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Urbanization, Life Style Changes and the Incidence/In-Hospital Mortality of Acute Myocardial Infarction in Japan

– Report From the MIYAGI-AMI Registry Study –

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Background: It remains to be examined whether urbanization and lifestyle changes are associated with the incidence and mortality from acute myocardial infarction (AMI) in Japan.

Methods and Results: A total of 19,921 AMI patients (male/female 14,290/5,631) registered by the MIYAGI-AMI Registry Study from 1988 to 2009 were divided into 2 groups according to their residences; inside (urban area, n=7,316) and outside (rural area, n=11,402) of Sendai City. From 1988 to 2009, the incidence of AMI (/100,000 persons/year) increased more rapidly in the rural area (24.2 to 51.4) than in the urban area (31.3 to 40.8) ($P<0.001$), with rapid aging in both areas. Moreover, from 1998 to 2009, the age-adjusted incidence of AMI in young (<44 years) and middle-aged (45–64 years) male patients (both $P<0.05$) in the rural area increased significantly, along with a markedly increased prevalence of dyslipidemia ($P<0.001$). Although in-hospital mortality from AMI decreased in both areas over the last 20 years (both $P<0.001$), it remained relatively higher in female than in male patients and was associated with higher age of the onset, longer elapsing time for admission and lower prevalence of primary coronary intervention in female patients in both areas.

Conclusions: These results demonstrate that urbanization and lifestyle changes have been associated with the incidence and mortality from AMI, although sex differences still remain to be improved. (*Circ J* 2012; **76:** 1136–1144)

Key Words: Acute myocardial infarction; Aging; Life-style; Risk factors; Sex

The incidence and mortality from coronary artery disease (CAD) has been declining in the United States and European countries.[1–4] These declines have been attributed to the control of risk factors (eg, hypertension, dyslipidemia and smoking) and the improvement in critical care (eg, coronary revascularization therapy).[5–7] In contrast to the Western countries, in Japan, a highly developed and racially homogeneous country that is rapidly aging, total cholesterol levels and the prevalence of obesity have been increasing as a result of lifestyle Westernization influence since the 1960s.[8,9] However, the mortality from CAD has been declining and has remained much lower compared with other Western countries from 1960 to 2000.[9–11] Importantly, there are some differences in lifestyle between people living in rural and urban areas in Japan. Indeed, it was reported that people in urban areas had greater intakes of fat and cholesterol than those in rural areas in Japan.[8] However, only a few studies have previously addressed the difference in the incidence and mortality from CAD between the rural and urban areas in Japan.[8,12]

In order to explore the annual trend for acute myocardial infarction (AMI) in Japan, we have been conducting the MIYAGI-AMI Registry Study for more than 30 years since 1979, where almost all AMI patients in the Miyagi prefecture have been prospectively registered.[10,13,14] The Miyagi prefecture, which is located in northeastern Japan, includes Sendai City, one of the 19 government-designed cites, and has a typical balance of urban and rural areas in Japan. Sendai City merged with neighboring municipalities in 1987–1988 and the population of Sendai City increased to 1,008,130 in 2000, which accounted for approximately 40% of the population of

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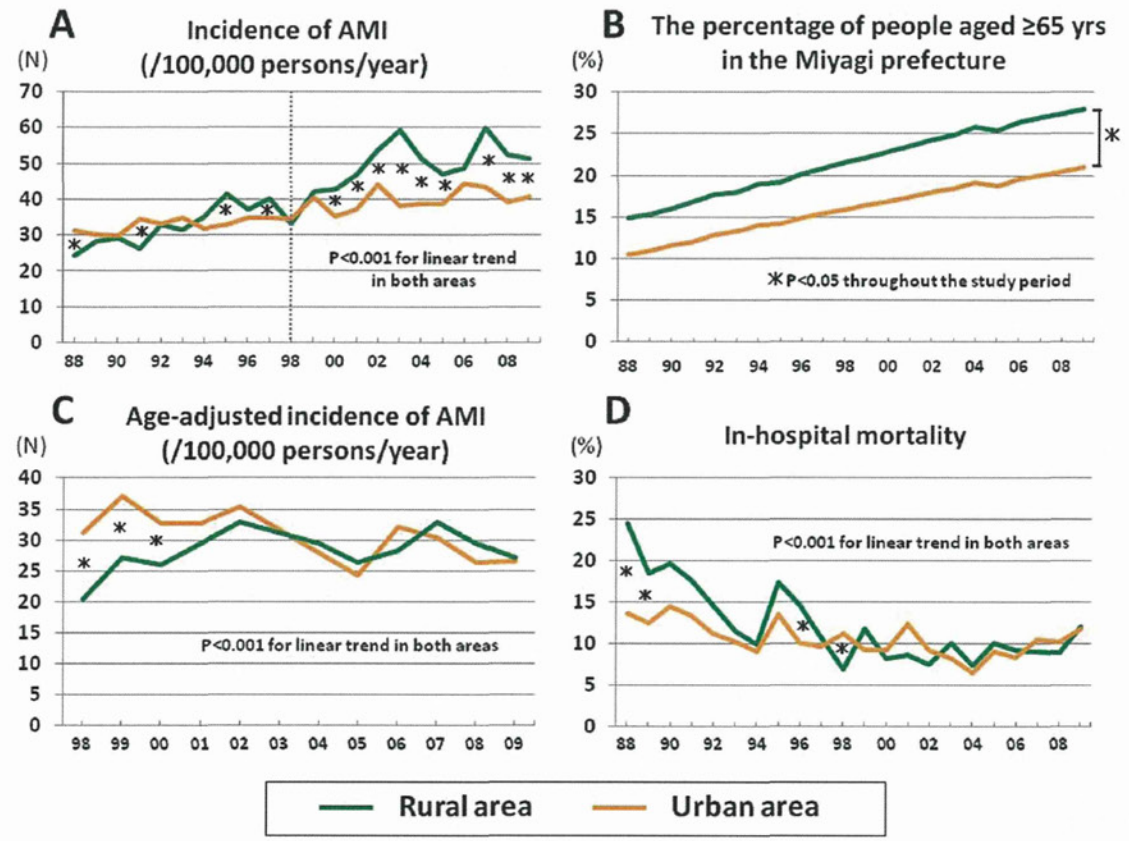

Figure 1. The 20-year trend of acute myocardial infarction (AMI) in the Miyagi prefecture. (**A**) The incidence of AMI (/100,000 persons/year) has significantly increased in both areas (both P<0.001) with a greater extent in the urban area over the last 20 years, accompanied with rapid aging in both areas (**B**). (**C**) The age-adjusted incidence of AMI (/100,000 persons/year) in the rural area increased significantly (P<0.001), whereas that in the urban area decreased significantly (P<0.001) in the recent 10 years (between 1998 and 2009). (**D**) In-hospital mortality (%) decreased and remained at a low level in the past 10 years in both areas. *P<0.05 for the difference between the rural and the urban areas.

the Miyagi prefecture, which was 2,365,320 in 2000. The population density of Sendai City (1,279/km^2 in 2000) has been much higher than that of any other parts of the Miyagi prefecture (209/km^2 in 2000).[15]

In the present study, we examined whether urbanization and lifestyle changes were associated with the incidence and mortality from AMI, with special reference to the difference between the urban and rural areas in our MIYAGI-AMI Registry Study.

Methods

The MIYAGI-AMI Registry Study

The MIYAGI-AMI Registry Study is a prospective, multicenter and observational study. As previously reported,[10,13,14] this registry was established in 1979 and all 43 hospitals with a coronary care unit and/or cardiac catheterization facility in the Miyagi prefecture have been participating (**Appendix 1**). In the Miyagi prefecture, almost all AMI patients are transferred to one of those participating hospitals via the emergency medical service. This study was approved by the Institutional Review Broad of Tohoku University Graduate School of Medicine under the condition that personal data are protected at all times.

In the MIYAGI-AMI Registry Study, the diagnosis of AMI and decision to use reperfusion therapy were made by individual cardiologists in charge. Diagnosis of AMI was made based on the WHO-MONICA criteria.[16] Briefly, it was based on the finding of typical severe chest pain accompanied by abnormal ECG changes and increased serum levels of cardiac enzymes (ie, creatine phosphokinase, aspartate amino transferase and lactate dehydrogenase). Coronary thrombolysis was performed with intravenous administration of urokinase (480–960×10^3 IU for 30 min) or alteplase (290–435×10^3 IU/kg for 60 min) or with intracoronary administration of urokinase (maximum 960×10^3 IU) or alteplase (maximum 6.4×10^6 IU). Rescue percutaneous coronary intervention (PCI) was performed when thrombolysis was unsuccessful. Primary PCI has been widely performed in the Miyagi prefecture since 1992, as reported previously.[10,13,14]

The registration form of the MIYAGI-AMI Registry includes the date and time of symptom onset, age, sex, pre-hospital management (eg, use of ambulance, time interval from the onset of symptoms to admission), infarction site, coronary risk factors (hypertension, diabetes mellitus, dyslipidemia and smoking), reperfusion therapies (eg, thrombolysis and/or PCI), and in-hospital outcome (eg, in-hospital mortality). In our MIYAGI-AMI Registry Study, we have revised the registra-

Table. Clinical Characteristics and Outcome of the Study Population

	Rural area			P value for trend	Urban area			P value for trend
	1998–2001 (n=2,145)	2002–2005 (n=2,699)	2006–2009 (n=2,807)		1998–2001 (n=1,529)	2002–2005 (n=1,508)	2006–2009 (n=1,682)	
Male								
Age (years)	66.2±12.4*	67.0±12.9*	66.7±12.7	0.373	65.0±12.7	65.2±12.9	65.9±12.9	0.046
Age-adjusted incidence of AMI (/10^5 persons/year)								
All	42.3±3.8*	47.2±3.2	47.3±2.5	0.274	55.1±4.7	49.3±10.9	47.9±4.1	0.163
<45 years old	4.9±0.9	5.8±0.7	6.9±1.2	0.018	5.1±0.7	5.7±0.5	6.0±2.7	0.460
45–64 years old	66.6±6.3*	83.2±5.5	88.9±14.9	0.016	91.2±4.9	85.9±21.0	83.7±8.2	0.402
65–74 years old	170.2±32.9	186.3±39.2	179.3±17.8	0.679	228.2±18.1	208.1±56.3	180.1±15.6	0.065
≥75 years old	253.5±47.0*	261.1±62.9	250.8±33.4	0.937	355.0±48.0	277.8±73.4	308.0±19.7	0.207
Hypertension (%)	46.1	59.5*	60.9	<0.001	48.2	54.3	63.0	<0.001
Diabetes mellitus (%)	27.5	32.9	29.5*	0.265	30.6	31.6	34.1	0.070
Dyslipidemia (%)	22.4*	34.1*	41.4	<0.001	32.2	39.0	42.0	<0.001
Smoking (%)	40.6	42.1	40.6	0.956	44.0	41.8	38.6	0.008
In-hospital mortality (%)	7.6	6.8	7.8	0.832	8.8	5.7	8.7	0.997
Female								
Age (years)	74.1±9.7	76.1±11.1	75.3±11.4	0.017	74.4±10.4	74.6±12.0	75.3±11.4	0.224
Age-adjusted incidence of AMI (/10^5 persons/year)								
All	11.5±2.4*	13.6±1.1	13.2±1.0	0.202	15.1±1.2	11.9±2.0	12.4±2.4	0.077
<45 years old	0.2±0.4	0.4±0.2	0.7±0.5	0.114	0.2±0.2	0.5±0.3	0.5±0.7	0.297
45–64 years old	10.5±4.2	13.7±3.1	18.1±4.1	0.102	10.1±1.6	11.0±2.2	16.1±7.1	0.102
65–74 years old	54.5±1.8*	65.0±8.4	56.4±4.4	0.602	84.5±5.8	55.3±6.5	48.9±9.1	<0.001
≥75 years old	100.8±17.4*	135.7±14.9	120.8±7.9	0.076	165.9±13.9	131.4±19.4	129.8±17.2	0.016
Hypertension (%)	55.8	69.3	67.5	<0.001	60.2	63.5	65.0	0.137
Diabetes mellitus (%)	29.3	36.1	35.1	0.032	32.5	33.2	34.5	0.510
Dyslipidemia (%)	25.8	30.9	38.6	<0.001	31.0	37.1	37.7	0.039
Smoking (%)	8.9	6.6*	10.6	0.163	12.1	13.4	14.1	0.383
In-hospital mortality (%)	12.3	11.1	14.5	0.254	14.4	15.3	14.1	0.892

Values are mean±SD or n (%). *P<0.05 for the difference between rural and urban areas. AMI, acute myocardial infarction. Study population was divided into 2 groups according to the residence: inside (urban area) and outside Sendai City (rural area).

tion form gradually over the last 30 years. Thus, although the incidence of AMI and related data (time of onset, age and sex) are available for the last 30 years, the date on the pre-hospital management, infarction site, coronary risk factors, reperfusion therapies, duration of hospitalization and in-hospital outcome are only available for the last 10–20 years, which were analyzed in the present study.

Data Analysis

In the present study, we have registered a total of 19,921 patients with AMI (male/female 14,290/5,631) over the last 20 years after the municipal merger in 1988. In particular, we have focused on the patients registered between 1998 and 2009 (total, 12,491; male/female, 8,969/3,522), who were divided into 2 groups according to their residences; inside (urban area, n=4,719) and outside Sendai City (rural area, n=7,651), after excluding the patients whose residences were unknown (n=159). We also divided the total observational period of 12 years into the 3 periods: 1998–2001, 2002–2005 and 2006–2009. To calculate the sex- and age-adjusted incidence of AMI (/100,000 person/years), we applied the direct standardization method using the age distribution of the Japanese population from the 2000 census,[5] as the standard population. In addition, in order to clarify the age-specific trend, we categorized the age at AMI onset into the 4 groups: ≤44 (young), 45–64 (middle-aged), 65–74 (old) and ≥75 years old (high-old).[15]

Results are expressed as mean±SD. Linear trends were examined for continuous variables by using analysis of variance (ANOVA) with repeated measures or the Jonckheere-Terpstra trend test as appropriate, and for categorical variables by using the chi-square test for trend. Differences in mean values were examined with a t-test, Mann-Whitney test or chi-square test as appropriate. Multiple logistic regression analysis was used to examine determinants of risk factor prevalence in AMI patients. Variables used for analysis included: sex, age at onset of AMI (per 10 years), study periods (1998–2001, 2002–2005 and 2006–2009), residence (rural vs. urban), and other risk factors. The odds ratios (ORs) and 95% confidence intervals (95%CI) were calculated. A P-value less than 0.05 were considered to be statistically significant. All statistical analyses were performed using the statistical software SPSS version 18 for Windows.

Results

Over the last 20 years, the incidence of AMI (/100,000 persons/year) significantly increased in both the rural and the urban areas in the Miyagi prefecture (2.1- and 1.3-fold, respectively, both P<0.001) (**Figure 1A**). Furthermore, the extent of the increase in AMI incidence was greater in the rural area than in the urban area, finally exceeding that in the urban area after 2000. These changes were accompanied with rapid aging

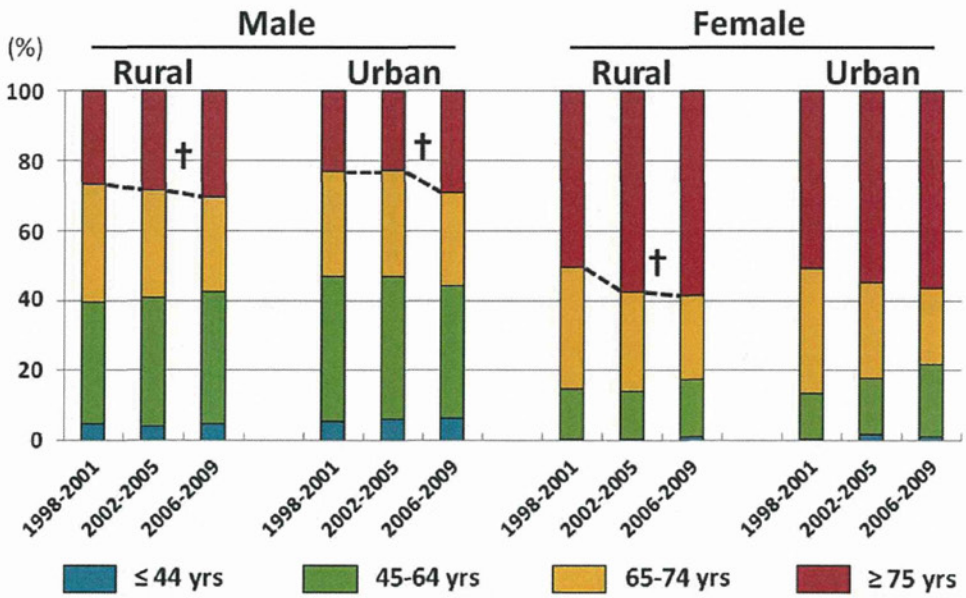

Figure 2. Age-distribution of acute myocardial infarction (AMI) patients. The percentage of high-old patients (≥75 years old) was markedly higher in female patients than in the patients in the rural and urban areas and has been increasing significantly in male patients in both areas and rural female patients. †P<0.05 for linear trend.

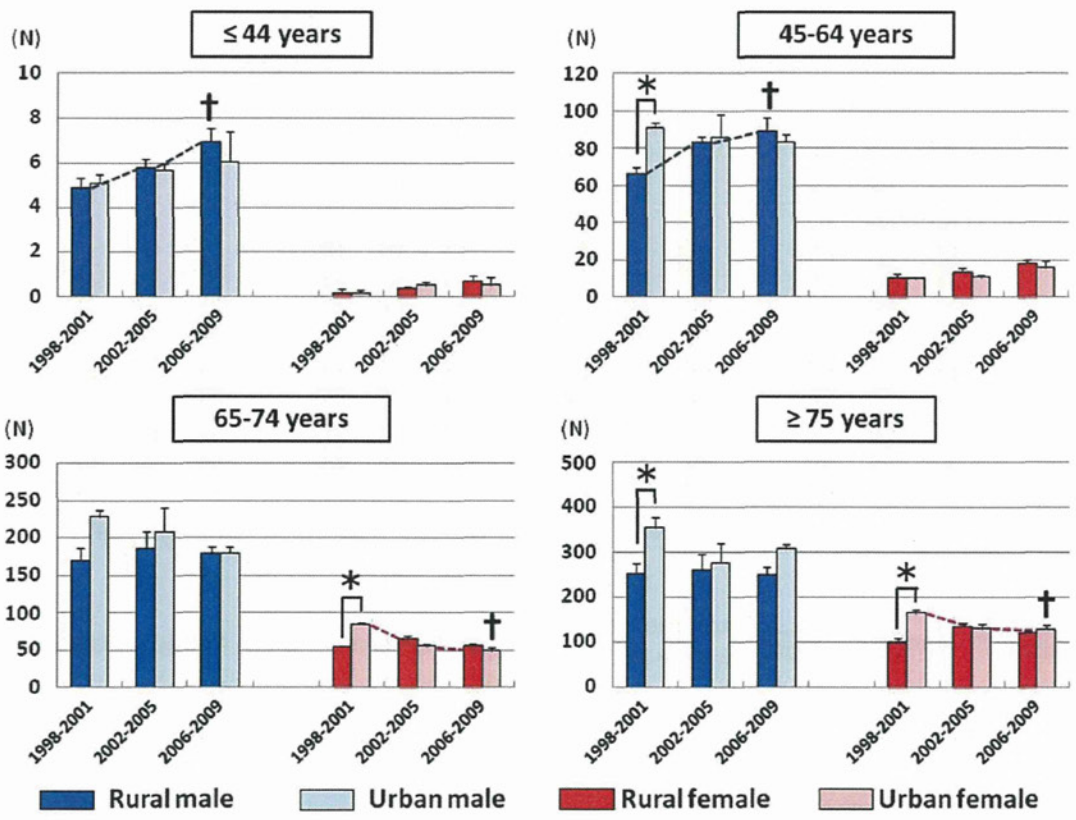

Figure 3. Age-specific incidence of acute myocardial infarction (AMI) (/100,000 persons/year). The significant increase in the age-adjusted incidence of AMI was noted in <44 and 45–64 year old rural male patients, and the significant decrease was noted in 65–74 and >75 year old urban female patients. Values are presented as mean±SE. *P<0.05 for the difference between rural and urban areas. †P<0.05 for linear trend.

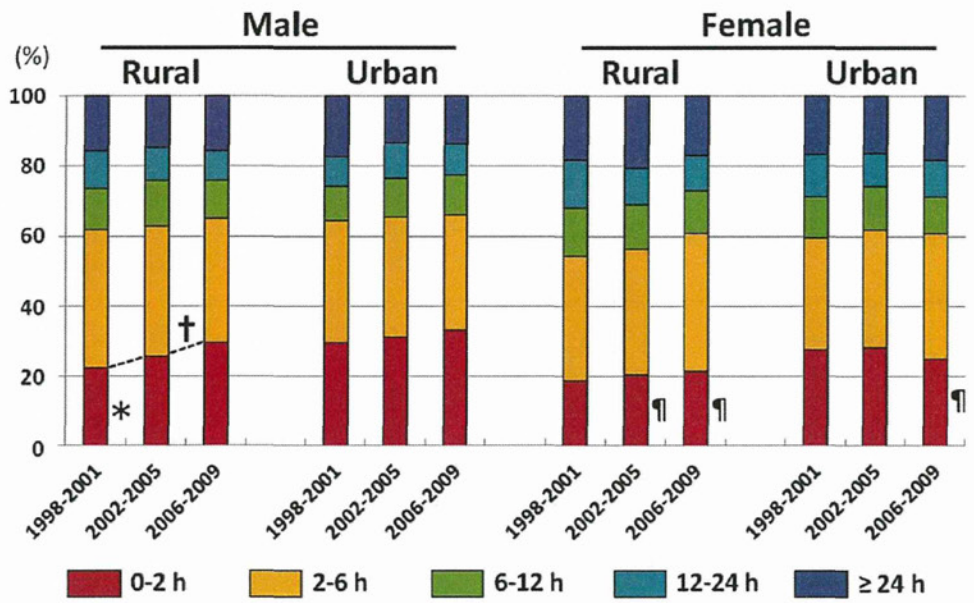

Figure 4. Time interval from the onset of symptoms to hospitalization. The percentage of patients with less than 2 h of elapsing time for hospitalization has significantly increased in rural male patients. The percentage was significantly lower in female patients than in male patients in both areas in 2006–2009. *P<0.05 for the difference between rural and urban areas. ¶P<0.05 for the difference between the sexes in the same rural or urban areas. †P<0.05 for a linear trend.

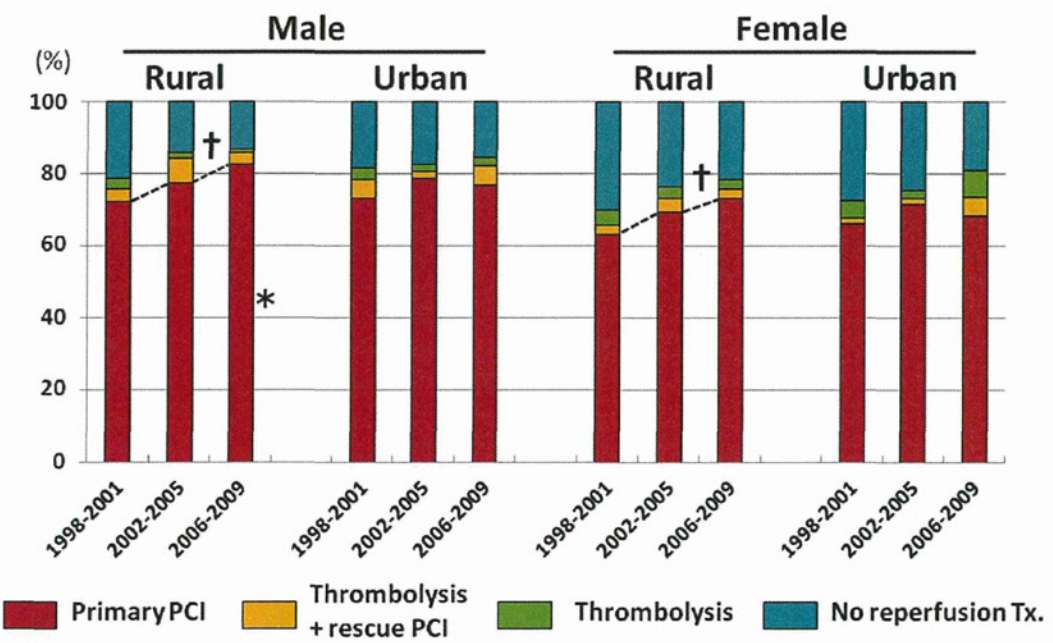

Figure 5. Prevalence of reperfusion therapy for acute myocardial infarction (AMI). The prevalence of primary percutaneous coronary intervention (PCI) steadily increased in the rural area in both sexes. Importantly, the prevalence of PCI was approximately 10% lower in female patients than in male patients in both rural and urban areas. *P<0.05 for the difference in male patients between rural and urban areas. †P<0.05 for linear trend.

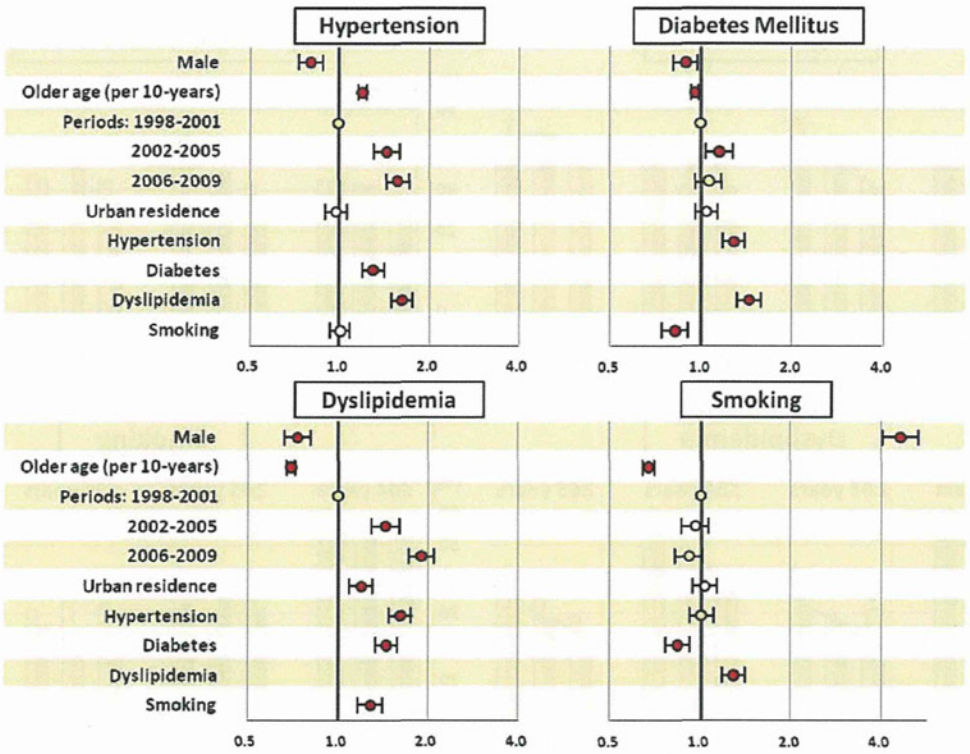

Figure 6. Multivariate analysis of coronary risk factors in acute myocardial infarction (AMI) patients. During the study periods, the prevalence of hypertension and dyslipidemia significantly increased in AMI patients. Hypertension was associated with older age but not with residence, whereas dyslipidemia was associated with younger age and urban residence. Smoking was associated with male sex and younger age. The odds ratios and 95% confidence intervals for factors are shown with red circles for P<0.05.

in both areas in the Miyagi prefecture (**Figure 1B**). Following age adjustment (**Figure 1C**), the incidence of AMI in the rural area increased significantly (P<0.001), whereas that in the urban area decreased significantly (P<0.001) in the recent 10-year period (between 1998 and 2009). In contrast, in-hospital mortality significantly decreased in both areas (both P<0.001), but to a greater extent in the rural area (0.5-fold in the rural area and 0.9-fold in the urban area) (**Figure 1D**). In 1998–2001, there was no significant difference in in-hospital mortality between the rural and urban male patients (P=0.263), and in-hospital mortality remained low (~8%) from 1998–2001 to 2006–2009 in both the rural and urban male patients (rural: P=0.832; urban: P=0.997) (**Table**). Importantly, in-hospital mortality of the female patients in both the rural and the urban areas remained doubled compared with the male patients during the study period (**Table**).

The clinical characteristics of the AMI patients in the present study are shown in **Table**. The female patients were approximately 10 years older than the male patients and approximately a half of them were ≥75 years-old in 1998–2001 in both areas, with a significant further increase in the rural area (male, P<0.001; female, P<0.001) and such a trend in the urban area (male, P=0.054; female, P=0.176) (**Figure 2**). In 1998–2001, the age-adjusted incidence of AMI was significantly lower in the rural area than in the urban area for both sexes (male, P=0.019; female, P=0.035) (**Table**). However, the difference between the 2 areas became insignificant in 2006–2009 for both sexes (male, P=0.824; female, P=0.530). When investigating the age-specific trend, the significant increase in the age-adjusted incidence of AMI was noted in the young (<44 years-old) and middle age (45–64 years-old) male patients only in the rural area (young, P=0.018; middle age, P=0.016), and the significant decrease was noted in the old (65–74 years-old) and high-old (>75 years-old) female patients in the urban area (old, P<0.001; high-old, P=0.016) (**Table**, **Figure 3**).

Regarding the time from the onset of AMI to admission, the percentage of the patients with less than 2 h of elapsing time at admission was significantly lower in the rural area than in the urban area for the male patients in 1998–2001 (P<0.001) (**Figure 4**). However, the difference became insignificant in 2006–2009 (P=0.051), accompanied with the significant increase in the percentage in the rural area (rural, P<0.001; urban, P=0.082). Importantly, in the rural female patients, the percentage of patients with less than 2 h of elapsing time at admission remained at a low level (~20%), and the difference between the sexes in the rural area became greater from 1998–2001 (P=0.086) to 2006–2009 (P<0.001). In contrast, the difference between the sexes in the urban area was significant in 2006–2009 (P=0.04). Moreover, the prevalence of primary PCI in the female patients was lower by ~10% compared with the male patients in both areas (**Figure 5**). In the male patients, the prevalence of primary PCI significantly increased only in the rural area from 1998–2001 to 2006–2009 (rural, P<0.001; urban, P=0.054), and a similar trend was also noted in the female patients (rural, P<0.001; urban, P=0.176).

Multivariate analysis of the coronary risk factors in AMI patients showed that the prevalence of hypertension and dys-

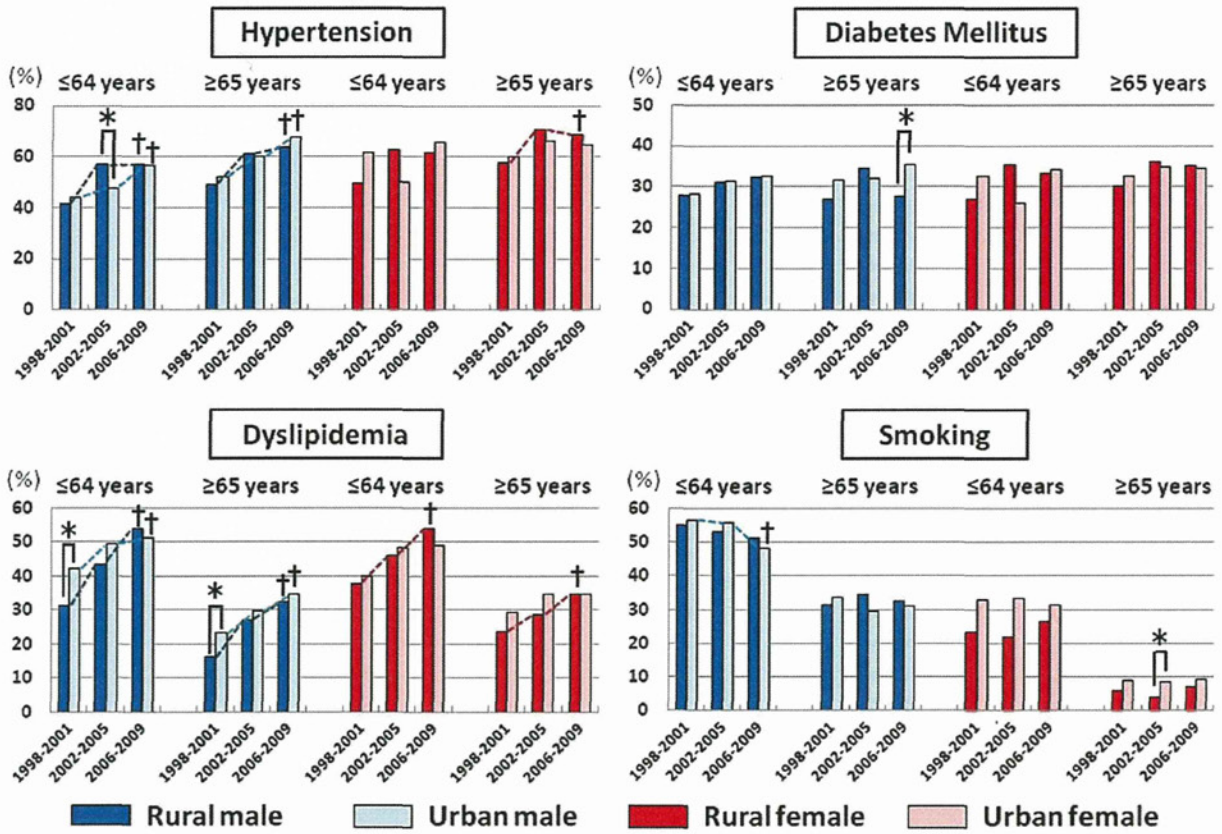

Figure 7. Prevalence of coronary risk factors in acute myocardial infarction (AMI) patients. The prevalence of hypertension and dyslipidemia had a trend of increase in both rural and urban areas. In particular, the prevalence of dyslipidemia in rural male patients aged ≤64 years markedly increased. *P<0.05 for the difference between rural and urban areas. †P<0.05 for linear trend.

lipidemia significantly increased and that of diabetes tended to increase (**Figure 6**). Hypertension was associated with older age but not with residence, whereas dyslipidemia was associated with younger age and urban residence. Although the prevalence of dyslipidemia in the male patients was significantly lower in the rural area than in the urban area in 1998–2001, it significantly increased in the rural area and the difference between the 2 areas became insignificant in 2006–2009 (**Table**). Moreover, the progressive increase in the prevalence of dyslipidemia was noted in both areas for both sexes with a more sharp increase in the rural area (**Figure 7**). Smoking was associated with male sex and younger age, but not with residence (**Figure 6**), and the prevalence of smoking largely remained unchanged in both areas for both sexes (**Figure 7**).

Discussion

The novel findings of the present study were that the incidence of AMI increased more rapidly in the rural area than in the urban area, with rapid aging in both areas. Moreover, the incidence of AMI in the rural male patients ≤64 years-old was increased along with the marked increase in the prevalence of dyslipidemia in Japan. Although in-hospital mortality from AMI markedly decreased in both areas over the last 20 years, it remained relatively high in female patients than in male patients in both areas. To the best of our knowledge, this is the first study that demonstrates the association between urbanization, life-style changes and the incidence and mortality of AMI in the largest number of patients in Japan.

Comparison of the Incidence of AMI Between Rural and Urban Areas

Although in the United States and European countries, the incidence of CAD has been declining in the last decades,[1,2,4] the present study demonstrates that the incidence of AMI has been rapidly increasing in both the rural and urban areas over the last 20 years, with a more noted increase in the former than in the latter. However, this tendency has disappeared following age adjustment in recent years only in the urban area, which implied that the increased tendency in the incidence of AMI in the rural area might be not be associated with rapid aging alone in recent years.

There were few studies that addressed the difference in the incidence of CAD between rural and urban areas in Japan. The Akita-Osaka study is the community-based survey, where the residents of the Yao City, Osaka prefecture (an urban community with a total census population of 23,552 in 2000) and those of Ikawa Town, Akita prefecture (a rural community with a total census population of 6,116 in 2000) were compared during the period of 1964–2003.[12] In this study, significant increases in the age-adjusted incidence of AMI and sudden cardiac death were noted in Yao City (in male patients from 1980 to 2003) but not in Ikawa City in both sexes.[12] The present study confirmed the results of the Akita-Osaka study

in the rural and urban areas of the same Miyagi prefecture. The Yamagata AMI Registry study provided more recent data and an age-specific trend in the period of 1993–2007.[17] The population density of the Yamagata prefecture was 133/km^2 in 2000, which was comparable with that of the rural area in the present study.[15] In this study, the age-adjusted incidence of AMI in the male but not that in the female patients significantly increased. In particular, the male population who were younger than 65 years old showed a marked increase in AMI, a consistent finding with the present results for the rural area. These results indicate that the incidence of AMI has been increasing in the younger male population in the rural areas of Japan. Taken together, unlike the trend in Western countries, it appears that the incidence of AMI has been increasing in Japan to a greater extent in the rural area than in the urban area over the last 20 years and has been associated with rapid aging.

Decreasing In-Hospital Mortality and Improvement in Critical Care

In the present study, the in-hospital mortality from AMI significantly decreased in both the urban and the rural areas over the last 20 years. The present study also demonstrates that primary PCI was performed more frequently in the rural area than in the urban area, along with the shortening in the elapsing time from the onset to hospitalization. The recent progress in critical care might have beneficial effects, overcoming the rapid aging in AMI patients.

In the most recent 10 year period, the in-hospital mortality remained at a low level in male patients, whereas in female patients, the mortality remained doubled compared with the male patients in both the rural and the urban areas. It was previously reported that the poorer outcome of the female AMI patients could be caused by multiple factors, including higher age, higher risk profiles, longer elapsing time from the onset to hospitalization, higher incidence of Killip class ≥2, and less frequent use of primary PCI.[18–20] Indeed, in the present study, the female patients were approximately 10 years older than the male patients and half of them were older than 75 years and needed a longer time from the onset of AMI to hospitalization in the both areas in 2006–2009. These points might have limited the use of primary PCI with a resultant poor outcome for the female AMI patients in the present study.

Changes in the Prevalence of Coronary Risk Factors in AMI Patients

The WHO-MONICA studies, as well as several Japanese cohort studies, demonstrated that the incidence of cardiovascular diseases increased and were associated with the clustering of risk factors.[21–23] In the present study, the prevalence of hypertension and dyslipidemia in AMI patients significantly increased in both the rural and urban areas. Importantly, there was a significant difference in the prevalence of dyslipidemia between the rural and urban areas with a marked increase noted in the rural area, especially in those male patients aged ≤64 years. Indeed, previous studies demonstrated that dyslipidemia is an independent risk factor in male but not in female patients,[17,24] and in the Yamagata-AMI Registry study, the increased prevalence of dyslipidemia in the younger male patients with AMI was also associated with an increased incidence of AMI.[17] In the Miyagi prefecture, the intake of animal fat was significantly higher in the rural than in the urban area in 2000 (rural 20.7 g/day vs. urban 23.4 g/day, $P<0.05$).[25] Moreover, in Japan, fat intake and serum levels of total cholesterol were higher in the urban than in the rural areas in 1966; however, the difference in cholesterol levels between the 2 areas became smaller in 1966–1985 along with the influence of Westernization of food habits in the rural area.[8] Taken together, it might indicate that the increase in the incidence of AMI in younger male patients in the rural area was likely to be associated with the marked increase in the prevalence of dyslipidemia.

The present study also demonstrates the increase in the prevalence of hypertension in AMI patients. In the Tohoku district, including the Miyagi prefecture, the prevalence of hypertension was relatively higher compared with other parts of Japan,[12,26] and thus more careful and strict control of risk factors is needed.

The prevalence of smoking remained high not only in the urban areas but also in the rural areas. In particular, in the younger male patients, the prevalence of smoking (~50%) was higher compared with the general Japanese population (36.8% in males and 9.1% in females in 2008).[27] Importantly, in the younger urban female patients, it remained more than 30%; 3 times higher than in the general Japanese population.

Study Limitations

Several limitations should be mentioned for the present study. First, although in the Miyagi prefecture, almost all AMI patients are transferred to our participating hospitals via the established emergency medical system, we cannot completely confirm that all patients have been registered in our registry. Second, while the MIYAGI-AMI Registry Study has been conducted over 20 years, the diagnosis of AMI has been changing.[28] In the present study, the diagnosis was made on the basis of the WHO-MONICA criteria with creatine kinase (CK).[16] Indeed, troponins are widely used in recent clinical practice and are more sensitive and specific biomarkers of myocyte necrosis than CK,[29] which might affect the results. Third, this study is an observational study and cannot reach the cause-effect relationship. Moreover, we did not examine the prevalence of risk factors in control subjects and did not collect the data of medical treatment for prevention, thus we were unable to precisely estimate the influence of risk factors on the incidence of AMI. Finally, in the present study, we did not examine the long-term mortality but only examined in-hospital mortality. The increasing incidence of decreasing in-hospital mortality from AMI in the Japanese population has apparently resulted in the recent increase in the number of patients with ischemic heart failure, as recently demonstrated in our heart failure cohort study, the CHART-1 and the CHART-2 studies.[30,31] Thus, a more effective strategy to improve the management of post-infarction heart failure needs to be developed.

Conclusions

Our MIYAGI-AMI Registry Study demonstrates that urbanization and life-style changes have been associated with the incidence and mortality of AMI in Japan, although sex differences still remain to be improved.

Acknowledgments

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ORIGINAL ARTICLE
Vascular Biology and Vascular Medicine

Ezetimibe Improves Endothelial Function and Inhibits Rho-Kinase Activity Associated With Inhibition of Cholesterol Absorption in Humans

Kotaro Nochioka, MD, PhD; Shin-ichi Tanaka, BSc; Masanobu Miura, MD; Do.e Zhulanqiqige, MD, PhD; Yoshihiro Fukumoto, MD, PhD; Nobuyuki Shiba, MD, PhD; Hiroaki Shimokawa, MD, PhD

Background: Ezetimibe is an inhibitor of cholesterol absorption in the intestine. We examined whether ezetimibe improves endothelial function, and if so, what mechanisms are involved.

Methods and Results: Nineteen healthy subjects (male/female 14/5; mean age, 31±3 [SD] years-old) were randomized to receive ezetimibe (10 mg/day) or pravastatin (10 mg/day) for 4 weeks in a cross-over manner with a 4-week washout interval. Lipid profiles, flow-mediated dilatation (FMD) and Rho-kinase activity of circulating leukocytes (the extent of phosphorylation of myosin binding subunit, a Rho-kinase substrate) were examined. We also evaluated remnant-like particle cholesterol (RLP-C) known as an up-regulator of Rho-kinase and cholesterol absorption status by measuring cholestanol and campesterol/lathosterol ratio (CLR) (both absorption markers). Although ezetimibe and pravastatin equally reduced low-density lipoprotein cholesterol (E: –25% vs. P: –21%), the CLR was reduced by ezetimibe but was rather increased by pravastatin (E: –41% vs. P: +37%; P<0.01). Reduction in RLP-C by ezetimibe was greater compared with pravastatin (E: –33% vs. P: –14%; P<0.05). Importantly, ezetimibe significantly improved FMD (26%, P<0.05) and reduced Rho-kinase activity (–21%, P<0.05), whereas pravastatin had no such effects. A significant correlation was noted between the reduction in cholestanol and the improvement in FMD (P<0.05).

Conclusions: These results indicate that ezetimibe improves endothelial function and inhibits Rho-kinase activity associated with the inhibition of cholesterol absorption, suggesting novel anti-atherogenic effects of the agent in humans. (*Circ J* 2012; **76:** 2023–2030)

Key Words: Ezetimibe; Flow-mediated dilatation; LDL cholesterol; Rho-kinase

Ezetimibe is a lipid-lowering agent that selectively inhibits cholesterol absorption by binding to the Nieman-Pick C1 Like 1 (NPC1L1) protein.[1] Inhibition of cholesterol absorption in the small intestine reduces the events of myocardial infarction and death due to coronary artery disease in patients with prior myocardial infarction and hyperlipidemia, suggesting the beneficial effects of ezetimibe by lipid modification.[2] However, the ENHANCE trial (2008) evoked a controversy regarding the clinical benefit of ezetimibe for preventing atherosclerosis,[3] as ezetimibe (10 mg/day) or simvastatin (80 mg/day) for 24 months failed to suppress the progression of carotid intima/medial thickness in patients with an early stage of familial hypercholesterolemia.[3] Subsequently, however, the SHARP trial (2011) demonstrated the beneficial effects of a combination therapy with ezetimibe and simvastatin on the long-term prognosis of patients with chronic kidney disease.[4]

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Endothelial dysfunction is one of the initial steps of atherosclerosis.[5] Rho-kinase, a small GTP-binding protein, has been

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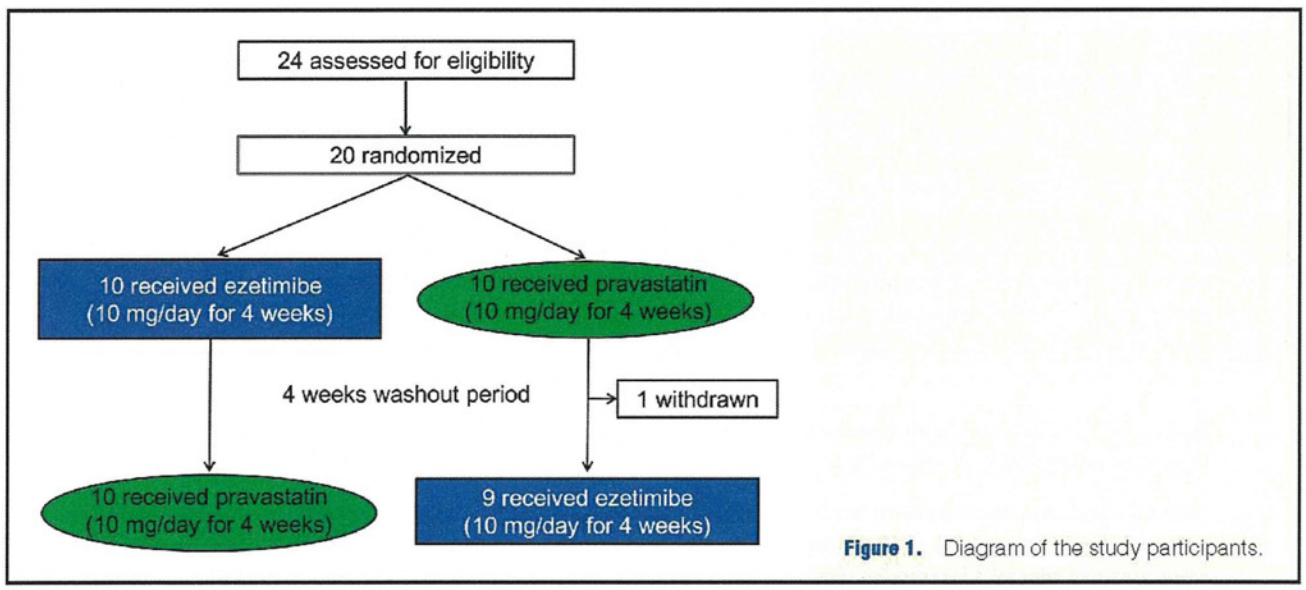

Figure 1. Diagram of the study participants.

Table 1. Baseline Characteristics of the Study Subjects

	Total (n=19)
Age (years)	30.9 (2.7)
Male (%)	14 (73.7)
Height (cm)	169.9 (7.8)
Weight (kg)	64.5 (10.8)
BMI (kg/m^2)	22.5 (2.3)
Waist circumference (cm)	79.8 (8.0)
Systolic BP (mmHg)	119.7 (11.4)
Diastolic BP (mmHg)	73.1 (7.3)
Heart rate (/min)	72.7 (11.7)
FBS (mg/dl)	92.5 (11.4)

Results are expressed as mean (SD).
BMI, body mass index; BP, blood pressure; FBS, fasting blood sugar.

implicated as one of the major causes of endothelial dysfunction and atherosclerosis.[6,7] Therefore, the inhibition of Rho-kinase might be a possible therapeutic option in preventing cardiovascular diseases.[6,7] Previous studies showed that ezetimibe inhibits intestinal cholesterol absorption and reduces the level of remnant-like particle cholesterol (RLP-C),[8] which is known to enhance Rho-kinase activity.[9] Furthermore, ezetimibe also inhibits absorption of oxidized cholesterol that is incorporated in atherogenic oxidized lipoproteins.[10]

In the present study, we thus evaluated whether ezetimibe improves endothelial function and inhibits Rho-kinase activity, in comparison with the effects of pravastatin that showed a similar low-density lipoprotein cholesterol (LDL-C) lowering effect, in humans.

Methods

Study Protocol

The study protocol was approved by the ethics committee of the Tohoku University Graduate School of Medicine and written informed consent was obtained from all subjects. The study conformed to the Declaration of Helsinki and was registered in the University Hospital Medical Information Network (UMIN000002946).

The present single center, randomized, cross-over study was performed at the Tohoku University Hospital in Sendai, Japan. A total of 20 healthy subjects were randomized to receive either ezetimibe (10mg/day) or pravastatin (10mg/day) for 4 weeks. After a 4-week washout period, the subjects were switched to take the alternate agent for an additional 4 weeks (**Figure 1**). It was confirmed that a 4-week wash out period was enough to restore the pre-treatment condition for both statin and ezetimibe.[11,12] The participants were requested not to change their life-style behavior including diet and physical exercise during the study period. The study medicines (ezetimibe and pravastatin) were purchased from Bayer-HealthCare Co (Osaka, Japan) and Daiichi-Sankyo Co (Tokyo, Japan), respectively. A venous blood sample was drawn and flow-mediated dilatation (FMD) was measured at 0, 4, 8, and 12 weeks after the randomization. Primary outcomes were the changes in FMD and Rho-kinase activity caused by each intervention and the differences between the 2 treatments. We also examined the relationships between the outcomes and the following parameters: serum levels of total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), and RLP-C. We also measured cholesterol absorption markers (cholestanol, campesterol and sitosterol), a cholesterol synthesis marker (lathosterol) and the campesterol/lathosterol ratio (CLR), high-sensitivity C-reactive protein (hsCRP) and the homeostasis model assessment of insulin resistance (HOMA-IR).

Study Subjects

Healthy individuals aged ≥20 years old were enrolled in the present study. Individuals who had at least 1 of the following conditions were excluded: (1) a history of receiving lipid-lowering medicines; (2) active diseases; (3) taking any kind of medicines or supplements; (4) former or current smokers; (5) liver and/or renal dysfunction; and (6) eating disorder.

All participants were recruited by posted notices on bulletin boards in the Tohoku University from December 2009 to August 2010. We screened 24 subjects and excluded 4 subjects; 2 subjects for liver dysfunction and dyslipidemia, 1 for a high hsCRP level due to a sinus infection, and 1 for being a current smoker. There were no significant side-effects related to ezetimibe or pravastatin, but 1 subject showed a low LDL-C level

Table 2. Effects of Ezetimibe and Pravastatin on Lipid Profile, Cholesterol Absorption/Synthesis Markers, hsCRP and HOMA-IR

	Ezetimibe (10 mg/day)				Pravastatin (10 mg/day)			
	Baseline	After 4 weeks	%Change	P value	Baseline	After 4 weeks	%Change	P value
Fasting lipids (mg/dl)								
Total cholesterol	199 (31)	164 (24)	−17.2	<0.001	201 (37)	177 (26)	−12.2	<0.001
LDL-C	121 (24)	90 (18)	−25.4	<0.001	120 (27)	95 (19)	−20.8	<0.001
HDL-C	60 (15)	57 (12)	−5.1	0.04	61 (16)	63 (15)	3.0	0.15
TG	85 (34)	83 (22)	−3.0	0.72	84 (25)	76 (30)	−9.0	0.23
Cholesterol absorption marker (μg/ml)								
Cholestanol	3.0 (0.8)	2.4 (0.5)	−37.5	0.001	3.1 (0.7)	2.7 (0.8)	−14.0	0.001
Campesterol	5.6 (2.8)	3.5 (2.3)	−37.4	<0.001	5.4 (3.0)	5.3 (3.4)	−0.03	0.63
Sitosterol	3.5 (2.0)	2.3 (1.5)	−35.0	<0.001	3.6 (2.0)	3.3 (1.9)	−10.0	0.16
Cholesterol synthesis marker (μg/ml)								
Lathosterol	3.6 (1.3)	4.3 (1.7)	20.2	<0.001	3.5 (1.3)	2.4 (0.9)	−33.5	<0.001
RLP-C (mg/dl)	3.9 (1.5)	2.6 (1.0)	−33.2	0.002	3.6 (1.1)	3.1 (0.8)	−14.2	0.07
hsCRP (mg/dl)	0.4 (0.7)	0.3 (0.3)	−21.0	0.58	0.5 (0.8)	0.2 (0.1)	−58.8	0.13
HOMA-IR	2.0 (0.8)	2.1 (1.1)	8.6	0.30	1.9 (0.8)	1.8 (0.9)	−6.3	0.10

Results are expressed as mean (SD).
hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment-insulin resistance; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride ; RLP-C, remnant-like particles cholesterol.

(56 mg/dl) at 4 weeks after the treatment with pravastatin and was excluded from the study. Thus, the final analyses were conducted for the 19 subjects who completed the study protocol (**Figure 1**). There were no significant changes in bodyweight, blood pressure or heart rate after each treatment during the study period.

Biochemical Analysis

Serum levels of TC, LDL-C, HDL-C and TG were determined by using enzymatic methods at the central laboratory of the Tohoku University Hospital. Serum was obtained by centrifugation at 1,400 g for 30 min at 4°C and the aliquots were carried immediately to the Special Reference Laboratory Inc., Tokyo, in containers at –80°C for the assays of RLP-C, cholestanol, campesterol, sitosterol, and lathosterol. RLP-C was measured by using the immunoadsorption method (RLP MIXER J-100, Otsuka Electronics, Japan) and cholestanol, campesterol, sitosterol and lathosterol were measured by using a gas chromatograph (GC-17A, Shimadzu, Kyoto, Japan). The HOMA-IR was calculated by using the international formula: fasting glucose (mmol/L)×fasting insulin (mU/L)/22.5.[13]

Rho-Kinase Activity Assay

Rho-kinase activity was evaluated by Western blot analysis using circulating leukocytes.[12,14] The activity was quantified by calculating the ratio of the levels of the phosphorylated myosin-binding subunit (p-MBS) and the total MBS (t-MBS), substrates of Rho-kinase.[12,14] Circulating leukocytes were isolated from blood samples as previously described.[12,14] Cell extracts were loaded on 7.5% SDS-PAGE gel and immunoblotted for the detection of Rho-kinase activity, using rabbit polyclonal anti-phospho-myosin phosphatase 1 (MYPT-1) (Thr696) (Upstate) for p-MBS and mouse monoclonal anti-MYPT1 (BD Biosciences) for t-MBS.[12,14] All Western blot analyses were performed by 1 technician in a blinded manner. Because the bands of p-MBS were not detected in 2 samples, analyses were performed for the remaining 17 subjects.

Measurement of FMD

Examinations of FMD were performed using UNEXEF18G (UNEX, Aichi, Japan)[15] by 1 trained ultrasonographer in a blinded manner. The subjects were prohibited from diet, exercise, or caffeine/alcohol intake at least 9 h before the examination. The procedure was performed in a quiet, dark, temperature-controlled examination room according to the guidelines of the International Brachial Artery Reactivity Task Force.[16] Briefly, the right brachial diameter was measured at rest after 10 min of bed rest and during reactive hyperemia above the antecubital fossa with a 10-MHz linear array transducer.[16] Reactive hyperemia was induced by inflating a sphygmomanometiric cuff, which is equipped in UNEXEF18G, on the proximal portion of the arm at least 50 mmHg above the systolic blood pressure to occlude arterial inflow for 5 min. End-diastolic images of the brachial artery were obtained and diameters were measured with R-wave synchronized automated edge-detection software automatically.[15] The values of FMD were calculated with the following formula:

(diameter at peak hyperemia − diameter at rest)/(diameter at rest) × 100.[16]

Statistical Analysis

All continuous variables are reported as mean ± standard deviation (SD) unless otherwise stated. We assessed the differences in measured parameters before and after the treatment within each arm using the paired t-test. We also compared the changes caused by each intervention between the 2 arms using an unpaired t-test. The Spearman rank correlation test was used to evaluate the statistical significance in the correlation between the changes in FMD or Rho-kinase activity and those in LDL-C, RLP-C or cholestanol after each treatment. We performed all analyses using IBM SPSS Statistics 18.0 (New York, NY, USA). A 2-sided P value of <0.05 was considered to be statistically significant.

Results

Baseline Characteristics and Changes in Lipid Profiles, hsCRP, and HOMA-IR

Table 1 shows the baseline characteristics of the study sub-

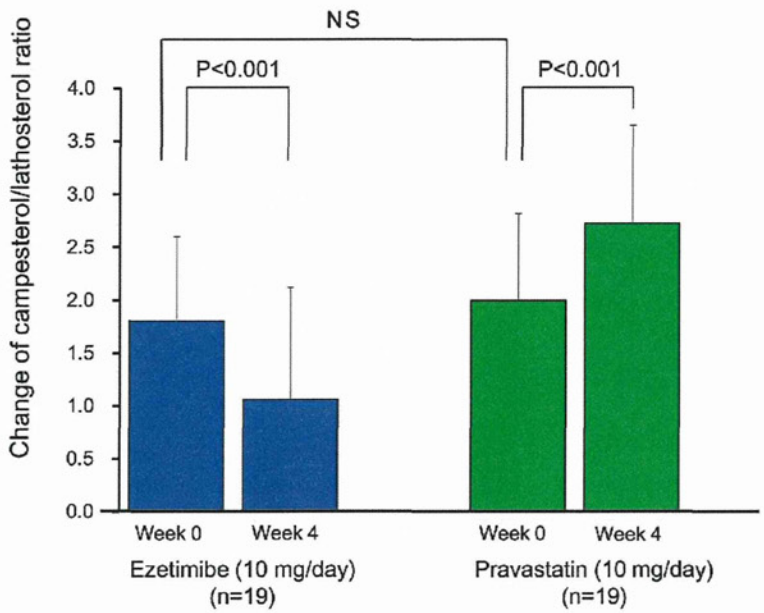

Figure 2. Changes of the campesterol/lathosterol ratio before and after a 4-week treatment with ezetimibe (10mg/day) or pravastatin (10mg/day). A higher level reflects increased cholesterol absorption.

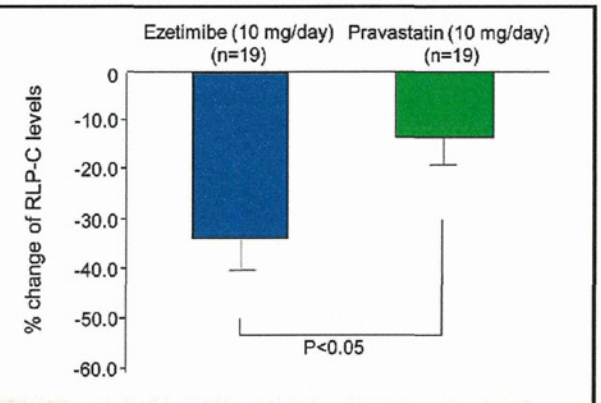

Figure 3. Changes of remnant-like lipoprotein cholesterol (RLP-C) before and after a 4-week treatment with ezetimibe (10mg/day) or pravastatin (10mg/day).

jects. The mean age was 30.9±2.7 years and males accounted for 73.7%. No participants had a body mass index (BMI) ≥25.0kg/m^2. **Table 2** shows the effects of ezetimibe (10mg/day) (E) or pravastation (10mg/day) (P) on lipid profiles after the 4-week treatment. There was no significant difference in the lipid profiles at baseline between the ezetimibe and pravastatin treatment arms. Both ezetimibe and pravastatin caused a comparable extent of reduction in LDL-C after each treatment (E: –25.4% vs. P: –20.8%). A mild but significant reduction in HDL-C was observed in the ezetimibe treatment (E: –5.1% vs. P: +3.0%), whereas there was no significant reduction in TG in either treatment (E: –3.0% vs. P: –9.0%). Ezetimibe reduced RLP-C to a greater extent compared with pravastatin (E: –33.2% vs. P: –14.2%; P<0.05). Both ezetimibe and pravastatin tended to decrease hs-CRP levels, but the changes did not reach statistical significance in either group. HOMA-IR also did not show significant changes after the treatment, suggesting that neither ezetimibe nor pravastatin altered insulin sensitivity during the 4-week study period (**Table 2**).

Changes in Cholesterol Absorption and Synthesis

The changes in cholesterol absorption/synthesis markers are shown in **Table 2**. Ezetimibe decreased cholesterol absorption markers, such as cholestanol, campesterol and sitosterol levels. Lathosterol, a cholesterol synthesis marker, was increased by ezetimibe, but decreased by pravastatin. Ezetimibe significantly decreased CLR (–41.1%, P<0.001), whereas pravastatin significantly increased it (+36.5%, P<0.001; **Figure 2**).

Changes in RLP-C

Figure 3 shows the effects of ezetimibe and pravastatin on RLP-C levels. Ezetimibe significantly decreased the RLP-C level (–1.3±0.5mg/dl, P<0.01), whereas pravastatin was without the effect (–0.5±0.3mg/dl, NS), and the extent of the reduction was significantly greater with ezetimibe treatment (E: –33.2% vs. P: –14.2%; P<0.05; **Figure 3**).

Effect of Ezetimibe and Pravastatin on Rho-Kinase Activity

Figure 4 shows the changes in Rho-kinase activity of circulating leukocytes after each treatment. Baseline Rho-kinase activity did not differ significantly between the 2 treatment arms (E: 1.03±0.44 vs. P: 0.97±0.28). Importantly, after the 4-week treatment, ezetimibe significantly inhibited the Rho-kinase activity (–21%, P<0.05), whereas pravastatin was without the effect (+6.2%, NS).

Effect of Ezetimibe and Pravastatin on FMD

Figure 5 shows the effect of ezetimibe and pravastatin on FMD. No significant difference in baseline FMD was observed between the 2 treatment arms (E: 6.8±2.6% vs. P: 7.5±2.1%). FMD was significantly improved by ezetimibe (+25.5%, P<0.05), whereas pravastatin was without the effect (+4.1%, NS).

Correlations Between LDL-C, RLP-C, Cholestanol, Rho-Kinase Activity, and FMD

There were no significant correlations between the changes in Rho-kinase activity and those in LDL-C, RLP-C, or cholestanol level after the treatments (**Figures 6A–C**). Similarly, no

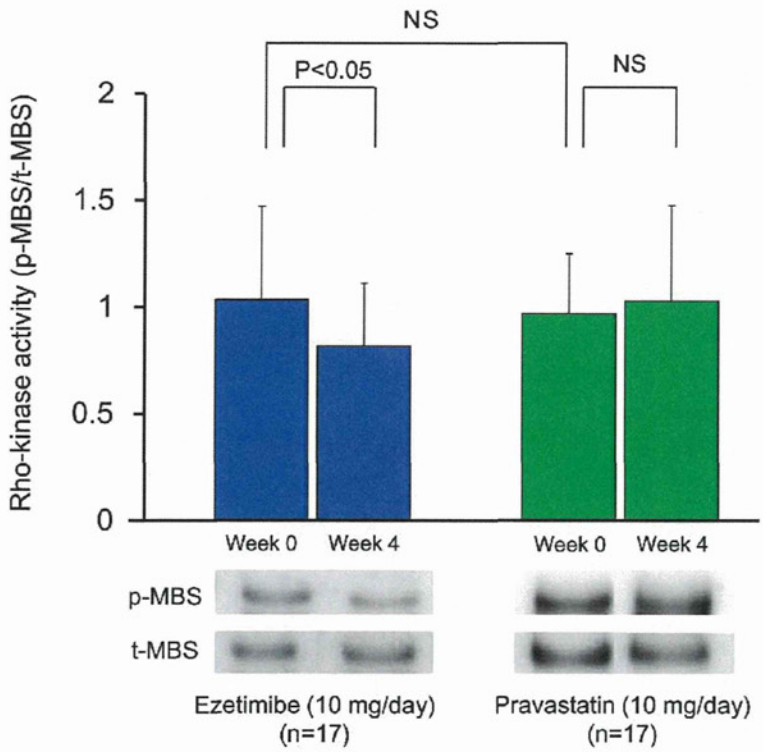

Figure 4. Rho-kinase activity of circulating leukocytes before and after a 4-week treatment with ezetimibe (10mg/day) or pravastatin (10mg/day). p-MBS, phosphorylated myosin-binding subunit; t-MBS, total myosin-binding subunit.

significant correlation was noted between the changes in FMD and those in LDL-C or RLP-C (**Figures 6D–E**). Importantly, however, there was a significant correlation between the changes in FMD and those in cholestanol levels (**Figure 6F**). No significant correlation was noted between the changes in FMD and those in Rho-kinase activity (**Figure 6G**).

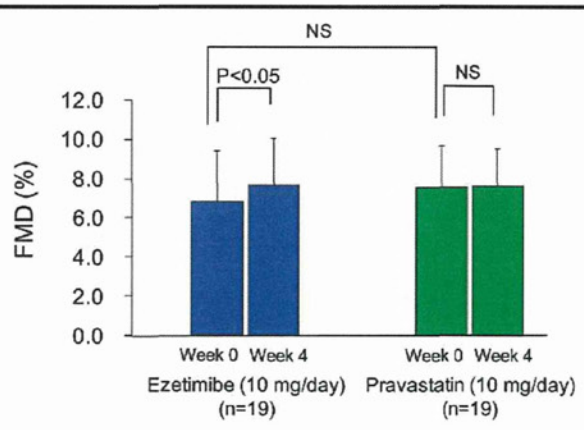

Figure 5. Changes in flow-mediated dilatation (FMD) before and after a 4-week treatment with ezetimibe (10mg/day) or pravastatin (10mg/day).

Discussion

In the present study, we were able to demonstrate that the treatment with ezetimibe (10mg/day for 4 weeks) significantly inhibited the Rho-kinase activity of circulating leukocytes in healthy subjects and that the ezetimibe treatment significantly enhanced FMD, where the changes in FMD were significantly associated with those in the serum cholestanol level, a cholesterol absorption marker. These results suggest that ezetimibe has novel anti-atherogenic effects associated with its inhibition of cholesterol absorption, which could explain, at least in part, the beneficial effects of the agent in the SHARP trial.[4]

Effects of Ezetimibe on FMD

In the present study, ezetimibe significantly improved FMD in healthy volunteers. Because FMD has been demonstrated as an established surrogate marker of endothelial function,[16-18] the beneficial effect of ezetimibe on endothelial function might exert anti-atherogenic effects in humans. In contrast, pravastatin did not significantly improve FMD, although both treatments caused a comparable extent of LDL-C reduction in the present study. We consider that the difference in the effect on endothelium function between ezetimibe and pravastatin was mainly caused by the difference in the alteration in cholesterol absorption/synthesis status as follows.

In the present study, we evaluated the cholesterol absorption/synthesis status by examining the surrogate markers, cholestanol and plant sterols (campesterol, lathosterol and sitosterol) levels, as it is difficult to directly examine the cholesterol absorption/synthesis status in humans. Interestingly, we noted the significant correlation between the change in FMD and that in cholestanol level, a cholesterol absorption marker. Cholestanol is the 5α-saturated derivative of cholesterol and serum cholestanol levels usually remain constant.[19] The cholestanol production is generally at a low level (<20mg/day),[19] and serum cholestanol is absorbed from the gut in humans.[19,20] It was reported that in middle-aged normal males, high levels of serum cholestanol reflect the status with high cholesterol ab-

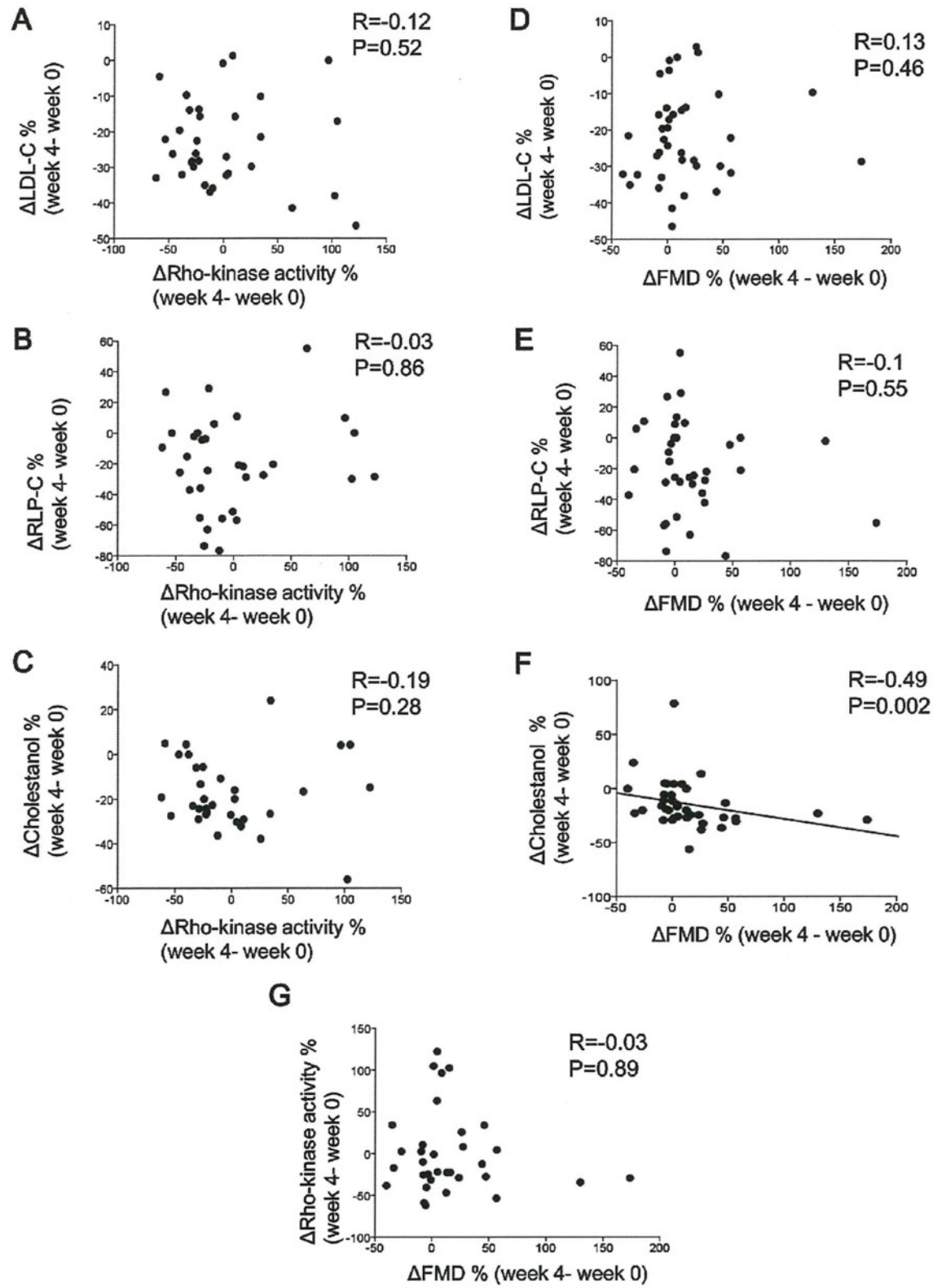

Figure 6. Correlations between changes in Rho-kinase activity of circulating leukocytes and those in low-density lipoprotein cholesterol (LDL-C) (**A**), remnant-like lipoprotein cholesterol (RLP-C) (**B**), cholestanol (**C**), and correlations between changes in flow-mediated dilatation (FMD) and those in LDL-C (**D**), RLP-C (**E**), cholestanol (**F**), and Rho-kinase activity (**G**). A linear regression line was plotted to visualize the statically significant relationship (**F**).

sorption and low cholesterol synthesis.[21] Thus, the serum cholestanol level has been used as a surrogate marker of cholesterol absorption. Indeed, it was previously reported that the baseline serum cholestanol level is the predictor of recurrent coronary events in patients with coronary artery disease.[22]

In the present study, CLR as a marker of cholesterol absorption/synthesis status,[21] was also decreased by ezetimibe but was rather significantly increased by pravastatin. It was previously shown that ezetimibe inhibits the absorption of dietary oxidized cholesterol and reduces its incorporation into lipoproteins.[10] Although the direct assay of atherogenic cholesterols, such as circulating oxidized cholesterol, was not performed in the present study, it is highly possible that ezetimibe improved endothelial function through inhibition of atherogenic cholesterol absorption in the intestine.

The present study results are in contrast to those reported by Landmesser et al, as they reported that simvastatin (10 mg/day for 4 weeks) significantly improved FMD, whereas ezetimibe was without the effects in chronic heart failure patients.[23] It should be noted that they enrolled patients with established heart failure and lower LDL-C levels (simvastatin group, 106± 8 mg/dl; ezetimibe group, 109±6 mg/dl), whereas we enrolled normal volunteers in the present study. Furthermore, simvastatin is a lipophilic statin that has a more potent LDL-C lowering effect compared with a hydrophilic statin, pravastatin.

Possible Mechanisms of Rho-Kinase Inhibition by Ezetimibe in Humans

In the present study, ezetimibe significantly inhibited the Rho-kinase activity of circulating leukocytes in healthy volunteers, independent of LDL-C. It was reported that the Rho-kinase of circulating leukocytes is the primary determinant of leukocyte recruitment to the vessel wall and is the critical mediator of neointimal proliferation following vascular injury.[24] Thus, the inhibitory effect of ezetimibe on Rho-kinase activity might be one of the important mechanisms of its anti-atherogenic effects.

We consider that the possible mechanism of the inhibitory effect of ezetimibe on Rho-kinase activity involves, at least in part, the reductions in serum atherogenic cholesterols. Indeed, in the present study, the extent of the reduction in serum RLP-C level was significantly greater by ezetimibe compared with pravastatin. We have previously demonstrated that RLP-C enhances Rho-kinase activity in the coronary artery both in vitro and in vivo.[9] Thus, the reduction in RLP-C by ezetimibe might also exert a beneficial effect on endothelium function. In the present study, ezetimibe significantly reduced serum levels of circulating plant sterols (campesterol and sitosterol), biomarkers of cholesterol absorption, and increased those of lathosterol, a marker of cholesterol synthesis. It also was reported that increased serum levels of cholesterol absorption were significantly associated with cardiovascular diseases in the Framingham Offspring study.[25]

In the present study, however, no significant correlation was noted between the changes in Rho-kinase activity of circulating leukocytes and those in lipid profiles, but a significant correlation was noted between improvement of endothelial function and the extent of inhibition of cholesterol absorption. These findings could be explained by the fact that the baseline levels of RLP-C and plant sterols in the present normal volunteers were much lower than in patients with atherosclerosis,[9,26,27] which could have masked the association between atherogenic cholesterols and Rho-kinase activity. Furthermore, Rho-kinase activity could be upregulated not only by RLP-C or plant sterols but also by other mechanisms (eg, angiotensin-II and IL-1β).[28,29]

The present study findings might be in contrast to those reported by Liu et al, where they found in patients with dyslipidemia that simvastatin (40 mg/day for 4 weeks) significantly improved endothelial function and inhibited the Rho-kinase activity of circulating leukocytes, whereas ezetimibe (10 mg/day for 4 weeks) was without the effect.[30] Although the reason for the discrepancy between the present study and that by Liu et al remain to be elucidated, it is conceivable that the different dose of statins (pravastatin 10 mg/day vs. simvastatin 40 mg/day), the different study subjects (normal volunteers vs. patients with dyslipidemia) and the carry-over effects of statins in patients with cardiovascular diseases[31] might be involved.

Study Limitations

Several limitations should be mentioned for the present study. First, in the present study, we only enrolled normal healthy volunteers and thus the present study findings remain to be confirmed in patients with dyslipidemia and/or cardiovascular diseases. Furthermore, we cannot exclude the possibility that non-healthy subjects were included in the present study population. Second, we only examined the 4-week treatment with ezetimibe or pravastatin and the longer period of treatment would have provided more definite effects of the agents, especially on lipid profiles and endothelial function. Finally, we did not directly measure serum levels of atherogenic cholesterols, and the direct measurement of those profiles would reveal more detailed aspects of the anti-atherogenic effects of ezetimibe.

Conclusions

In the present study, we were able to demonstrate that ezetimibe improves endothelial function and inhibits Rho-kinase activity in humans, suggesting novel anti-atherogenic effects of the agent. The present study findings also suggest that the beneficial effect of ezetimibe is mediated by its inhibitory effects on absorption of atherogenic cholesterol.

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Disclosures

Conflict of interest: None declared.

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FASTTRACK
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The Great East Japan Earthquake Disaster and cardiovascular diseases

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Aims While previous studies reported a short-term increase in individual cardiovascular disease (CVD) after great earthquakes, mid-term occurrences of all types of CVDs after great earthquakes are unknown. We addressed this important issue in our experience with the Great East Japan Earthquake (11 March 2011).

Methods and results We retrospectively examined the impact of the Earthquake on the occurrences of CVDs and pneumonia by comparing the ambulance records made by doctors in our Miyagi Prefecture, the centre of the disaster area, during the periods of 2008–11 ($n = 124\,152$). The weekly occurrences of CVDs, including heart failure (HF), acute coronary syndrome (ACS), stroke, cardiopulmonary arrest (CPA), and pneumonia were all significantly increased after the Earthquake compared with the previous 3 years. The occurrences of ACS and CPA showed the rapid increase followed by a sharp decline, whereas those of HF and pneumonia showed a prolonged increase for more than 6 weeks and those of stroke and CPA showed a second peak after the largest aftershock (7 April 2011). Furthermore, the occurrence of CPA was increased in the first 24 h after the Earthquake, followed by other diseases later on. These increases were independent of age, sex, or residence area (seacoast vs. inland).

Conclusion These results indicate that the occurrences of all types of CVDs and pneumonia were increased in somewhat different time courses after the Earthquake, including the first observation of the marked and prolonged increase in HF, emphasizing the importance of intensive medical management of all types of CVDs after great earthquakes.

Keywords Earthquake • Cardiovascular disease • Heart failure • Tsunami

Introduction

On 11 March 2011, the Great East Japan Earthquake hit the northeast part of Japan with a magnitude of 9.0 on the Richter scale, which was one of the largest ocean-trench earthquakes ever recorded in Japan (*Table 1*).[1] The Earthquake caused huge damage, including 15 861 dead, 3018 missing persons, and 388 783 destroyed houses as of 6 June 2012.[2] It forced many people (~400 000) to be evacuated to temporary accommodation, such as public halls, gymnastic halls, and scholastic institutions in Northeast Japan. Since the Earthquake occurred with its epicentre located at 38° latitude, 19 min North, and 142° longitude, 22 min East, our Miyagi Prefecture with a population of 2 348 165 was the closest area to the epicentre (*Figure 1A*), where there was the largest amount of damage and number of victims, including 9512 dead, 1581 missing persons, and 232 553 destroyed houses as of 8 May 2012,[2,3] and most of the damage was observed in the seacoast area, including 9506 dead (95.8%), 1578 missing persons (99.8%) and 222 880 destroyed houses (95.8%).

It has been previously reported that the occurrences of acute coronary syndrome (ACS), stroke, pulmonary embolism, and takotsubo cardiomyopathy were increased after the large earthquakes in Japan (*Table 1*).[4–9] Furthermore, it has been reported that the occurrences of sudden cardiac death and haemodynamically unstable ventricular tachyarrhythmias were increased after the Northridge Earthquake in California, USA, and the Wenchuan Earthquake in China, respectively (*Table 1*).[10] Thus, the previous reports have revealed that the occurrences of

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Table 1 Past major earthquakes and cardiovascular diseases

Place of earthquake (country)	Year	Month	Magnitude	Temperature on onset day (°C) (high/low)	No. of deaths	No. of injured	Diseases increased	Periods of increased occurrences after each earthquake
Northridge (USA)[11]	1994	January	6.7	19/9	57	5400	Sudden deaths	On the day of the earthquake
Hanshin-Awaji (Japan)[8,9,13]	1995	January	7.3	8/1.4	6434	43 792	AMI, pneumonia	AMI: from 1st to 4th week Pneumonia: first month
Indian Ocean (Indonesia)	2004	December	9.1	32/25	Over 220 000	130 000	No data available	No data available
Mid-Niigata (Japan)[6,7]	2004	Octorber	6.8	26.4/22	68	4805	Takotsubo cardiomyopathy, PE, sudden deaths	Takotsubo cardiomyopathy: from 1st to 3rd week, PE and sudden deaths: first week
Wenchuan (China)[17]	2008	May	7.9	25.0/17	69 197	18 222	VT/VF	From 1st to 3rd day
East Japan (Japan)	2011	March	9.0	6.2/-2.5	15 845	5894	HF, ACS, stroke, CPA, pneumonia	See text

AMI, acute myocardial infarction; PE, pulmonary artery embolism; ACS, acute coronary syndrome; VT/VF, ventricular tachycardia/ventricular fibrillation; HF, heart failure; CPA, cardiopulmonary arrest.

various cardiovascular diseases (CVDs) were increased after large earthquakes. However, these studies reported only the short-term occurrence of individual CVD and the longer-term occurrences of all types of CVDs after great earthquakes remain to be elucidated.

In the present study, we thus addressed this important issue by comparing the ambulance records made by medical doctors in our Miyagi Prefecture, the centre of the disaster area, during the periods of 2008–11. The present study demonstrates for the first time the marked and prolonged increase in the occurrence of heart failure (HF), in addition to other CVDs, which has not been reported previously.

Methods

This study was a collaboration study with the Miyagi Medical Association and the Fire Departments of the Miyagi Prefecture. The Ethics Committees of Tohoku University Hospital approved this study protocol.

Study population

We enrolled all ambulance transport records in the Miyagi Prefecture from 11 February to 30 June in each year of 2008–11 ($n = 124\,152$), from 4 weeks before to 16 weeks after 11 March, in order to reveal the effects of the Earthquake on the occurrence of CVDs. In Japan, medical doctors in the emergency rooms routinely make the diagnoses of transported patients at the initial visit. These reports were collected and stored in the fire departments that operate the emergency medical system. We were able to obtain all the medical records from the 12 fire departments in the Miyagi Prefecture. In our prefecture, the 12 fire departments routinely transfer patients to the 57 hospitals with emergency rooms that are registered by the prefecture. Among these 57 hospitals, 56 (98%) have an echocardiography machine, 57 (100%) have full-time physicians, and 38 (67%) have full-time cardiologists. It has been reported that the diagnostic accuracy of ACS in the emergency room is 83.4% in Japan.[10] Based on the records, we examined the weekly occurrences of HF, ACS, stroke, cardiac pulmonary arrest (CPA), and pneumonia and compared them with those in the previous 3 years (2008–10). Furthermore, we examined the daily occurrences of the diseases for a week before and after the Earthquake.

To access the impact of the Earthquake and the aftershocks on the occurrence of the diseases, we counted the number of earthquakes with a seismic intensity of 1 or greater on the Japanese scale, which were observed in the Miyagi Prefecture during the study period (Japan Meteorological Agency: http://www.jma.go.jp/jma/index.html). We defined the municipalities facing the Pacific Ocean as the seacoast area where the Tsunami directly attacked and the remaining inner area as the inland area (*Figure 1B*).

Definition of the diseases

We obtained all diagnoses from the ambulance records, which were made by attending doctors in emergency rooms based on physical examination, ECG, chest X-ray, echocardiography, and laboratory findings including the blood gas test. When definitive diagnoses are made by doctors in the emergency rooms, they write the diagnoses on the ambulance records; however, when definitive diagnoses are not confirmed in the emergency rooms, they write tentative diagnoses or only symptoms, which are defined as undiagnosed cases in the present study. We have excluded such undiagnosed cases from the analyses in the present study.

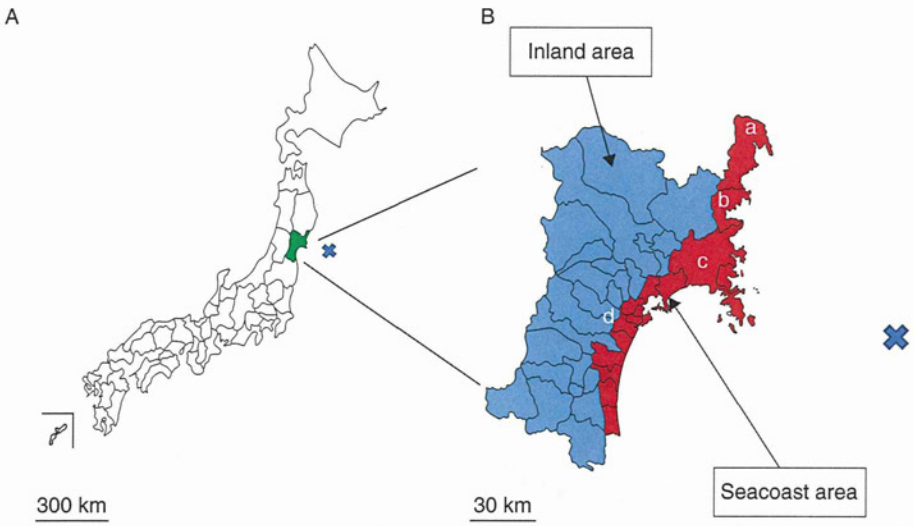

Figure 1 Locations of the epicentre of the Great East Japan Earthquake and the Miyagi Prefecture. (*A*) The epicentre of the East Japan Earthquake (cross) was under the Pacific Ocean about 150 km east of the Miyagi Prefecture. (*B*) The municipalities in the Miyagi Prefecture facing the Pacific Ocean were defined as the seacoast area (shown in red) and the other area as the inland area (shown in blue). Many cities in the coast area, including Kesennuma (a), Minami-sanriku (b), Ishinomaki (c), and Sendai (d) were seriously damaged by the Tsunami.

Acute coronary syndrome was defined as acute myocardial infarction or unstable angina, stroke as intracranial haemorrhage, cerebral infarction or subarachnoid haemorrhage, and CPA as cardiopulmonary resuscitation performance regardless of the causes. In each year, we calculated 'the rate of definitive diagnosis at admission in the emergency rooms (%)', which means the percentage of cases with definitive diagnosis made by doctors among all transported cases.

Statistical analysis

To assess the differences in the occurrences of CVDs and pneumonia before and after the Earthquake between 2011 and the previous 3 years, we applied the Poisson regression model to the daily occurrences in 2008–11 with the 'dummy' variables which indicate the individual weeks in 2011.[11] First, we defined the dummy variable of each week after 11 March 2011 that takes a value of 1 or 0, indicating whether or not the sample was observed in the corresponding week. Then, we fitted the Poisson regression model with all dummy variables to explain the daily occurrences of the CVDs. Finally, we selected effective dummies of significant weeks by the backward elimination stepwise regression method. Furthermore, we calculated odds ratio with the 4-week occurrence of the disease in 2011 before and after the Earthquake in the following subgroups; young (<75 years old) and old (≥75 years old) patients, male and female, and the inland and seacoast residence areas. We used Fisher's exact test for the subgroup analyses. Continuous variables are expressed as mean ± SD. All statistical analyses were performed using R 2.15.0 (www.r-project.org/). All *P*-values were two-sided, and *P*-values of <0.05 were considered to be statistically significant.

Results

The total number of ambulance transports in the period of 11 February to 30 June in 2008, 2009, 2010, and 2011 was 28 709, 28 069, 30 645, and 36 729, respectively. When compared with the previous 3 years (2008–10), the number of ambulance transports in 2011 peaked on Day 2 (12 March) followed by a gradual decline (see Supplementary material online, *Figure S1*). The rate of definitive diagnosis at admission in the emergency rooms made by attending doctors was 56.7% (16 265/28 709 cases), 56.6% (15 873/28 069 cases), 56.2% (17 217/30 645 cases), and 55.5% (20 400/36 729 cases), respectively. Thus, the rate of definitive diagnosis at admission in the emergency rooms was comparable among the 4 years studied. The prevalence of male sex was also comparable among the 4 years (51.9, 51.3, 51.4, and 51.8%, respectively). The age of all transported patients in 2011 (61.2 ± 25.3 years old) was significantly higher than those in the previous 3 years (57.6 ± 26.5 years old in 2008; 58.3 ± 26.2 years old in 2009; 59.3 ± 26.4 years old in 2010, all $P < 0.001$); however, the age of patients with each disease in 2011 was comparable with that in the previous 3 years (2008–10) (data not shown).

Importantly, the weekly occurrences of the five diseases examined, including HF, ACS, stroke, CPA, and pneumonia, were all significantly increased soon after the Earthquake (*Figure 2A–F*; see Supplementary material online, *Table S1*). The occurrence of CPA was significantly increased after the Earthquake even after excluding the non-cardiopulmonary cases (*Figure 2D* and *E*). Furthermore, in the time-course analyses of daily occurrences, we were able to demonstrate the significant increase in the occurrence of CPA on the day of the Earthquake even after excluding the non-cardiopulmonary cases, while the increased occurrences of other diseases were noted a few days after the Earthquake (*Figure 3A* and *C–F*). Also, the occurrence of ACS did not peak during the first 7 days (*Figure 3B*). In the subanalysis of the patients with stroke, a significant increase in the occurrence was noted only