

Decreased Serum Ghrelin Levels in Patients with Acute Myocardial Infarction

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Ghrelin is a novel growth hormone-releasing peptide isolated from the stomach and possesses various cardioprotective effects, including energy balance improvement and regulation of autonomic nervous system activity. We investigated the changes in serum ghrelin levels and its association with cardiac function and myocardial infarct size in patients with acute myocardial infarction (AMI). Forty-seven consecutive patients were divided into the following 4 groups: 16 patients with AMI, 12 patients with unstable angina pectoris (UAP), 13 patients with stable angina pectoris (SAP), and 6 control patients. Serum levels were measured with the ELISA kit. Compared to the control (72 ± 26 fmol/mL), SAP (69 ± 47 fmol/mL), and UAP (72 ± 31 fmol/mL) groups, serum ghrelin levels on admission were significantly lower in the AMI group (27 ± 12 fmol/mL, $P < 0.01$). After admission, the serum ghrelin level gradually increased (30 ± 15 fmol/mL on day 2 and 39 ± 18 fmol/mL on day 7) and became significantly higher on day 14 (49 ± 28 fmol/mL, $P < 0.01$), compared to the level on admission. In patients with AMI, the ratio of day 14 to admission serum ghrelin levels, an index of AMI-related acute changes in ghrelin, correlated positively with peak creatine phosphokinase levels ($R = 0.72$, $P < 0.01$) and the double products ($R = 0.60$, $P < 0.01$) and inversely with left ventricular ejection fraction ($R = -0.53$, $P < 0.05$). In conclusion, serum ghrelin levels are significantly decreased in association with myocardial infarct size and cardiac function.

Keywords: acute myocardial infarction; coronary artery disease; desacyl ghrelin; ghrelin; hormone
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Introduction

In patients with acute myocardial infarction (AMI), one of the presenting symptoms is loss of appetite (Patel et al. 2004). Increased sympathetic activity in AMI has also been documented and may contribute to the progression of myocardial injury and the development of lethal ventricular tachyarrhythmia (Schömig 1990).

Ghrelin is a novel growth hormone (GH)-releasing peptide, originally isolated from the stomach, which has been identified as an endogenous ligand for the GH secretagogue receptor (GHS-R) (Kojima et al. 1999). GHS-R mRNA is found not only in the hypothalamus and pituitary gland but also in the heart and blood vessels (Gnanapavan et al. 2002). Significant evidence for a cardiovascular func-

tion of ghrelin and its role in the regulation of autonomic activity has been reported (Soeki et al. 2008; Kishimoto et al. 2012; Mao et al. 2012). Although recent experimental studies have demonstrated that administration of ghrelin could protect against ischemia and reperfusion injury, attenuate post-infarction ventricular dysfunction and remodeling, and improve the prognosis of myocardial infarction and heart failure (Nagaya et al. 2001; Chang et al. 2004; Schwenke et al. 2008; Soeki et al. 2008; Mao et al. 2012), the role of ghrelin in the clinical setting remains unknown.

The present study was therefore designed to investigate the changes in serum ghrelin levels and its association with cardiac function and myocardial infarct size in AMI patients.

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Methods

Study Subjects

We investigated 89 patients with chest pain who suspected of coronary artery disease and admitted to the Coronary Care Unit in the National Cerebral and Cardiovascular Center (Suita, Osaka, Japan) between March and May 2003 in a prospective manner. Approval to conduct the study was obtained from the ethics committee of the National Cerebral and Cardiovascular Center. After 42 patients with left main trunk stenosis, cardiogenic shock, and severe kidney dysfunction (serum creatinine ≥ 1.5 mg/dL) and without informed consent were excluded, a total of 47 patients were included in the present study and divided into the following four groups; AMI ($n = 16$), unstable angina pectoris (UAP, $n = 12$), stable angina pectoris (SAP, $n = 13$) and control without significant ($\geq 50\%$ stenosis) coronary artery stenosis ($n = 6$). All patients with AMI, UAP or SAP were successfully treated with percutaneous coronary intervention (PCI). The diagnosis of AMI was based on the following criteria; chest pain for more than 30 min; at least 0.1 mV of ST segment elevation in two adjacent electrocardiogram leads; admission to hospital within 24 hours of the onset of symptoms. SAP was defined as the presence of coronary artery disease with stable chest symptoms. UAP was defined as the presence of new-onset angina, angina at rest or angina of increasing frequency without the elevation of cardiac enzyme. All patients had no evidence of active infection, gastric ulcer, or other primary cachectic state such as cancer or thyroid disease, and severe liver disease. From all patients written informed consent was obtained with measurements of body mass index (calculated as body weight in kilograms divided by the square of the height in meters), blood pressure and heart rate.

Blood Sampling and Assay for Serum Ghrelin

In the control and SAP groups, blood samples were taken from the antecubital vein on the morning after an overnight fast. In the UAP and AMI groups, blood samples were taken on admission and before treatment. In the AMI group, blood samples were also taken on day 2, day 7, and day 14. Serum samples were prepared as previously described (Akamizu et al. 2005). Serum levels of unacylated ghrelin (desacyl ghrelin) were measured with the commercially available desacyl-ghrelin ELISA kit according to the manufacturer's protocol (Mitsubishi Kagaku, Tokyo, Japan). The lower limit of detection for desacyl ghrelin in this assay system is 12.5 fmol/mL. The intra- and inter-assay coefficients of variation are 3.7% and 8.1% for desacyl ghrelin, respectively (Akamizu et al. 2006).

Cardiac Catheterization, Laboratory and Echocardiographic Measurements

As reported previously (Kataoka et al. 2010), selective coronary angiography was performed in multiple projections after intracoronary administration of nitroglycerin (0.125-0.25 mg). In patients treated with PCI, all procedural decisions, including device selection and intravascular ultrasound, were made at the discretion of the individual PCI operator. Intravenous heparin (5,000 IU) and intracoronary nitroglycerin (0.5 mg) were administered before PCI. Procedural success was defined as residual stenosis $< 50\%$ without major complications (i.e., AMI, need for bypass surgery or repeat PCI, or death). Following PCI, left ventriculography was performed to measure end-diastolic and end-systolic volumes and ejection fraction (LVEF) using the area-length method (QLV-CMS version 5.0, MEDIS Medical

Imaging Systems, Leiden, the Netherlands) (Sheehan et al. 1986). In addition, creatine phosphokinase (CPK) and CPK-MB levels were measured on admission and at 3-hour intervals until CPK and CPK-MB levels peaked. Glycemic and lipid profile, and c-reactive protein (CRP) levels were also measured. Echocardiographic analysis was performed to determine left ventricular dimension and fractional shortening at admission and follow-up period.

Statistical Analysis

Continuous variables are expressed as means \pm s.d. Comparisons of clinical parameters among the 4 groups were performed by one-way analysis of variance. Correlation coefficients between serum ghrelin levels and clinical variables were calculated by linear regression analysis. $P < 0.05$ was considered to indicate statistical significance.

Results

Baseline Characteristics

There were no significant differences in age, sex, body mass index, and the prevalence of coronary risk factors among the 4 groups (Table 1). Also, heart rate, blood pressure and laboratory data except CRP were comparable among the 4 groups (Table 2). LVEF was lower and CRP levels were higher in the AMI group than other 3 groups (Tables 1 and 2), whereas the number of diseased vessels and the percentage of PCI with stent use were comparable among the SAP, UAP, and AMI groups. In the AMI group, elapsed time from the onset of AMI was 7 ± 5 hours and peak CPK levels were $3,088 \pm 3,489$ U/L. The infarct site was anterior in 9 patients, inferior in 4 patients, and posterior in 5 patients. Medical treatment following AMI includes anti-platelet agents ($n = 16$), angiotensin-converting enzyme inhibitors ($n = 11$), β -blockers ($n = 2$), statins ($n = 5$) and anti-diabetic agents ($n = 4$).

Serial Changes in Serum Ghrelin Levels and Left Ventricular Function

The serum ghrelin level on admission was significantly lower in the AMI group (27 ± 12 fmol/ml) (Fig. 1), whereas it was comparable among the non-AMI groups, i.e., the control (72 ± 26 fmol/ml), SAP (69 ± 47 fmol/ml), and UAP (72 ± 31 fmol/ml) groups. The serum ghrelin levels were rather not associated with risk factors including high density lipoprotein cholesterol, low density lipoprotein cholesterol, total cholesterol, triglyceride and glucose levels in the overall patients.

Fig. 2 plots the time of the first and second blood sampling and serum ghrelin levels in patients with AMI. Fig. 3 shows the serial changes in serum ghrelin levels in the AMI group. The serum ghrelin level gradually increased (30 ± 15 fmol/mL on day 2 and 39 ± 18 fmol/mL on day 7) and became significantly higher on day 14 (49 ± 28 fmol/mL, $P < 0.01$), compared to the level on admission. The serum ghrelin level gradually increased and became significantly higher on day 14, compared to the level on admission. In addition, the ratio of serum ghrelin levels on day 7 and day

Table 1. Baseline Clinical Characteristics.

	Control	SAP	UAP	AMI
Male / Female	5 / 1	12 / 1	9 / 3	15 / 1
Age, years	66 ± 5	66 ± 6	64 ± 5	67 ± 10
BMI, kg/m ²	23 ± 2	22 ± 2	23 ± 2	23 ± 2
HT, n (%)	3 (50)	8 (61)	11 (92)	9 (56)
DM, n (%)	3 (50)	9 (69)	7 (58)	9 (56)
HLP, n (%)	3 (50)	9 (69)	7 (58)	9 (56)
Smoker, n (%)	1 (17)	3 (23)	3 (25)	8 (50)
OMI, n (%)	0 (0)	1 (8)	1 (8)	2 (13)
LVEF, %	56 ± 7*	54 ± 4*	54 ± 7*	42 ± 7
Stent use, n (%)	0*	11 (85)	9 (75)	13 (81)

AMI, acute myocardial infarction; BMI, body mass index; DM, diabetes mellitus; HLP, hyperlipidemia; HT, hypertension; LVEF, left ventricular ejection fraction; OMI, old myocardial infarction; SAP, stable angina pectoris; UAP, unstable angina pectoris.

The control group consists of patients without coronary artery disease.

* $P < 0.05$ vs. the AMI group.

Table 2. Vital Signs and Laboratory Data at Admission.

	Control	SAP	UAP	AMI
Heart rate, /min	64 ± 11	68 ± 11	69 ± 16	68 ± 15
Systolic BP, mmHg	151 ± 12	147 ± 20	158 ± 22	141 ± 34
Diastolic BP, mmHg	82 ± 8	76 ± 10	88 ± 11	82 ± 15
Glucose level, mg/dl	205 ± 75	120 ± 31 [†]	132 ± 44 [†]	154 ± 58
IRI, μ U/ml	–	6.4 ± 4.3	4.7 ± 2.8	5.3 ± 1.5
CRP level, mg/dl	0.4 ± 0.4	0.3 ± 0.2*	0.5 ± 1.0	3.0 ± 4.1
BUN, mg/dl	16 ± 2	16 ± 3	15 ± 3	14 ± 4
Cre, mg/dl	0.9 ± 0.1	0.8 ± 0.1	0.75 ± 0.16	0.8 ± 0.2
T-chol, mg/dl	206 ± 19	197 ± 34	185 ± 29	203 ± 15
HDL, mg/dl	38 ± 6	45 ± 9	44 ± 10	41 ± 13
LDL, mg/dl	120 ± 16	129 ± 15	186 ± 125	121 ± 23
TG, mg/dl	242 ± 52	149 ± 73 [†]	130 ± 51 [†]	114 ± 73 [†]

BP, blood pressure; BUN, blood urea nitrogen; Cre, creatinine; CRP, c-reactive protein; HDL, high density lipoprotein cholesterol; IRI, immunoreactive insulin; LDL, low density lipoprotein cholesterol; T-chol; total cholesterol; TG, triglyceride.

* $P < 0.05$ vs. the AMI group, [†] $P < 0.05$ vs. Control group.

14 to that on admission was significantly increased in comparison with that on admission (Fig. 4).

In patients with AMI, serum ghrelin levels on day 14 divided by that on admission correlated positively with peak creatinine phosphokinase levels ($R = 0.72$, $P < 0.01$) and inversely with LVEF ($R = -0.53$, $P < 0.05$) (Fig. 5A and B). This index of AMI-related acute changes in ghrelin levels also correlated positively with the double products (systolic blood pressure multiplied by heart rate on admission; mmHg/min) ($R = 0.60$, $P < 0.05$), but not with body mass index (Fig. 6A and B) and CRP levels (at baseline and maximum levels, and those differences).

Table 3 shows left ventricular function evaluated by echocardiography at admission and the follow-up period in SAP, UAP and AMI groups. Fractional shortening was sig-

nificantly ($P < 0.05$) lower at admission and remained tended to be reduced ($P = 0.08$) at the follow-up in AMI group than SAP and UAP groups. The index of AMI-related acute changes in ghrelin did not correlated with changes in left ventricular dimension between the baseline and the follow-up.

Discussion

The major findings of the present study using a sensitive ELISA kit are: (1) serum ghrelin levels are significantly lower on admission in AMI patients than control patients with no coronary artery disease and patients with SAP or UAP; (2) ghrelin levels gradually recovered during the 2 weeks following AMI; and (3) the ratio of day 14 to admission serum ghrelin levels, an index of AMI-related acute

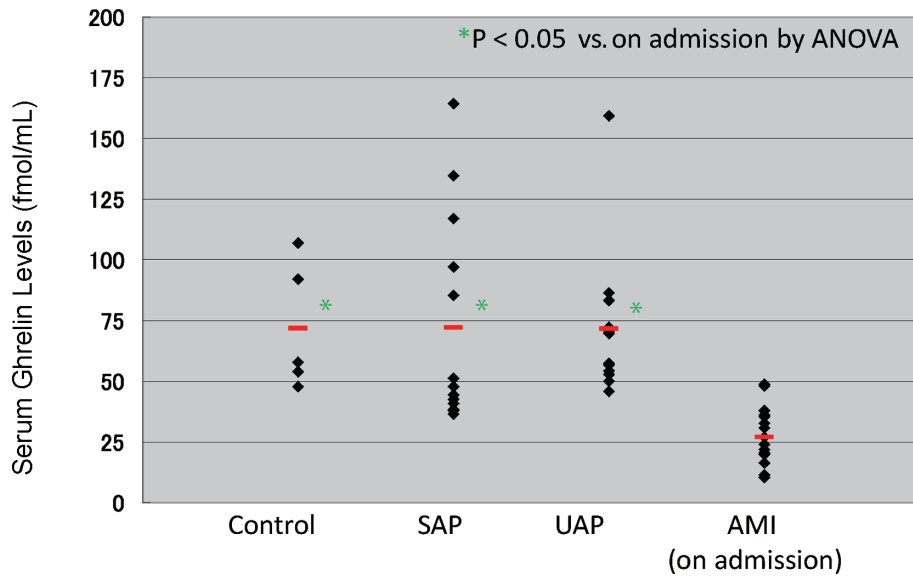


Fig. 1. Decreased serum ghrelin levels in patients with acute myocardial infarction. Control, no coronary artery diseases ($n = 6$); SAP, stable angina pectoris ($n = 13$); UAP, unstable angina pectoris ($n = 12$); AMI, acute myocardial infarction ($n = 16$). Plots show data for individual patients and the red bars shows the mean. $*P < 0.05$ vs. AMI (data on admission).

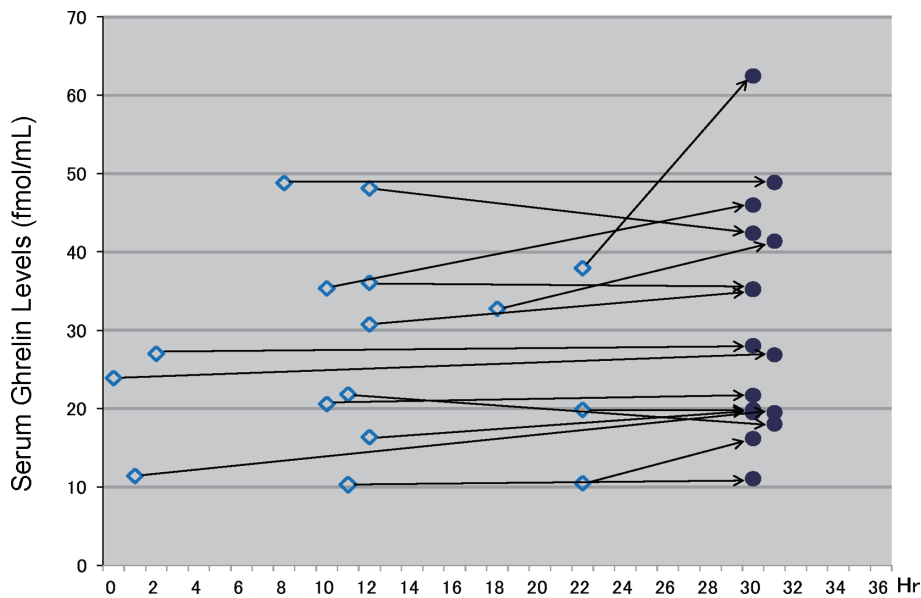


Fig. 2. Serum ghrelin levels at the first (on admission; open diamonds) and second blood sampling points (on day 2; closed circles) in patients with AMI. The abscissa indicates military time. For example, 0 and 12 hours indicates the midnight and the noon, respectively. Arrows indicate the time lag between admission and the second blood sampling of day 2. The second blood sampling was performed in fasting condition. Hr, hour.

changes in ghrelin levels, correlated positively with peak CPK levels and the double product, and inversely with LVEF.

Decreased Serum Ghrelin Levels in Patients with AMI

Ghrelin, a 28-amino acid peptide, was isolated from the human and rat stomach and identified as an endogenous

ligand for the GHS-R (Kojima et al. 1999). The ghrelin system is ubiquitously expressed and participates in the regulation of appetite, energy, bodyweight, metabolism of glucose and fat, as well as modulation of gastrointestinal function and cell proliferation or apoptosis (Zhang et al. 2010; Kishimoto et al. 2012). In addition, mounting experimental evidence suggests that ghrelin has a close relation-

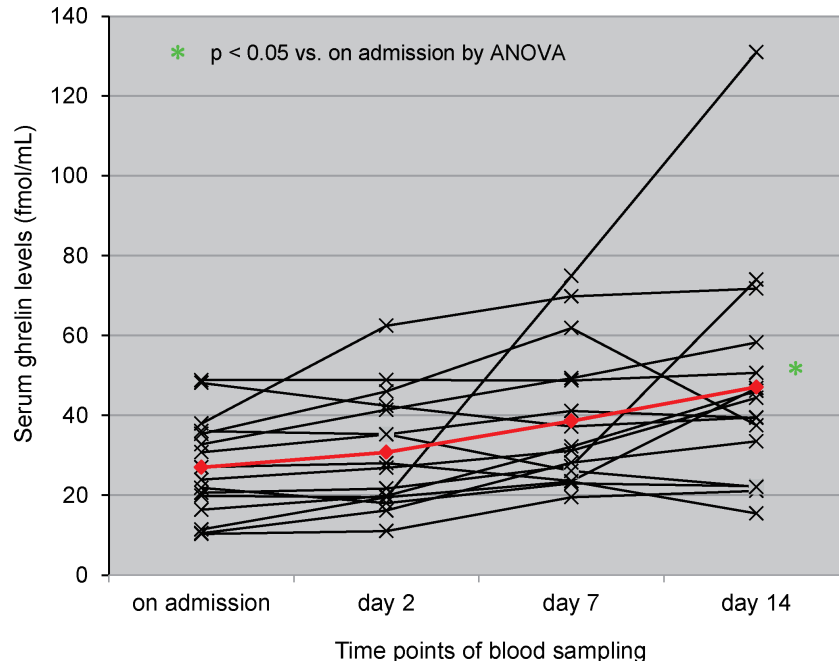


Fig. 3. Gradual increase in serum ghrelin levels in AMI patients. Plots show serial serum ghrelin levels for individual patients on admission, day-2, -7, and -14. Red diamonds show the mean. **P* < 0.05 vs. data on admission.

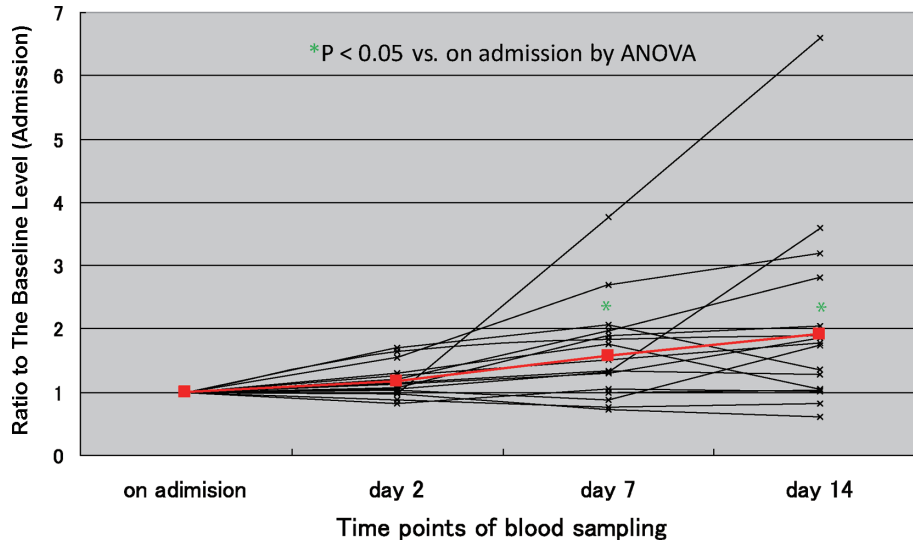


Fig. 4. Serum ghrelin levels increased linearly over time in AMI patients. Plots show the ratio of day 7 to admission serum ghrelin levels and the ratio of day 14 to admission serum ghrelin levels for individual patients. Red squares show the mean. **P* < 0.05 vs. data on admission.

ship with the cardiovascular system (Zhang et al. 2010; Kishimoto et al. 2012).

In the previous study with only one-point, desacyl ghrelin non-specific measurement, serum ghrelin levels decreased in AMI patients in comparison with the healthy control, although there were no data of time interval between the onset of AMI and blood sampling (Kadoglou et

al. 2010). Therefore, this is the first clinical study investigating serial changes in serum ghrelin levels in patients following AMI. Interestingly, serum ghrelin levels were significantly decreased at the onset of myocardial infarction and recovered gradually over weeks to levels comparable with non-AMI patients with stable or unstable angina or no coronary artery disease.

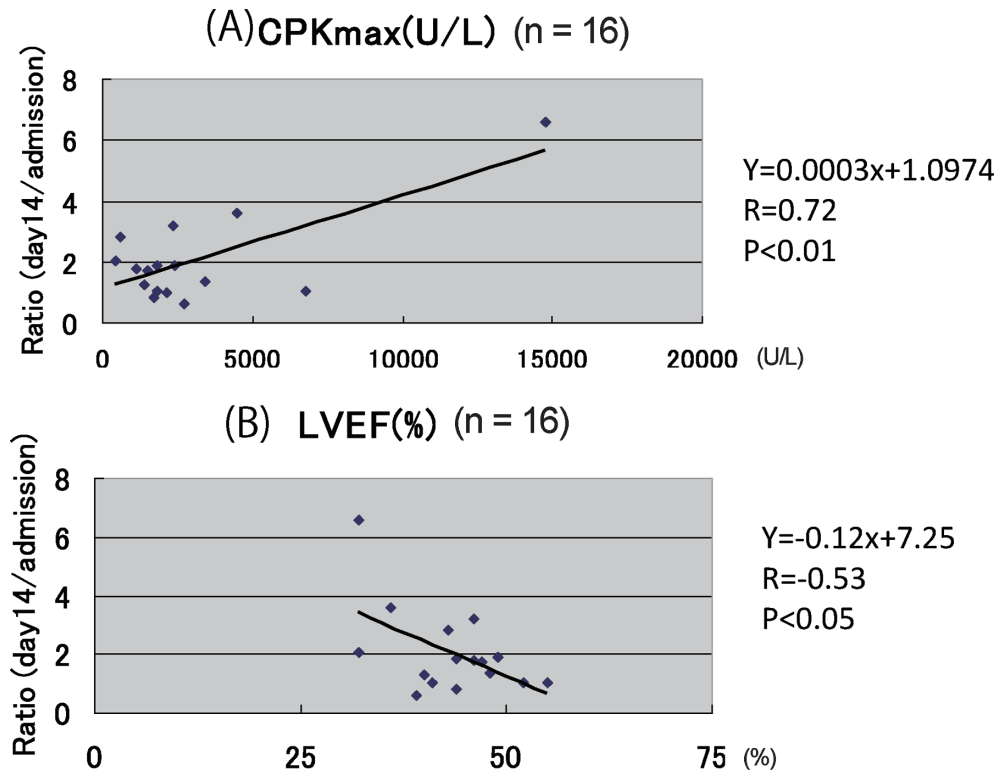


Fig. 5. Correlation between ghrelin and creatine phosphokinase (CPK), ghrelin and left ventricular ejection fraction (LVEF). (A) Positive correlation between AMI-related suppressive changes in ghrelin and CPK levels and (B) inverse correlation with LVEF. AMI-related suppressive changes indicate the ratio of day 14 to admission serum ghrelin levels.

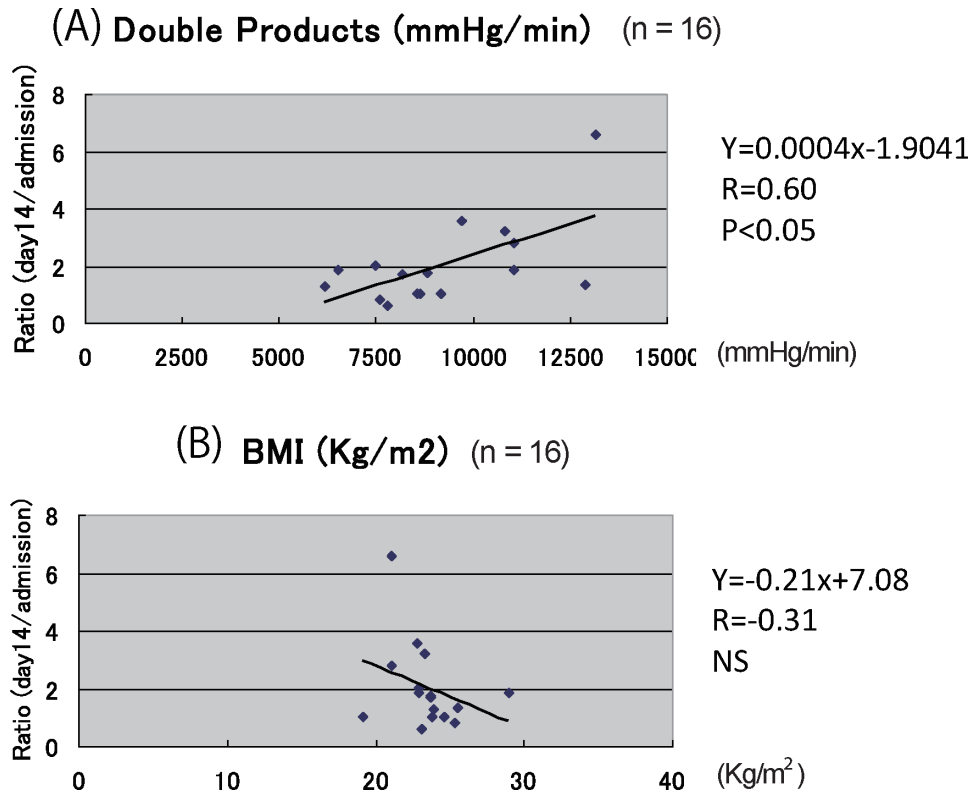


Fig. 6. Correlation between ghrelin and double products, ghrelin and body mass index (BMI). (A) Positive correlation between AMI-related suppressive changes in ghrelin and the double products, but (B) not with BMI.

Table 3. Echocardiographic Data at Admission and during the Follow-up Period.

	SAP	UAP	AMI
Echo-data at admission			
Dd, mm	48 ± 3	47 ± 6	48 ± 6
Ds, mm	32 ± 4	30 ± 9	36 ± 8
FS, %	33 ± 6*	37 ± 10*	25 ± 8
Echo-data during the follow-up period			
Days after the admission (median)	1,740	1,521	1,033
Dd, mm	47 ± 4	46 ± 1	47 ± 8
Ds, mm	30 ± 6	30 ± 4	33 ± 7
FS, %	36 ± 6	34 ± 7	30 ± 8

Echo, echocardiography; Dd, end-diastolic dimension; Ds, end-systolic dimension; FS, fractional shortening.

* $P < 0.05$ vs. the AMI group.

Association of Changes in Ghrelin Levels with Myocardial Infarct Size and Cardiac Function

The present study also demonstrated that the ratio of day 14 to admission serum ghrelin levels, an index of AMI-related acute changes in ghrelin, correlated positively with peak CPK levels and the double product, and inversely with LVEF. Although these findings do not address a cause-and-effect relationship, several possibilities could be discussed. Friberg and colleagues (2000) reported that GH secretion was stimulated in patients with AMI, especially in patients with severe cardiac damage (Friberg et al. 2000). Since ghrelin is a GH-releasing peptide, increased GH levels may decrease ghrelin secretion due to the negative feedback loop of GH.

Decreased ghrelin levels may be related in part to extensive myocardial damage in AMI patients (Fig. 5A and B). It is reported that administration of ghrelin protects the heart against ischemia and reperfusion injury via ERK1/2 and PI3K/Akt-dependent pathways (Chang et al. 2004). Also, in an animal model of AMI, ghrelin effectively inhibits cardiac sympathetic activity, prevents ventricular tachyarrhythmias, and reduces mortality (Schwenke et al. 2008; Soeki et al. 2008). In the present study, ghrelin levels were well preserved in patients with lower double products (Fig. 6A).

Considering these cardioprotective effects of ghrelin, its exogenous administration may represent a new approach for the treatment of AMI with severely depressed left ventricular function. Indeed, in patients with heart failure, Nagaya et al. (2004) demonstrated that exogenous ghrelin administration induces beneficial hemodynamic effects via reducing cardiac afterload and increasing cardiac output without an increase in heart rate.

Limitations

The interpretation of our results should take into consideration several limitations. First, the number of study patients is small. Second, the mechanism by which AMI suppresses ghrelin secretion remains unknown because this

was not a mechanistic study. Therefore, larger study is needed for the confirmation of our results in the future. Third, in all of the UAP and AMI groups, the first blood samples were not taken after the overnight fast. Possibility remains whether difference from the control and SAP groups with fasted blood samples may affect the result. However, Fig. 2 shows that serum ghrelin levels were low and comparable between the first (obtained at admission) and the second sampling (obtained in fasting condition) except one patient.

Conclusion

The present study demonstrates that serum ghrelin levels are significantly decreased in association with myocardial infarct size and cardiac function in patients with AMI. Taking the various cardioprotective actions of ghrelin into account, the present findings support further study of ghrelin administration as a therapeutic modality for AMI patients.

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Conflict of Interest

The authors declare no conflict of interest.

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