase after AMI, however, are not solely limited to stress hyperglycemia but can extend to glycemic excursions, which include acute glucose changes in both directions. Less is known regarding the association between glycemic fluctuation during the early phase of AMI and the extent of myocardial salvage.
Monocytes in human peripheral blood are heterogeneous. Differential expression of CD14 and CD16 allows monocytes to be divided into subsets: CD14+CD16− monocytes express C-C motif chemokine receptor 2 (CCR2), whereas CD14++CD16+ monocytes express C-X3-C motif chemokine receptor 1 (CX3CR1).

CD14+CD16− monocytes give rise to recruited macrophages and dendritic cells, in response to a pro-inflammatory stimulus, whereas CD14+CD16+ monocytes give rise to resident tissue cells. Previously, we explored the impact of monocyte subset on myocardial salvage following AMI and found that peak CD14+CD16− monocyte level is correlated with the extent of myocardial salvage after AMI.

The purpose of the present study was to investigate the effect of glucose fluctuations, using the continuous glucose monitoring system (CGMS), on myocardial salvage in patients with successfully recalibration of primary AMI.

Methods

Patients

A total of 68 patients with AMI were enrolled in the study. AMI was diagnosed as: (1) chest pain within 24 h before admission that lasted for >30 min and was not relieved by sublingual nitroglycerin; (2) ST-segment elevation and/or abnormal Q-wave on electrocardiogram (ECG); and (3) elevated serum creatine kinase level. Exclusion criteria were as follows: (1) AMI for >24 h from onset; (2) a history of myocardial infarction; (3) evidence of malignant disease; (4) systemic inflammatory conditions requiring any anti-inflammatory drugs; (5) lack of cardiovascular magnetic resonance imaging (CMR), due to renal dysfunction (serum creatinine ≥1.5 mg/dl), claustrophobia, dyspepsia, metallic implant or tattoo; or (6) unwillingness to participate. CGMS was used in all included patients unless refusal. All patients received coronary angiography on admission and then underwent percutaneous coronary intervention (PCI) using coronary stents and had final thrombolyis in myocardial infarction (TIMI) flow grade 3 after PCI. Patients were routinely treated with heparin, isosorbide dinitrate, clopidogrel, aspirin, and an angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker. All patients gave informed consent to participate in the trial.

Clinical Parameters

The assessed clinical parameters were age, gender, and coronary risk factors (smoking, hypertension, diabetes mellitus, hyperlipidemia, and obesity). The diagnostic criteria for coronary risk factors were as follows: hypertension, blood pressure >140/90 mmHg and/or a history of anti-hypertensive medication; diabetes mellitus, fasting plasma glucose >126 mg/dl; casual plasma glucose >200 mg/dl, or a diabetic pattern based on the 75-g oral glucose tolerance test (OGTT); >126 mg/dl or OGTT ≥200 mg/dl; hyperlipidemia, serum total cholesterol level >220 mg/dl or serum triglyceride level >150 mg/dl; obesity, body mass index >25 kg/m².

Blood Sampling and Analysis

Peripheral blood samples were collected from all subjects as soon as possible after admission and on days 2, 3, 4, and 5 days after the onset of AMI. Plasma samples were collected in ethylenediamine tetra-acetic acid (EDTA) anticoagulant. Urinary samples were collected from all subjects as soon as possible after admission. Plasma and urinary samples were stored at −80°C until required for analysis.

A 2-h OGTT was performed in 23 patients who had no previous diagnosis of diabetes and/or glycated hemoglobin (HbA1c) <6.5% at 2 weeks after the onset of AMI. Patients drank a solution containing 75 g dextrose, and blood samples were obtained at 0, 30, 60, and 120 min. Serum glucose level and active glucagon-like peptide-1 (GLP-1; 7-36 amide) from each OGTT sample and immunoreactive insulin level from 0 and 30 min were measured. Blood used for GLP-1 measurement was collected in EDTA-coated tubes (1.5 μl/ml) containing apropin (40 μl/ml) and an inhibitor of dipeptidyl peptidase (DPP)-4 (10 μl/ml; Millipore, Billerica, MA, USA). Blood was centrifuged and plasma was subsequently stored in aliquots at −80°C until required for analysis. Immunoassay kits for active GLP-1 were obtained from Millipore. Active GLP-1 response was evaluated using the area under the GLP-1 concentration-time curve (AUCGLP-1) from 0 to 120 min. The homeostasis model assessment of insulin resistance (HOMA-R) index was calculated using the following formula: fasting glucose (mg/dl)×fasting insulin (μU/ml)/405. The insulogenic index was calculated using the following formula: (insulin_min– insulin_max)/(glucose_max–glucose_min). All other laboratory analyses were performed in the central laboratory of the hospital.

Urinary 8-isoprostane, measured as an index of oxidative stress, was analyzed using a commercially available enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA).

Continuous Glucose Monitoring

Subcutaneous interstitial glucose level was monitored over a period of 2 consecutive days using the fourth-generation CGMS (Medtronic iPro2). A sensor was inserted approximately 24 h after the onset of AMI. Data were downloaded and glucose profiles were evaluated based on the blood glucose data collected. The CGMS was applied to the abdominal area. Blood glucose was measured using the finger-stick test, at least 4 times per day (at mealtimes and at bedtime). Standardized meal was started on the day of the onset of AMI, which contained 6,698 kJ (15% protein, 23% fat and 62% glucose). I.v. drip injection with glucose solution was not used during the first 72 h after AMI.

Assessment of Glycemic Variability

Intra-day glycemic variability was assessed as the mean amplitude of glycemic excursion (MAGE), which was calculated as described by Service et al12 by measuring the arithmetic mean of differences between the consecutive peak and nadir if the differences were greater than the standard deviation around the mean glucose level; measurements in the peak-to-nadir or nadir-to-peak directions were determined by the first qualifying excursion.

Cytometry

For cytometric analysis, monoclonal antibodies against CD14 (fluorescein isothiocyanate [FITC]-conjugated, clone M5E2; BD Bioscience, San Jose, CA, USA) and CD16 (phycoerythrin [PE]-CyTM5-conjugated, clone 3G8; BD Bioscience) were used as described previously.10-13 Matched-isotype antibodies (FITC-conjugated mouse IgG2a isotype, clone G155-178, and PE-CyTM5 mouse IgG1 isotype, clone MOPC-21; BD Bioscience) were used as negative controls. Blood (100 μl) was incubated for 30 min at room temperature in the dark. For erythrocyte lysis and leukocyte fixation, 1 ml of lysis solution was added (BD FACS Lyse, Lysing Solution; Becton Dickinson, Editorial p67
Late gadolinium enhancement (LGE) imaging covering the whole ventricle was acquired 10–15 min after i.v. injection of 0.1 mmol/kg gadolinium-diethylenetriamine penta-acid (Magnevist, Schering, Berlin, Germany). A 3-D inversion-recovery turbo gradient echo sequence was used, and images were obtained during an end-expiratory breath-hold. Scan parameters were as follows: TR, 4.1 ms; TE, 1.25 ms; flip angle, 15°; field of view, 350 × 350 mm; partial echo; matrix, 224 × 256; and spatial resolution, 1.56 × 2.24 × 10 mm³ reconstructed to 0.68 × 0.68 × 5 mm³. The inversion time (200–300 ms) was optimized to null the normal myocardium. The slice positions for both T2W and LGE acquisitions matched those of the cine images.

**CMR Data Analysis**

All analyses were performed by consensus of 2 blinded observers (Y.O. and A.S.) on an off-line workstation (View Forum, Philips Medical Systems). The extent of the area at risk (T2W hyperintense lesion) and the extent of the area of LGE were quantified at the same slice location with the maximum extent of T2 signal abnormality using the following formula as previously reported: (area of high signal/total slice area) × 100. Therefore, we provided data regarding CD14+CD16−, CD14++CD16+, and CD14−CD16+ subsets in this study. Leukocytes were counted using an automated Coulter Counter (Beckman Coulter, Miami, FL, USA).

**Non-Invasive CMR Protocol**

CMR was performed using a 1.5-T clinical scanner (Intera Achieva, Philips Medical Systems, Best, The Netherlands) equipped with a 5-element cardiac phased-array coil for signal reception 7 days after the onset of AMI, as previously described. During the procedure, the patient was monitored with a continuous single-lead ECG, repeated blood pressure measurement, and pulse oximetry. With the patient in the supine position, contiguous short-axis cine images covering the left ventricle (LV) from base to apex were acquired using a standard steady-state free-precession sequence. A breath-hold T2-weighted (T2W) sequence was then applied with short-T1 inversion recovery for fat saturation. Imaging parameters were: repetition time (TR), 2 R–R intervals; echo time (TE), 90 ms; slice thickness, 8 mm; field of view, 35 μm; and matrix, 256 × 512 in 3 short-axis slices (basal, midventricular, and apical). Each slice was obtained during an end-expiratory breath-hold of 12–15 s depending on the patient’s heart rate.

Cytometric analysis was performed in a flow cytometer (BD FACS AriaTM; Becton Dickinson) using BD FACSDiva (Becton Dickinson). Monocytes were first gated in a forward scatter/sideward scatter (FSC/SSC) dot-plot, and 2-color fluorescence was measured within the monocyte gate (Figure 1). CD14+CD16− cells were defined as monocytes expressing CD14 but not CD16, CD14+CD16+ cells were defined as monocytes expressing CD16 and either a high level of CD14 (CD14++CD16+), a low level of CD14 (CD14−CD16−), or a level of CD14 (CD14++CD16+). The recently updated classification of monocyte heterogeneity acknowledges the existence of 3 monocyte subsets, that is, classical monocytes (CD14++CD16−), intermediate monocytes (CD14++CD16+), and non-classical monocytes (CD14+CD16+). Therefore, we provided data regarding CD14+CD16−, CD14++CD16+, and CD14−CD16− subsets in this study. Leukocytes were counted using an automated Coulter Counter (Beckman Coulter, Miami, FL, USA).
Glucose Fluctuation and Myocardial Salvage After AMI

Results

Patient Characteristics

Of 68 eligible patients with ST-segment elevation myocardial infarction, 60 patients underwent CMR. Reasons for not undergoing CMR were renal dysfunction (n=4), long-term intensive care >7 days, congestive heart failure (n=4), claustrophobia (n=1) and having a tattoo (n=1). Of the 60 patients who had CMR, 36 consecutive patients were enrolled in this study and 24 patients were excluded. The reasons for exclusion were previous myocardial infarction (n=11), recent myocardial infarction >24 h (n=9), refusal to participate (n=3) and inflammatory disease (n=1). In all 36 study subjects, 7 patients were treated with statins before admission.

We did not find any significant relationship between glucose fluctuation, monocyte subsets and anti-diabetic medications and statins in the present subjects. No patients used insulin and any anti-inflammatory drugs before admission. The median

systolic wall motion in the infarct region. The change in regional wall motion was defined as percent increase in LV wall motion during systole compared with diastole. Myocardial segments showing LGE at day 7 were defined as the infarct region. For an assessment of infarct size, LV myocardium with LGE volumes was quantified. For analysis of the change in regional wall motion, the 2 most basal and 2 most distal slices were excluded, because short-axis images at these levels preclude a reliable segmental evaluation owing to the presence of the LV outflow tract and a small diameter, respectively.

Statistical Analysis

All data are expressed as mean±SD unless stated otherwise. For statistical analysis of categorical MSI variables, parameters were evaluated on univariate regression. A multivariate regression model was used to determine predictors of MSI. Those variables that had P<0.01 on univariate analysis and clinically influential factors for myocardial salvage were included in the multiple regression analysis: MAGE, CD14+CD16− monocytes, reperfusion time and peak creatine kinase-myocardial band (CK-MB). All statistical analyses was performed using SPSS version 11.0 (SPSS, Chicago, IL, USA). The authors had full access to the data and take responsibility for its integrity. P<0.05 was considered significant.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-diabetes mellitus</th>
<th>Diabetes mellitus</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67±9.9</td>
<td>68±8.4</td>
<td>0.64</td>
</tr>
<tr>
<td>Men</td>
<td>17 (71)</td>
<td>10 (83)</td>
<td>0.56</td>
</tr>
<tr>
<td>Culprit vessel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>10 (42)</td>
<td>4 (33)</td>
<td>0.83</td>
</tr>
<tr>
<td>LCX</td>
<td>4 (16)</td>
<td>3 (25)</td>
<td></td>
</tr>
<tr>
<td>RCA</td>
<td>10 (42)</td>
<td>5 (42)</td>
<td></td>
</tr>
<tr>
<td>Coronary risk factor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>20 (83)</td>
<td>11 (92)</td>
<td>0.70</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>17 (71)</td>
<td>8 (67)</td>
<td>0.86</td>
</tr>
<tr>
<td>Current smoking</td>
<td>19 (79)</td>
<td>6 (50)</td>
<td>0.17</td>
</tr>
<tr>
<td>Family history</td>
<td>5 (21)</td>
<td>2 (17)</td>
<td>0.86</td>
</tr>
<tr>
<td>Obesity</td>
<td>8 (33)</td>
<td>7 (58)</td>
<td>0.24</td>
</tr>
<tr>
<td>Medication on admission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACEI or ARB</td>
<td>5 (21)</td>
<td>3 (25)</td>
<td>0.86</td>
</tr>
<tr>
<td>β-blocker</td>
<td>2 (8)</td>
<td>1 (8)</td>
<td>1.00</td>
</tr>
<tr>
<td>CCB</td>
<td>6 (25)</td>
<td>6 (50)</td>
<td>0.24</td>
</tr>
<tr>
<td>Statin</td>
<td>3 (13)</td>
<td>4 (33)</td>
<td>0.33</td>
</tr>
<tr>
<td>Aspirin</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>0.70</td>
</tr>
<tr>
<td>Anti-diabetic medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>–</td>
</tr>
<tr>
<td>Biguanide</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>–</td>
</tr>
<tr>
<td>α-GI</td>
<td>0 (0)</td>
<td>4 (33)</td>
<td>–</td>
</tr>
<tr>
<td>DPP-4 inhibitor</td>
<td>0 (0)</td>
<td>6 (50)</td>
<td>–</td>
</tr>
<tr>
<td>Insulin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>–</td>
</tr>
<tr>
<td>Reperfusion time (min)</td>
<td>419±327</td>
<td>446±357</td>
<td>0.99</td>
</tr>
<tr>
<td>Max CK (IU/L)</td>
<td>2,788±2,391</td>
<td>3,651±3,794</td>
<td>0.73</td>
</tr>
<tr>
<td>Max CK-MB (IU/L)</td>
<td>284±214</td>
<td>313±223</td>
<td>0.62</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.5±0.3</td>
<td>7.5±1.5</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Data given as mean±SD or n (%). *P<0.05. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; CK, creatine kinase; CK-MB, creatine kinase-myocardial band; HbA1c, glycated hemoglobin; LAD, left anterior artery; LCX, left circumflex artery; RCA, right coronary artery.
Figure 2. Impact of glycemic fluctuation on myocardial salvage. Measurement of myocardial salvage index was evaluated 7 days after acute myocardial infarction. Patients with (A) little glycemic variability (mean amplitude of glycemic excursion [MAGE]=57 mg/dl, <median: 72 mg/dl) and (B) intense glycemic variability (MAGE=121 mg/dl, ≥median: 72 mg/dl). (A-1,B-1) Hyperintense areas indicating extent of the areas at risk on T2-weighted, fast spin-echo imaging. (A-2,B-2) Infarcted areas, indicated by late gadolinium enhancement-bright images. Myocardial salvage index was determined as follows: (area of high signal/total slice area)×100.
Glucose Fluctuation and Myocardial Salvage After AMI

Significantly associated with MAGE in the non-diabetes group and the diabetes group (r=0.19, P=0.21 and r=−0.09, P=0.68). The serum glucagon and insulin levels on admission were evaluated in 33 patients. There was no significantly relationship between these parameters, glucagon and insulin, and glucose fluctuation (r=−0.16, P=0.45 and r=0.15, P=0.53, respectively).

Hypoglycemia (<70 mg/dl) was observed on the CGMS record in 37% of patients (n=13). There was no significant relationship between hypoglycemia and MSI (P=0.093).

Monocyte Subsets, Acute-Phase MSI
Peripheral whole blood samples were obtained at admission and days 2–5 and used to analyze the 3 distinct monocyte subsets (CD14+CD16−, CD14++CD16+ and CD14−CD16+). As shown in Figure 4, the proportion of CD14+CD16− monocytes was significantly associated with MSI (r=−0.40, P=0.02) and MAGE (r=0.39, P=0.02).

Urinary 8-Isoprostane and Glycemic Variability
Urinary 8-isoprostane, an oxidative stress marker, was measured in all study subjects. There was a significant relationship between urinary 8-isoprostane and MAGE (r=0.35, P=0.038).

Active GLP-1, MAGE and Acute-Phase MSI
Changes in active GLP-1 level in the OGTT were used because fasting active GLP-1 level cannot be used to predict increments after OGTT for the evaluation of incretin secretion.20 AUCGLP-1 was significantly negatively correlated with MAGE (r=−0.42, P=0.047), and was also significantly positively associated with

MAGE was 72 mg/dl.

Of the 36 patients enrolled in this study, OGTT was performed in 23 patients at 2 weeks after the onset of AMI. The reasons for exclusion were diabetes (n=8) and refusal to participate (n=5). A total of 17% (n=4) and 30% (n=7) of these 23 patients were diagnosed with diabetes and impaired glucose tolerance, respectively.

Effects of Glycemic Variability on Acute-Phase MSI
We investigated the relationship between glycemic variability and MSI 7 days after AMI onset on CMR as the difference between the areas of myocardium at risk (T2W hyperintense lesion) and the areas of LGE. MSI in a representative patient with low glycemic variability (MAGE=57 mg/dl, <median: 72 mg/dl) and in a representative patient with high glycemic variability (MAGE=121 mg/dl, ≥median: 72 mg/dl) is shown in Figure 2.

MAGE was significantly negatively associated with MSI (r=−0.49, P=0.01; Figure 3). In the non-diabetes group and the diabetes group, the negative correlation with MAGE was significant (r=−0.46, P=0.025 and r=−0.63, P=0.028, respectively). In accordance with our previous findings,6 MAGE was significantly lower in patients with stress hyperglycemia (admission blood glucose level ≥180 mg/dl, n=15) than in those without stress hyperglycemia (admission blood glucose level <180 mg/dl, n=21; 47.2±11.7% vs. 53.7±13.2%, P=0.046, respectively). HOMA-R index and insulinogenic index were not significantly associated with MSI (r=−0.37, P=0.12 and r=0.22, P=0.35, respectively). As unexpected, the relationship between MAGE and stress hyperglycemia was not significant (P=0.11). In addition, glucose tolerance and HbA1c level was not significantly associated with MAGE in the non-diabetes group and the diabetes group (r=0.19, P=0.21 and r=−0.09, P=0.68). The serum glucagon and insulin levels on admission were evaluated in 33 patients. There was no significantly relationship between these parameters, glucagon and insulin, and glucose fluctuation (r=−0.16, P=0.45 and r=0.15, P=0.53, respectively). Hypoglycemia (<70 mg/dl) was observed on the CGMS record in 37% of patients (n=13). There was no significant relationship between hypoglycemia and MSI (P=0.093).

Monocyte Subsets, Acute-Phase MSI and MAGE
Peripheral whole blood samples were obtained at admission and days 2–5 and used to analyze the 3 distinct monocyte subsets (CD14+CD16−, CD14++CD16+ and CD14−CD16+). As shown in Figure 4, the proportion of CD14+CD16− monocytes was significantly associated with MSI (r=−0.40, P=0.02) and MAGE (r=0.39, P=0.02).

Urinary 8-Isoprostane and Glycemic Variability
Urinary 8-isoprostane, an oxidative stress marker, was measured in all study subjects. There was a significant relationship between urinary 8-isoprostane and MAGE (r=0.35, P=0.038).

In addition, urinary 8-isoprostane was significantly negatively associated with MSI (r=−0.33, P=0.047; Figure 5).

Active GLP-1, MAGE and Acute-Phase MSI
Changes in active GLP-1 level in the OGTT were used because fasting active GLP-1 level cannot be used to predict increments after OGTT for the evaluation of incretin secretion.20 AUCGLP-1 was significantly negatively correlated with MAGE (r=−0.42, P=0.047), and was also significantly positively associated with
Figure 4. Peripheral whole blood samples were obtained at admission and days 2–5 and used to analyze the 3 distinct monocyte subsets (CD14⁺CD16⁻, CD14⁺CD16⁺ and CD14⁻CD16⁻). The relative proportion of CD14⁺CD16⁻ monocytes was significantly associated with myocardial salvage index ($r=-0.40$, $P=0.02$).

Figure 5. Relationship between urinary 8-isoprostane and glycemic variability. There was a significant relationship between urinary 8-isoprostane, an oxidative stress marker, and myocardial salvage index ($r=-0.33$, $P=0.047$).
Glucose Fluctuation and Myocardial Salvage After AMI

We showed previously that stress hyperglycemia in patients without diabetes was an independent predictor of impairment of myocardial salvage. Glycemic disorders in AMI, however, are not limited to stress hyperglycemia but can also include glycemic excursions with acute glucose changes in both directions. We have found a significant relationship between glycemic fluctuation and the degree of myocardial salvage. Although we did not address the underlying mechanisms in terms of the relationship between them, inflammation and oxidative stress may be involved. We have also shown that peak CD14+CD16− monocyte level is significantly negatively correlated with myocardial salvage after AMI and positively correlated with glucose fluctuation. The mechanisms via which CD14+CD16− monocytes enhance injury, however, remain unclear. Previous studies have shown after adjustment for acute LVEF. We showed previously that stress hyperglycemia in patients without diabetes was an independent predictor of impairment of myocardial salvage. Glycemic disorders in AMI, however, are not limited to stress hyperglycemia but can also include glycemic excursions with acute glucose changes in both directions. We have found a significant relationship between glycemic fluctuation and the degree of myocardial salvage. Although we did not address the underlying mechanisms in terms of the relationship between them, inflammation and oxidative stress may be involved. We have also shown that peak CD14+CD16− monocyte level is significantly negatively correlated with myocardial salvage after AMI and positively correlated with glucose fluctuation. The mechanisms via which CD14+CD16− monocytes enhance injury, however, remain unclear. Previous studies have shown

Multiple Regression Analysis
A multivariate regression model was used to determine predictors of MSI. Those variables that had P<0.1 on univariate analysis and clinically influential factors for myocardial salvage were included in the multiple regression analysis: MAGE, CD14+CD16− monocytes, urinary 8-isoprostane, AUCGLP-1, reperfusion time and peak CK-MB (Table 2). In multiple regression analysis, MAGE, CD14+CD16− monocytes and AUCGLP-1 appeared to be independent predictors of MSI (P=0.030, P=0.043 and P=0.048, respectively). No other clinical factors affected MSI.

Discussion
Blood glucose level continuously fluctuates in the human body. We have shown here for the first time that glycemic fluctuation assessed using MAGE is significantly associated with the impairment of myocardial salvage in patients with primary AMI. Although the detailed mechanisms of the association between glucose fluctuation and the impairment of MSI recovery remain unclear, glycemic variability during the acute phase provides important clues to the pathogenesis of myocardial salvage following recanalization of AMI.

Independent of diabetic status, the occurrence of hyperglycemia during AMI is associated with a particularly high risk for adverse clinical outcomes. Stress hyperglycemia with AMI is associated with an increased risk for in-hospital mortality in patients with and without diabetes. Ishihara et al noted a significant correlation between high glucose level on admission and impaired pre-discharge LV ejection fraction (EF), even after adjustment for acute LVEF. We showed previously that stress hyperglycemia in patients without diabetes was an independent predictor of impairment of myocardial salvage. Glycemic disorders in AMI, however, are not limited to stress hyperglycemia but can also include glycemic excursions with acute glucose changes in both directions. We have found a significant relationship between glycemic fluctuation and the degree of myocardial salvage. Although we did not address the underlying mechanisms in terms of the relationship between them, inflammation and oxidative stress may be involved. We have also shown that peak CD14+CD16− monocyte level is significantly negatively correlated with myocardial salvage after AMI and positively correlated with glucose fluctuation. The mechanisms via which CD14+CD16− monocytes enhance injury, however, remain unclear. Previous studies have shown

<table>
<thead>
<tr>
<th>Table 2. Independent Factors for Myocardial Salvage Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate</td>
</tr>
<tr>
<td>Univariate</td>
</tr>
<tr>
<td>MAGE (mg/dl)</td>
</tr>
<tr>
<td>CD14+CD16− monocytes (%)</td>
</tr>
<tr>
<td>Urinary 8-isoprostane</td>
</tr>
<tr>
<td>AUCGLP-1 (pmol·h·L−1)</td>
</tr>
<tr>
<td>Reperfusion time (min)</td>
</tr>
<tr>
<td>Max CK-MB (IU/L)</td>
</tr>
</tbody>
</table>

*P<0.05 vs. MSI. AUCGLP-1, area under the GLP-1 concentration-time curve; Glp-1, glucagon-like peptide-1; MAGE, mean amplitude of glycemic excursion. Other abbreviation as in Table 1.
that monocyte chemotactic protein (MCP)-1, a ligand for CCR2, is markedly upregulated in an ischemic myocardium and is responsible for the recruitment of mononuclear cells into the injured myocardium.24,25 In addition, Dewald et al showed that MCP-(-/-) mice have significantly lower tumor necrosis factor (TNF)-a, interleukin (IL)-1β, and IL-6 messenger ribonucleic acid expression after 6 h of reperfusion with wild-type infarcts in a closed-chest model of reperfusion murine myocardial infarction.26 Taken together, monocytes recruited in the myocardium through CCR2/MCP-1 interactions might play a critical role in the impairment of myocardial salvage.

In addition, we have shown in this study that urinary 8-iso-prostane, an oxidative stress marker, was significantly associated with glycemic fluctuation. From this result, it might be expected that glycemic fluctuation induced oxidative stress, resulting in the impairment of myocardial salvage. Recent studies have indicated that glycemic variability has more deleterious effects on the development of cardiovascular complications in patients with diabetes than sustained hyperglycemia in the development of diabetic complications, because acute glucose swings activate the oxidative stress.7,8 Monnier et al showed that the urinary excretion rate of 8-iso-prostaglandin F2a, which is generated by free radical-mediated oxidation of arachidonic acid,9 was highly and positively correlated with glycemic variability assessed on MAGE. In addition, Esposito et al showed that circulating levels of cytokines, including IL-6, IL-18 and TNF-a, increased as the blood glucose level increased but immediately returned to normal as glucose levels returned to normal.10 Such responses of cytokines to acute elevation of blood glucose were completely prevented by inhibition of the anti-oxidant glutathione. These findings suggest that glycemic variability exaggerates inflammation via an oxidative mechanism closely linked to myocardial salvage. Therefore, we speculate that glucose variability-induced excessive oxidative stress, leading to preferential recruitment of CD14(+)/CD16(-) monocytes, may result in impairment of myocardial salvage. Further studies are needed to verify this hypothesis.

The efficacy of cardioprotective strategies can be quantified by myocardial salvage as an indicator of therapeutic benefit, because the extent of myocardial salvage is an independent predictor of clinical outcome.31,32 Comparing the extent of LGE with the extent of T2W AAR, it is possible to determine the proportion of myocardium that has been salvaged. The T1W and T2W CMR approaches for quantification of AAR utilize non-contrast, early and late gadolinium enhancement techniques. The technical progress, high spatial resolution, and potential for retrospective quantification of the AAR make CMR the most appropriate technique for assessment of myocardial salvage. Here we showed that MSI correlated negatively with the level of MAGE (r=-0.49, P=0.01), indicating that glucose excursion may be an important contributing factor to myocardial salvage after AMI. Because the present results show that glucose fluctuations during the acute phase of AMI affect myocardial salvage, glucose variability may be a suitable target for the treatment of glycemic disorders in patients with AMI. Two classes of incretin-related drugs, GLP-1 analogs and DPP-4 inhibitors, could prevent glucose variability. It has been reported that both GLP-1 analogs and DPP-4 inhibitors, could prevent glucose variability. Here, we measured active GLP-1 after OGTT and found that the level was significantly correlated with MSI, suggesting cardioprotective effects. In addition to the effects of GLP-1 on glucose variability, there is also increasing evidence to support a number of direct effects on the heart that may have the potential to influence cardiovascular outcome. Nikolaidis et al showed that GLP-1 infusion improved regional and global LV function in patients with AMI and severe dysfunction after successful primary angioplasty.33 Potential mechanisms of cardioprotection include the effect of myocardial energy metabolism on the potential benefits associated with a shift toward increased glucose utilization. The pre-clinical and clinical evidence regarding the effects of incretin on the heart has been demonstrated. GLP-1 is likely to have attractive pharmacological properties as a cardioprotective agent because it has a short half-life and there is minimal risk of hyperglycemia. Additional basic research and clinical studies are necessary to further address the cardioprotective efficacy of a GLP-1 agonist.

### Study Limitations

Several study limitations should be considered in the interpretation of the results. First, the results were prospective in terms of patient enrollment but observational in nature. Thus, the present results cannot provide a mechanistic explanation for the improvement in MSI 7 days after AMI that was associated with glycemic fluctuations. Second, we performed CMR assessment of MSI 7 days after reperfusion. The timing of determination of an area of myocardial edema is very important, and this may have affected the results. Wright et al evaluated the influence of the time delay between PCI and imaging assessment of AAR in patients with reperfused AMI using T2W CMR.15 In their study, CMR was performed between 1 and 20 days after PCI (mean and median 4 days). They found no correlation between T2W AAR and the delay between PCI, and CMR did not cause systematic differences in the measured T2W AAR or myocardial salvage. In addition, myocardial edema has been related to be a tissue footprint of the AAR, lasting for at least 7 days after AMI, providing an extended window to evaluate myocardial salvage. Botker et al recommended that the optimal time to evaluate myocardial salvage during a cardiac examination is 1–2 weeks after infarction, because the final infarct size on LGE CMR remains almost constant after 1 week, and AAR remains stable for at least 7 days.36 But we cannot exclude the possibility that earlier acquisition would have influenced the extent of myocardial salvage. Further investigation of the optimal timing of measurement of T2W AAR or myocardial salvage is needed in serial studies of individual patients. Finally, the study population was relatively small. It remained unclear what is the predictor of increase glucose fluctuation due to small sample size.

In conclusion, glycemic variability is associated with impairment of myocardial salvage in patients with successful recanlization of AMI, suggesting that the manipulation of glucose variability from peak to nadir could provide a potential therapeutic target for salvaging ischemic damage.

### Acknowledgments

This work was supported in part by Grant-in-Aid for Scientific Research (C), Japan (No. 23391058).

### Disclosures

No conflict of interest.

### References


3. Page 178