Because of the westernization of our lifestyle, this aging population will endure more chronic medical conditions, such as cardiovascular disease, dyslipidemia, diabetes mellitus, chronic kidney disease, and the metabolic syndrome (MetS). MetS is a constellation of multiple risk factors, such as central obesity, dyslipidemia, elevated glucose, and elevated blood pressure, in which insulin resistance is the main underlying disorder.[1] Because the degree of insulin resistance increases with age, elderly are at a higher risk to develop cardiometabolic disorders.[2] At the same time, elderly with MetS are supposed to have a higher risk of cardiovascular disease.[3] In elderly, the diagnosis of MetS is also related to a more pronounced cognitive decline and, thus, disability.[4] Therefore, the identification and treatment of patients with MetS would be an important approach to reduce morbidity and impairment in elderly.

In 2000, we conducted lipid survey in various districts in Japan.[5] In this survey, we found that the level of triglyceride increased in middle-aged men along with the increased body mass index (BMI) compared with the data in 1990.[6] However, the BMI did not change in the elderly population in spite of a small increase in triglyceride levels. Although MetS is a risk factor for cardiovascular disease in middle-aged and elderly people and, therefore, a public health problem, it is still unknown whether the same diagnostic criteria can be applied to both groups.

In the last few years, several expert groups have attempted to set forth simple diagnostic criteria to be used in clinical practice to identify patients with MetS. The committee of International Diabetes Federation (IDF) adopted waist circumference as the surrogate marker for central obesity as an essential component of this syndrome,[7] whereas the National Cholesterol Education Program Adult Treatment Panel III (ATP III) criteria required no single factor for diagnosis, but instead, required the presence of at least three out of five components for the diagnosis.[1] In Japan, the committee has established the diagnostic criteria under the same principle as the IDF criteria, except the cutoff for high glucose as 110 mg/dL instead of 100 mg/dL.[8] The cutoff of waist circumference for central obesity was adopted as 85 cm or greater in men and 90 cm or greater in women in Japanese criteria, although the Asian cutoff of waist circumference is 90 cm or greater in men and 80 cm or greater in women. Recently, several groups have shown that the Asian cutoff for the waist circumference is better than that of the Japanese.[9–12] Furthermore, Hata et al.[13] have shown that MetS defined by the Japanese criteria, with the modification of a waist circumference of 90 cm or greater in men and 80 cm or greater in women, is a better predictor of each ischemic stroke subtype in the Japanese population. Therefore, modification of the Japanese criteria for MetS might be necessary in the future.

The purpose of this study was to examine the prevalence of MetS in the Japanese elderly population and to compare the prevalence of MetS and comorbidities with those in the middle-aged population. We also compared the prevalence of MetS by modified ATP III, IDF, and Japanese diagnostic criteria.

2. Methods

2.1. Designs and data collection

The Research Group on Serum Lipid Level Survey 2000 in Japan asked the members of 36 institutes from various areas around Japan to join this survey. The project was designed to produce representative data of serum lipid levels in the civilian Japanese population. The subjects were people receiving annual health examinations in general community, companies, and schools, and not those visiting hospitals. Among the total number of 12,839 participants, we measured the waist circumferences of 3264 people aged 20–79 years (men, 1917 and women, 1357). In this study, we examined the prevalence of MetS in subjects aged 40–79 years (men, 1425 and women, 941) and compared the prevalence of MetS along with each metabolic abnormality according to the Japanese and ATP III criteria. The Ethics Committee in Kyoto University School of Medicine approved this study. Oral informed consent was obtained from all the participants.

2.2. Laboratory methods

All serum and plasma samples were obtained in the fasting state. All lipid and other analyses were conducted on venous blood samples within 1 week of collection at Bio Medical Laboratories (BML) (Saitama, Japan). Serum cholesterol and triglyceride levels were measured by enzymatic assay. High-density-lipoprotein (HDL) and low-density-lipoprotein cholesterols were measured enzymatically by a kit from Daiichi Kagaku Co. Ltd. (Tokyo, Japan). The results of lipid analyses in the four surveys were indirectly standardized according to the criteria of the Centers for Disease Control and Prevention (CDC) Lipid Standardization Program.[14] Thus, the cholesterol levels in these five surveys appear to be comparable. Plasma glucose was determined enzymatically, and hemoglobin A1c (HbA1c) was determined by a kit from Kyowa Medex Co. Ltd. (Tokyo, Japan). Serum insulin was determined by immunoradiometric assay (Abbott Diagnostics Division, Abbot Park, IL). Waist circumference at the umbilical level was measured in the late exhalation phase in standing position.

2.3. Definition of MetS

According to the definition released by ATP III, published in 2008, we analyzed the prevalence of MetS. We modified the criteria by using the Asian cutoff of waist circumference (90 cm for men and 80 cm for women). Other differences are fasting glucose greater than or equal to 100 mg/dL and HDL cholesterol less than 50 mg/dL in women. MetS of modified ATP III criteria was defined as the presence of at least three abnormalities among central obesity, hypertriglyceridemia, low HDL cholesterolemia, high blood pressure, and fasting high glucose. We also analyzed using the Japanese diagnostic criteria of the MetS in 2005, defining MetS as the presence of two or more abnormalities in the presence of central obesity (waist circumference: 85 cm or more in men and 90 cm or more in women). Three abnormalities are as follows: (1) triglycerides greater than or equal to 150 mg/dL and/or HDL cholesterol less than 40 mg/dL or under treatment for this type of dyslipidemia; (2) systolic blood pressure greater than or equal to 130 mmHg and/or diastolic blood pressure greater than or equal to 85 mmHg, or under treatment for high blood pressure; (3) fasting glucose greater than or equal to 110 mg/dL or under treatment for diabetes (Table 1). Furthermore, we used modified IDF criteria for comparison. People treated with lipid-lowering drugs who had normal triglyceride and HDL cholesterol in this study were excluded, because we could not obtain data whether they were treated for hypercholesterolemia or hypertriglyceridemia.

2.4. Data analysis

The results were expressed as mean value ± standard deviation. Differences in means were evaluated by unpaired t test, Mann–Whitney test, or analysis of variance, when appropriate. The categorical variables were compared by chi-square test. The analysis was performed by the Statistical Package for Social Sciences (ver. 11.5; SPSS Japan Inc., Tokyo, Japan). A p value of 0.05 or less was considered to indicate a statistically significant difference.

Table 1
Comparison among Japanese, modified IDF, and modified ATP III criteria for metabolic syndrome

Definition of metabolic syndrome	Japanese (1) + any 2 or more of (2)–(4)	Modified ATP III for Asians 3 or more of (1)–(5)	Modified IDF for Asians (1) + any 2 or more of (2)–(5)
Components			
Central obesity (waist circumference)	(1) ≥85 cm (men), ≥90 cm (women)	(1) ≥90 cm (men), ≥80 cm (women)	(1) ≥90 cm (men), ≥80 cm (women)
High blood pressure	(2) ≥130/85 mmHg and/or antihypertensive medication	(2) ≥130/85 mmHg and/or antihypertensive medication	(2) ≥130/85 mmHg and/or antihypertensive medication
Fasting high glucose	(3) ≥110 mg/dL and/or antidiabetic medication	(3) ≥100 mg/dL and/or antidiabetic medication	(3) ≥100 mg/dL and/or antidiabetic medication
Dyslipidemia	(4) Triglyceride ≥150 mg/dL and/or HDL-C <40 mg/dL	(4) Triglyceride ≥150 mg/dL	(4) Triglyceride ≥150 mg/dL
		(5) HDL-C <40 mg/dL (men), <50 mg/dL (women)	(5) HDL-C <40 mg/dL (men), <50 mg/dL (women)

IDF = International Diabetes Federation; ATP III = Adult Treatment Panel III; HDL-C = high-density-lipoprotein cholesterol.

3. Results

Table 2 shows the prevalence of MetS in Japanese middle-aged and elderly men and women according to the Japanese and modified ATP III and IDF criteria. According to the Japanese criteria, the prevalence of MetS was higher in both elderly men and women (13.3% *vs.* 18.9% in men and 1.5% *vs.* 4.8% in women). In women, the prevalence of MetS was almost three fold higher in the elderly than that in the middle-aged group ($p < 0.01$ by chi-square test). When we apply the modified ATP III and IDF criteria, the prevalence of MetS was also increased in women of each group ($p < 0.01$ in ATP III and IDF criteria by chi-square test), and the three fold increase in MetS in elderly women was consistent among the three criteria. The increase of MetS prevalence in women by modified ATP III and IDF criteria compared with that by the Japanese criteria was also statistically significant ($p < 0.01$). Intriguingly, when we used modified IDF criteria, the prevalence of MetS in middle-aged and elderly men was similar to that using the Japanese criteria. However, the prevalence of MetS in women by modified IDF criteria was similar to that by modified ATP III criteria.

To assess the effect of aging on each metabolic component, we compared the prevalence of central obesity, dyslipidemia, high blood pressure, high fasting glucose, and MetS in each age group according to the Japanese and modified ATP III criteria. In men, the prevalence of MetS was similar in each age group; yet, more people satisfied the modified ATP III criteria than the Japanese criteria (Table 3). In women, the prevalence of MetS was about 5% in the elderly, and almost no subjects were diagnosed with MetS less than 65 years old by the Japanese criteria. According to the modified ATP III criteria, the prevalence of MetS in women also increased in their 60s and was almost the same as that of men older than 65 years. We found a big difference in the prevalence of central obesity diagnosed by Japanese and Asian criteria for waist circumference in both genders. Thus, it is critical which cutoff is used to diagnose MetS.

The prevalence of central obesity in men was almost constant according to the Japanese or Asian criteria of waist circumference, although the prevalence seemed to be decreased in their 70s. The prevalence of central obesity in women increased toward menopause and remained almost the same after their 50s. However, when we used the Asian criteria, the prevalence of central obesity in women further increased in their 70s. The prevalence of dyslipidemia was almost constant in men among each age group and increased toward menopause in women. However, the prevalence reached a plateau after 55 years of age. As expected, the prevalence of dyslipidemia was higher in women according to the ATP III criteria than that by the Japanese criteria. The prevalence of high blood pressure increased with age both in men and in women. Intriguingly, the prevalence of high blood pressure did not show further increase after 60 years of age in both genders. The prevalence of high fasting glucose increased after 50 years of age in men and after 65 years of age in women. Thus, the prevalence of MetS and related components are associated with age, especially with menopause in women. We also compared the number of the MetS traits by dividing the cohort into three groups: from 40 to 49 years (young middle age); from 50 to 64 years (old middle age); and from 65 to 79 years (elderly). As shown in Figure 1, the prevalence of the subjects with indicated numbers of MetS components according to the modified ATP III criteria is quite similar in men of all age groups. However, in older women, the number of MetS components increased, which is consistent with the data in Table 2.

Next, we compared the demographic characteristics of men and women diagnosed with MetS by the modified ATP III criteria. As shown in Table 4, age and total, HDL, and low-density-lipoprotein cholesterol were higher, and waist circumference, triglyceride, diastolic blood pressure, remnant-like particle (RLP) cholesterol, and fasting glucose were lower in women than in men.

We then compared the demographic characteristics of elderly and middle-aged men and women with MetS by modified ATP III criteria. As shown in Table 5, systolic blood pressure was higher in older-middle-aged and elderly than in younger-middle-aged group, and diastolic blood pressure was higher in older middle-aged group in both genders. HDL cholesterol was lower in younger-middle-aged men and non-HDL cholesterol was lower in elderly men. Triglyceride was lower in elderly men and women. Insulin levels decreased according to age in men. There were no statistical differences in the other components.

Table 2
Prevalence of metabolic syndrome in middle-aged and elderly Japanese

%	Male (1425)		p	Female (941)		p
	40–64 yr (1266)	65–79 yr (159)		40–64 yr (732)	65–79 yr (209)	
Japanese criteria	13.3	18.9	NS	1.5	4.8	<0.01
Modified Adult Treatment Panel III	19.0	21.4	NS	9.0	26.8	<0.01
Modified International Diabetes Federation	14.0	14.8	NS	8.2	23.9	<0.01

The numbers in parentheses indicate the number of subjects in each group. p Value, 40–64 yr *vs.* 65–79 yr.
NS = not significant.

Table 3
Prevalence of metabolic syndrome and metabolic components according to the Japanese and modified ATP III criteria in each age group in the Japanese population (%)

Criteria and metabolic components	Sex															
	Male (age group, yr), %								Female (age group, yr), %							
	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79
n	291	289	359	191	136	67	62	30	171	123	185	108	145	93	75	41
Japanese criteria																
Central obesity	49.1	56.4	54.0	57.1	55.1	61.2	48.4	43.3	5.3	11.4	16.8	16.7	11.7	16.1	14.7	17.1
Hypertriglyceridemia	33.7	43.6	31.2	28.8	33.1	25.4	25.8	13.3	6.4	7.3	15.1	22.2	20.7	20.4	18.7	14.6
Low HDL cholesterolemia	11.3	14.2	12.3	13.1	12.5	9.0	9.7	13.3	1.8	4.1	2.2	2.8	2.8	6.5	2.7	4.9
Dyslipidemia	36.4	46.0	34.5	33.5	36.0	31.3	30.6	20.0	7.6	8.9	15.1	22.2	22.1	21.5	20.0	19.5
High blood pressure	16.2	20.8	28.7	27.2	49.3	41.8	43.5	40.0	8.8	8.9	17.8	19.4	49.7	49.5	40.0	43.9
High fasting glucose	11.3	15.6	19.5	23.6	22.8	22.4	25.8	16.7	1.8	4.9	3.8	10.2	9.7	15.1	16.0	14.6
Metabolic syndrome	9.6	15.2	14.2	12.0	16.2	22.4	17.7	13.3	0.0	0.8	2.2	3.7	1.4	5.4	4.0	4.9
Modified ATP III criteria																
Central obesity	23.7	34.6	30.1	26.7	30.9	38.8	22.6	23.3	18.7	32.5	31.4	47.2	36.6	48.4	60.0	63.4
Hypertriglyceridemia	33.7	43.6	31.2	28.8	33.1	25.4	25.8	13.3	6.4	7.3	15.1	22.2	20.7	20.4	18.7	14.6
Low HDL cholesterolemia	11.3	14.2	12.3	13.1	12.5	9.0	9.7	13.3	8.2	15.4	15.7	12.0	17.9	23.7	24.0	19.5
Dyslipidemia	36.4	46.0	34.5	33.5	36.0	31.3	30.6	20.0	12.9	17.9	22.7	26.9	29.7	33.3	32.0	31.7
High blood pressure	16.2	20.8	28.7	27.2	49.3	41.8	43.5	40.0	8.8	8.9	17.8	19.4	49.7	49.5	40.0	43.9
High fasting glucose	34.7	38.4	43.2	44.0	39.7	41.8	40.3	36.7	8.8	15.4	14.6	18.5	24.8	29.0	33.3	26.8
Metabolic syndrome	14.1	22.8	19.2	16.2	25.0	22.4	19.4	23.3	1.8	5.7	9.2	13.9	16.6	26.9	30.7	19.5

ATP III = Adult Treatment Panel III; HDL = high-density lipoprotein.

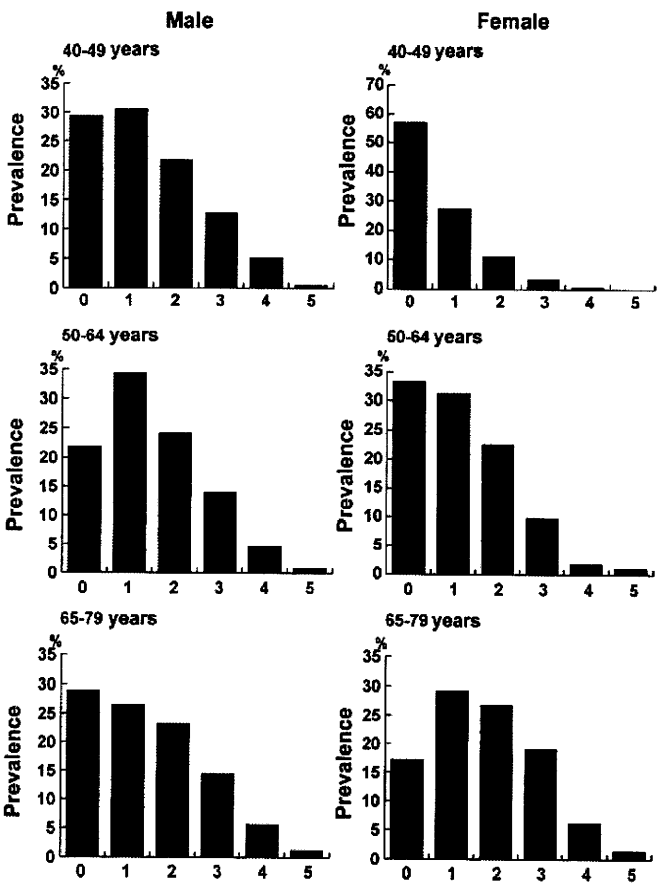

Fig. 1. Prevalence of the subjects with indicated numbers of metabolic syndrome components according to modified Adult Treatment Panel III criteria (central obesity, hypertriglyceridemia, low high-density-lipoprotein cholesterolemia, high blood pressure, high fasting glucose) in each age group of both genders.

Finally, we compared the prevalence of central obesity and other components among the people who satisfied the modified ATP III criteria according to their age in both genders. As shown in Table 6, the prevalence of hypertriglyceridemia and dyslipidemia in men was lower in the elderly, whereas the prevalence of high blood pressure increased in older-middle-aged and elderly men. In women, there was a tendency that the prevalence of hypertriglyceridemia was lower in elderly and that of high blood pressure increased according to age.

4. Discussion

In this study, we compared the prevalence of MetS in the Japanese middle-aged and elderly population by the Japanese and

Table 4
Demographic characteristics of men and women with metabolic syndrome

	Male ($n = 467$)		Female ($n = 201$)		*p*
	Mean	SD	Mean	SD	
Age, yr	53.4	8.8	61.9	9.4	<0.01
Body mass index	25.9	3.0	26.3	2.9	0.27
Waist circumference, cm	92.1	6.7	86.2	8.0	<0.01
Systolic blood pressure, mmHg	136	18	135	18	0.69
Diastolic blood pressure, mmHg	84	12.6	81	9.9	0.02
T-Cho, mg/dL	214	35.4	224	33.6	0.01
TG, mg/dL	197	155, 268	159	115, 194	<0.01
HDL-C, mg/dL	45.7	12.2	51.7	11.0	<0.01
Low-density-lipoprotein cholesterol, mg/dL	126	34.0	140	30.0	<0.01
Non-HDL-C, mg/dL	168	35.8	173	32.5	0.24
RLP-C, mg/dL	6.6	4.2, 10.7	4.3	3.2, 8.5	0.02
HbA1c, %	5.3	0.77	5.4	0.82	0.15
FBS, mg/dL	108	26	100	18.4	0.01
Insulin, μU/mL	8.3	4.7	8.0	4.4	0.52

TG and RLP-C are expressed as median (interquartile range). The difference was analyzed by unpaired *t* test except for TG and RLP-C. Mann–Whitney test was used for TG and RLP-C.
SD = standard deviation; HDL-C = high-density-lipoprotein cholesterol; T-cho = total cholesterol; TG = triglyceride; RLP-C = remnant-like particle cholesterol; HbA1c= hemoglobin A1c; FBS = fasting blood sugar.

Table 5
Demographic data of subjects with metabolic syndrome in each age group

	Sex	40–49 yr (M, 195; F, 27)		50–64 yr (M, 223; F, 102)		65–79 yr (M, 49; F, 72)		p
		Mean	SD	Mean	SD	Mean	SD	
Body mass index	M	26.1	3.0	25.9	3.1	25.5	2.5	0.55
	F	26.7	2.1	26.3	3.1	26.1	2.7	0.78
Waist circumference, cm	M	92.2	7.0	91.9	6.8	92.2	5.8	0.96
	F	87.9	8.7	85.6	9.1	86.3	6.7	0.65
Systolic blood pressure, mmHg	M	130	17.6	138	18.1	143	17.7	<0.01
	F	125	18.0	140	19.4	132	14.6	0.01
Diastolic blood pressure, mmHg	M	82	13.1	86	12.5	82	9.4	0.02
	F	80	11.2	84	10.9	79	7.8	0.04
T-Cho, mg/dL	M	219	34.9	212	35.6	203	33.6	0.06
	F	213	28.8	230	33.2	220	34.3	0.13
TG, mg/dL	M	272	154	234	242	171	71	0.03
	F	169	79.7	189	89.6	144	57.1	0.01
HDL-C, mg/dL	M	43.1	8.3	47.2	13.7	47.9	14.9	0.02
	F	48.3	9.6	52.9	11.5	51.4	10.7	0.38
Low-density-lipoprotein cholesterol, mg/dL	M	128	34.8	126	34.2	121	31.6	0.62
	F	131	26.2	142	28.9	140	31.1	0.51
Non-HDL-C, mg/dL	M	176	34.8	165	36.4	155	32.3	0.01
	F	165	33.5	178	31.0	169	33.6	0.26
HbA1c, %	M	5.1	0.7	5.4	0.9	5.4	0.6	0.14
	F	5.3	0.7	5.3	1.0	5.5	0.7	0.63
FBS, mg/dL	M	112	24.3	113	25.2	108	21.5	0.58
	F	100	13.6	106	22.5	106	19.7	0.61
Insulin, mU/mL	M	9.4	5.9	7.8	3.8	7.2	3.4	0.01
	F	7.9	2.0	8.1	3.6	8.0	5.6	0.98

p Value was analyzed by analysis of variance.
M = male; F = female; HDL-C = high-density-lipoprotein cholesterol; T-cho = total cholesterol; TG = triglyceride; RLP-C = remnant-like particle cholesterol; HbA1c= hemoglobin A1c; FBS = fasting blood sugar.

modified ATP III and IDF criteria. We showed that the prevalence of MetS was almost three fold higher by all the three criteria in elderly women than in middle-aged women, whereas there was almost no difference between middle-aged and elderly men. Consistent with our findings that the prevalence of MetS increased in elderly women compared with that in middle-aged population, other studies have also shown that the prevalence of MetS increases with increasing age.[15] Ford et al.[15] reported that the prevalence of MetS in subjects older than 60 years is approximately 40% in the Third Report of the National Cholesterol Education Program Expert Panel, in which they used the cutoff of 110 mg/dL for high fasting glucose and their criteria of waist circumference for central obesity. The prevalence of MetS in middle-aged population is approximately 25% in both genders, which is different from the result in our cohort. In Japan, Ishizaka et al.[16] and Aizawa et al.[17] have shown that the prevalence of MetS in men is approximately 20% in both middle-aged and elderly populations, whereas that in women is approximately 5% and 10% in middle-aged and elderly populations, respectively, although they used the original ATP III criteria and BMI instead of waist circumference. Tanaka et al.[18] also showed that the prevalence of MetS in Okinawa, a group of islands located in southwest of Japan, is approximately 30% in middle-aged and elderly men and 10% and 20% in middle-aged and elderly women, respectively, when they use ATP III criteria with the Japanese cutoff of waist circumference. Thus, the higher prevalence of MetS in middle-aged and elderly men than that in women is consistent in Japanese cohorts, although the prevalence is different with each diagnostic criterion.

Table 6
Prevalence of each metabolic abnormality in each age group in the subjects with metabolic syndrome

	Sex	Age group (yr)			p
		40–49	50–64	65–79	
Central obesity	M	74.5	72.1	82.4	0.47
	F	92.3	82.8	89.3	0.48
Hypertriglyceridemia	M	92.5	72.9	58.8	<0.01
	F	61.5	69	48.2	0.08
Low HDL cholesterolemia	M	39.6	34.3	32.4	0.61
	F	69.2	46.6	55.4	0.29
Dyslipidemia	M	94.3	77.9	73.5	<0.01
	F	76.9	79.3	71.4	0.62
High blood pressure	M	50.9	70.0	85.3	<0.01
	F	46.2	67.2	76.8	0.09
High fasting glucose	M	79.3	82.1	79.4	0.75
	F	46.2	65.5	64.3	0.41

The difference was analyzed by chi-square test.
M = male; F = female.

Among the metabolic components, the prevalence of central obesity and dyslipidemia increased with aging only in women, and that of high fasting glucose and high blood pressure increased in both genders (Table 3). The prevalence of dyslipidemia decreased in elderly men. Thus, middle-aged men tend to be more dyslipidemic, whereas elderly population tends to have a higher prevalence of central obesity, impaired glucose metabolism, and high blood pressure. Among the subjects diagnosed with MetS by modified ATP III criteria, systolic blood pressure increased with aging in both genders, whereas triglyceride and insulin decreased with aging in both genders, and insulin levels decreased with aging only in men (Table 5). The increased prevalence of high blood pressure in older middle-aged and elderly population is also confirmed in Table 6, although the *p* value was not statistically significant in women. Thus, blood pressure seems to have the strongest association with aging in both genders. The prevalence of central obesity did not increase with aging in the female subjects with MetS (Tables 5 and 6), whereas the prevalence of central obesity increased in female general population, as shown in Table 3. Thus, central obesity in women seems to be affected by aging, which is consistent with the results of other studies in Japan.[16,18] In men, the insulin levels decreased in the elderly in spite of the fact that FBS and HbA1c were not changed among the three groups, suggesting impaired insulin secretion in elderly men with MetS.

In this study, we used the Japanese and modified ATP III and IDF definitions to determine MetS. However, there was a large difference in the prevalence of MetS among the three definitions. This difference

is because of the fact that the Japanese definition requires the central obesity for its diagnosis as in the modified IDF criteria and has more stringent criteria for high fasting glucose for both genders and for HDL cholesterol for women. As shown in this study, the prevalence of MetS in elderly women by the Japanese criteria was very low. This is the reason why we used various analyses using modified ATP III criteria in this cohort. However, the Japanese guideline for MetS was established to identify patients with central obesity, who can reduce the risks by weight loss, whereas the ATP III criteria try to identify patients with multiple risk factors. Therefore, the Japanese criteria should be used to identify obese patients who can have a benefit by weight loss in middle-aged and elderly populations. However, in terms of risk prediction, there have been several reports discussing the cutoff levels of MetS components. Hata et al.[13] have shown significant associations between MetS defined by various criteria and the risk of ischemic stroke in the Hisayama study. In the study, they found that MetS was an independent risk factor for ischemic stroke when they used the modified Japanese criteria with Asian definition of central obesity. Another study from the same group showed that the optimal cutoff level of waist circumference to predict cardiovascular disease was 90 cm in men and 80 cm in women,[19] as we used in modified ATP III definition in this study. Sone et al.[11] also proposed to use the Asian cutoff for waist circumference to define central obesity from the data of Japan Diabetes Complication Study. In terms of the appropriate cutoff level of HDL cholesterol for the definition of MetS in Japanese women, not so many analyses have been done. In our study, the prevalence of low HDL cholesterolemia with the cutoff of 40 mg/dL was less than 5% and was approximately 20% with the cutoff of 50 mg/dL in elderly women. We previously showed that central obesity was significantly associated with low HDL cholesterolemia only when we used the cutoff of 50 mg/dL for women.[5] Therefore, further study is necessary to determine the appropriate cutoff level of HDL cholesterol in women.

In summary, we have shown the prevalence of MetS in Japanese elderly and middle-aged population using Japanese and modified ATP III and IDF criteria, and found the effect of aging on the prevalence only in women with either criterion. We also showed the effect of aging on each metabolic component in this cohort. Thus, aging is an important factor that affects the metabolic abnormality, and aging of the population would lead to the increase in the prevalence of MetS. Therefore, the development of better approaches to the prevention and management of MetS is necessary for successful aging in our society.

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Original Article

More Intensive Lipid Lowering is Associated with Regression of Coronary Atherosclerosis in Diabetic Patients with Acute Coronary Syndrome - Sub-Analysis of JAPAN-ACS Study

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Aim: We have shown that aggressive lipid lowering by pitavastatin and atorvastatin results in marked regression of atherosclerotic coronary lesions after acute coronary syndrome (ACS). The purpose of this study was to address the association of lipid levels after statin therapy with regression of atherosclerotic coronary lesions and major cardiovascular events in patients after ACS.
Methods: JAPAN-ACS is a prospective, randomized open-label study performed at 33 centers in Japan. Patients with ACS undergoing intravascular ultrasound (IVUS)-guided percutaneous coronary intervention (PCI) were randomly assigned to receive either 4 mg/day pitavastatin or 20 mg/day atorvastatin within 72 hours after PCI. IVUS image was obtained in 251 patients, including 73 diabetic patients. Lipid profiles at the end of the study were divided into quartiles and the association with the percent change in non-culprit coronary plaque volume (PV) was assessed in total and diabetic patients. We also studied whether baseline and follow-up levels of HDL-cholesterol are associated with restenosis after PCI.
Results: Decreasing LDL-cholesterol, non-HDL-cholesterol, LDL-C/HDL-C ratio, apolipoprotein B quartiles were associated with a progressively smaller plaque burden in total and diabetic patients. In diabetic patients, further reduction of these parameters was associated with a significantly greater reduction in PV. We also found that patients with lower HDL-cholesterol had a significantly higher incidence of target lesion revascularization.
Conclusions: Early intensive statin therapy in patients after ACS results in remarkable regression of coronary PV. Diabetic patients can have a benefit with more intensive therapy to achieve a lower target level in Japanese.

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Key words; Acute coronary syndrome, Plaque, Statin, Intravascular ultrasound, Diabetes mellitus

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Introduction

Accumulating evidence indicates that statins can reduce both cardiovascular morbidity and mortality in primary and secondary prevention, including patients

with acute coronary syndrome (ACS)[1-3]. Lowering LDL-cholesterol to even lower levels is associated with a further reduction in cardiovascular risk, as shown in the Treatment to New Target (TNT) Study[4] and in the Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) Study of secondary prevention[5]. These studies support the hypothesis that a lower LDL-cholesterol level can induce a greater risk reduction, at least in secondary prevention.

Moreover, many studies with surrogate endpoints show improvement of atherosclerosis by aggressively lowering LDL-cholesterol. Studies using intravascular ultrasound (IVUS) imaging demonstrate that statins attenuate the progression of atherosclerosis or even enable regression of atheromatous plaque[6, 7]. An IVUS study of patients with ACS also demonstrated that atorvastatin can reduce non-culprit coronary plaque in Japanese[8]; however, this was a relatively small trial conducted at a single center. Therefore, a larger multicenter study is expected to address the further roles of statins in patients with ACS.

We previously reported the results of the JAPAN-ACS (Japan Assessment of Pitavastatin and Atorvastatin in Acute Coronary Syndrome) study to address the role of statins in patients with ACS[9]. The JAPAN-ACS was performed as a prospective, randomized open-label parallel-group study with a blind endpoint evaluation at 33 centers, to comparatively examine the effect of 8- to 12-month treatment with pitavastatin and atorvastatin on the degree of coronary plaque regression in non-culprit lesions of the culprit vessel treated by PCI in patients with ACS. This study demonstrated the non-inferiority of pitavastatin 4 mg/day to atorvastatin 20 mg/day, with approximately 17% regression of the plaque volume (PV), suggesting that the effect of inducing plaque regression can be generalized to other statins with similar LDL-lowering effects with atorvastatin. However, in this study, diabetic patients showed less regression of coronary atheroma than non-diabetic patients in spite of similar LDL-cholesterol reduction by statins. In the sub-analysis of JAPAN-ACS we showed significant correlations between the percent change in PV and percent change of the LDL-cholesterol level or follow-up LDL-cholesterol level in diabetic patients[10]; however, a question remains whether there is an appropriate target lipid level to obtain the maximum effect on plaque regression. Therefore, in this sub-analysis of JAPAN-ACS we examined the association of lipid levels after statin therapy with the regression of atherosclerotic coronary lesions in diabetic and total patients after ACS. This analysis was performed in the entire patient population, using the full analysis set of the JAPAN-ACS study, as the regressive effect of the two statins was shown to be equivalent.

In this study we also asked whether baseline and follow-up levels of HDL-cholesterol are associated with restenosis or other cardiovascular events after PCI to show the effect of low HDL-cholesterolemia on coronary events, because previous studies have shown that low levels of HDL-cholesterol can predict major cardiovascular events[11-13].

Methods

Study Design

The present study is a post-hoc sub-analysis of the JAPAN-ACS (Japan Assessment of Pitavastatin and Atorvastatin in Acute Coronary Syndrome) study. JAPAN-ACS is a prospective, randomized open-label parallel group study with blind endpoint evaluation at 33 centers to examine the effect of 8–12 months treatment with pitavastatin versus atorvastatin in coronary plaque regression in non-percutaneous coronary intervention (PCI) sites of the culprit vessel in patients with ACS. The details of the study design have been reported previously[9, 14]. In brief, ACS patients selected in this study were over 20 years of age with hypercholesterolemia and had undergone successful PCI under IVUS guidance. They were found to have coronary plaques (more than 500 μm in thickness, or percent plaque area $\geq$20%) in the culprit vessel at least 5 mm from the PCI- treated lesions. ACS was defined as unstable angina pectoris, non-ST-elevation myocardial infarction (MI) or ST-elevation MI. The diagnosis of ACS was made based on the fulfillment of at least two of the following three criteria: 1) evidence of coronary ischemia on ECG, 2) increase ($\geq$2 times) in the serum creatinine phosphokinase (CK) or CK-MB levels, and/or troponin-T positivity, 3) presence of symptoms suggestive of ACS. Diabetes mellitus and other complications were diagnosed by the attending physicians. This study was conducted according to the 'Declaration of Helsinki', and with the approval of the institutional review boards of all 33 participating institutions. Written informed consent to participate was obtained from all of the patients enrolled.

Intravascular Ultrasound Procedure and Examination

Details of the intravascular ultrasound (IVUS) procedure and examination are documented elsewhere[9]. In brief, following IVUS-guided PCI for the culprit lesion in the patients with ACS, a final IVUS examination for analysis was performed in the culprit vessel. The IVUS catheter Atlantis SR Pro2 (Boston

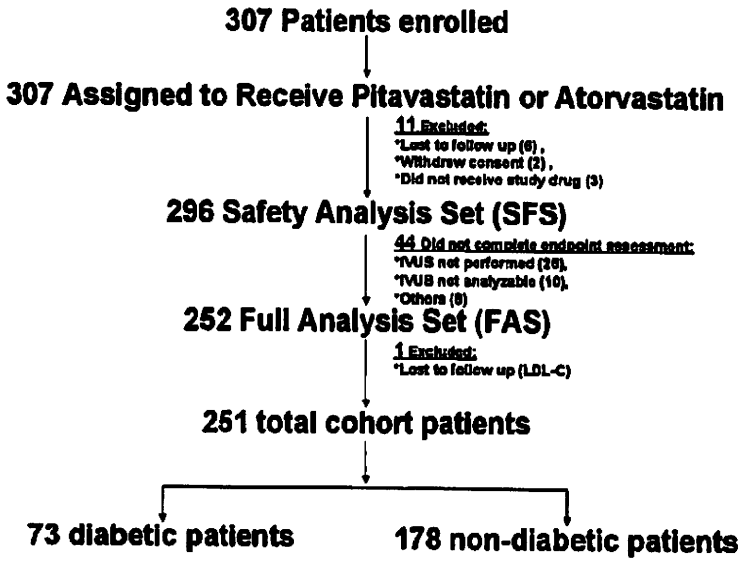

Fig. 1. Characteristics of Patients in JAPAN-ACS.

Scientific, Natik, USA) was used, and a motorized pullback device withdrew the transducer at 0.5 mm/sec. The consoles used were the ClearView or Galaxy 2 system (Boston Scientific, Natik, USA). The same imaging system with the same type of IVUS catheter was used for both the baseline and follow-up examinations

Two independent experienced investigators performed the quantitative IVUS analysis at the central laboratory. The target segment for analysis was identified as a non-PCI site of the culprit vessel (>5 mm proximal or distal to the PCI site) based on reproducible indices. Manual tracing was performed in every 0.1 mm cross-section and the software (echoPlaque2; INDEC systems Inc., Santa Clara, USA) automatically interpolated the tracings of 5 cross-sections between two manually traced images; therefore, the volume was calculated from each of the 0.017 mm-interval segments.

Blood Examination

Blood examinations for lipid levels were performed at baseline and 8–12 months follow-up. Lipid profiles were measured at SRL Co, Ltd. (Tokyo, Japan).

Statistical Analysis

We used the full analysis set (FAS) of data for primary analyses. Patient data were included in FAS if patients had ACS and measurable IVUS both at enrollment and follow-up. Because non-inferiority was shown between pitavastatin and atorvastatin, we combined the data of both groups and performed this subanalysis. LDL-cholesterol, non-HDL-cholesterol, LDL-C/HDL-C ratio, and apolipoprotein B (apo B) at the end of the study were divided into quartiles and the percentage change in PV in each quartile was compared in total and diabetic patients by ANOVA or t test when appropriate. The chi-square test was used for categorical variables. We also analyzed the association of baseline and follow-up HDL-cholesterol (over or equal to 40 mg/dL or less than 40 mg/dL) with the rate of restenosis in this cohort. The significance level was 5% two-sided (2.5% one-sided) and all statistical analyses were performed using the SAS System Release 9.1 (SAS institute, Cary, USA).

Results

Patient Population

The characterstics of patients in the present study are shown in **Fig. 1**. Between November 1, 2005 and October 31, 2006, 307 patients were enrolled at 33 centers in Japan, and 153 patients were randomly assigned to receive pitavastatin and 154 to atorvastatin. IVUS images qualifying for evaluation both at baseline and follow-up were obtained in 125 patients (82%) in the pitavastatin group and in 127 patients (82%) in the atorvastatin group. The median follow-up time with intraquartile range in the pitavastatin group was 9.3 (8.5–10.3) months and 9.6 (8.6–10.5) months in the atorvastatin group, respectively.

The details of baseline demographics and characteristics in this study have been reported elsewhere[9]. Among 251 total cohorts, 73 patients were diabetic.

There were no differences in the percent change of PV and LDL-cholesterol reduction between pitavastatin and atorvastatin groups in patients with or without diabetes. In diabetic patients, the percent change of PV was $-13.7 \pm 15.1\%$ ($p<0.001$, from baseline) in the pitavastatin group ($n=36$) and $-12.0 \pm 13.9\%$ ($p<0.001$, from baseline) in the atorvastatin group ($n=38$) ($p=0.7$, pitavastatin vs. atorvastatin). The percent change of PV was $-18.1 \pm 13.2\%$ ($p<0.001$, from baseline) in the pitavastatin group ($n=89$) and $-20.7 \pm 13.6\%$ ($p<0.001$, from baseline) in the atorvastatin group ($n=89$) in non-diabetic patients ($p=0.2$, pitavastatin vs. atorvastatin), while in diabetic patients, the percent change of LDL-cholesterol was $-35.7 \pm 21.1\%$ ($p<0.001$, from baseline) in the pitavastatin group ($n=35$) and $-37.6 \pm 22.6\%$ ($p<0.001$, from baseline) in the atorvastatin group ($n=38$) ($p=0.7$, pitavastatin vs. atorvastatin). The percent change of LDL-cholesterol was $-36.4 \pm 19.0\%$ ($p<0.001$, from baseline) in the pitavastatin ($n=89$) group and $-34.9 \pm 23.1\%$ ($p<0.001$, from baseline) in the atorvastatin group ($n=87$) in non-diabetic patients ($p=0.7$, pitavastatin vs. atorvastatin).

Table 1. Median and interquartiles of lipid profiles in total and diabetic patients

total cohort ($n=251$)			
Quartile	25%	50%	75%
LDL-C (mg/dL)	66	79	98
non HDL-C (mg/dL)	84	99	124
Apo B (mg/dL)	60	72	86
LDL-C/HDL-C	1.32	1.77	2.23
diabetic patients ($n=73$)			
Quartile	25%	50%	75%
LDL-C (mg/dL)	56.5	75	101.5
non HDL-C (mg/dL)	82	95	125.5
Apo B (mg/dL)	57	70	88
LDL-C/HDL-C	1.14	1.75	2.37

Association of Percent Change in Plaque Volume with Quartiles of Lipid Parameters

Table 1 shows 25th and 75th percentiles and medians in each lipid parameter in total and diabetic populations. According to these numbers we divided the total and diabetic patients into quartiles and compared the percent change of PV in each group (**Table 2**). Decreasing LDL-cholesterol, non-HDL-cholesterol, apo B, and LDL-C/HDL-C ratio quartiles were associated with a progressively larger percent change of PV

Table 2. Association of % change in plaque volume with quartile of lipid parameters

		quartile at follow up	1st	2nd	3rd	4th	p value
LDL-C	total	mean (range) [mg/dL]	53.2 (<66)	71.4 (66-79)	87.7 (79-98)	117.2 (98<)	
		% change in plaque volume (SD) [%]	−15.4 (12.7)	−20.3 (14.4)	−20 (13.0)	−14.2 (15.2)	0.03
	DM	mean (range) [mg/dL]	48.7 (<56.5)	69.1 (56.5-75)	88.6 (75-101.5)	119.4 (101.5<)	
		% change in plaque volume (SD) [%]	−16.5 (13.6)	−16.9 (14.5)	−10.6 (13.0)	−6.7 (15.2)	0.1
nonHDL-C	total	mean (range) [mg/dL]	70.0 (<84)	90.1 (84-99)	109.6 (99-124)	143.9 (124<)	
		% change in plaque volume (SD) [%]	−15.6 (12.8)	−18.5 (12.7)	−21.4 (14.0)	−14.0 (15.6)	0.01
	DM	mean (range) [mg/dL]	63.9 (<82)	87.4 (82-95)	111.6 (95-125.5)	149.9 (125.5<)	
		% change in plaque volume (SD) [%]	−16.2 (14.0)	−15.3 (14.6)	−12.4 (13.3)	−6.9 (15.4)	0.2
apoB	total	mean (range) [mg/dL]	50.5 (<60)	65.6 (60-72)	78.5 (72-86)	99.2 (86<)	
		% change in plaque volume (SD) [%]	−16.1 (12.4)	−19.2 (15.2)	−21.3 (11.7)	−13.2 (15.6)	0.006
	DM	mean (range) [mg/dL]	47.4 (<57)	62.6 (57-70)	78.8 (70-88)	99.6 (88<)	
		% change in plaque volume (SD) [%]	−16.3 (13.2)	−15.9 (14.3)	−14.6 (11.4)	−5.3 (15.9)	0.049
LDL-C/HDL-C	total	mean (range) [mg/dL]	1.02 (<1.32)	1.54 (1.32-1.77)	1.95 (1.77-2.23)	2.75 (2.23<)	
		% change in plaque volume (SD) [%]	−16.7 (14.5)	−19.5 (14.1)	−20.1 (13.1)	−13.6 (13.8)	0.03
	DM	mean (range) [mg/dL]	0.86 (<1.14)	1.48 (1.14-1.75)	1.95 (1.75-2.37)	2.86 (2.37<)	
		% change in plaque volume (SD) [%]	−18.6 (14.8)	−13.7 (14.6)	−14.2 (12.6)	−4.1 (13.0)	0.02

Table 3. Baseline characteristics of total cohort with quartiles of follow up LDL-cholesterol

Characteristic	1st (n=62)	2nd (n=62)	3rd (n=64)	4th (n=63)	p value
Age (years)	66.4±9.9	61.4±11.0	61.6±10.6	60.4±12.1	0.01
Male (%)	81	92	81	73	0.042
BMI (kg/m^2)	24.3±3.5	24.7±3.3	23.9±3.6	24.5±3.7	0.6
Waist circumference (cm)	86.7±8.6	88.1±7.7	86.3±9.0	87.2±10.6	0.7
Diabetes (%)	35	27	20	33	0.2
Hypertension (%)	65	69	59	57	0.5
Family history of CAD (%)	23	19	16	14	0.6
Smoking (%)	34	50	58	46	0.054
Alcohol drinker (%)	68	52	38	35	0.001
Culprit vessel (%)					
RCA	31	21	42	33	0.1
LAD	53	68	48	48	
LCx	16	11	8	19	
LMT	0	0	2	0	
BMS (%)	63	69	67	63	0.6
DES (%)	37	27	30	33	
Other than stent (POBA) (%)	0	3	3	3	
TC (mg/dL)	184.3±30.1	184.8±27.7	203.7±30.4	216.7±43.5	<0.0001
LDL-C (mg/dL)	117.2±27.7	122.2±23.9	138.3±27.3	152.2±37.3	<0.0001
TG (mg/dL)	106.0 (72.5, 139.8)	111.5 (67.0, 141.3)	120.0 (76.8,157.8)	106.0 (80.5, 154.5)	0.4[a]
HDL-C (mg/dL)	46.1±9.1	43.0±9.7	44.4±9.9	44.2±10.3	0.3
non-HDL-C (mg/dL)	137.3±27.9	142.0±26.0	159.3±29.2	171.4±38.4	<0.0001
LDL-C/HDL-C	2.6±0.8	3.0±0.8	3.3±0.9	3.5±0.8	<0.0001
Apo A-I (mg/dL)	116.3±20.0	106.8±17.3	111.2±18.9	109.3±20.6	0.047
Apo B (mg/dL)	92.6±20.4	97.6±18.3	110.6±20.1	116.0±26.2	<0.0001
Apo E (mg/dL)	4.3±1.4	4.0±1.1	4.1±1.0	4.3±1.1	0.4
Apo B/Apo A-I	0.82±0.22	0.93±0.22	1.02±0.23	1.08±0.25	<0.0001

TG is expressed as median and interquartile range, [a]: Wilcoxon/Kruskal-Wallis test

in total and diabetic patients. The difference was significant in all parameters of the total cohort, while the difference was significant only in apo B and LDL-C/HDL-C in diabetic patients. We also analyzed baseline demographics of each quartile according to follow-up LDL-cholesterol (**Table 3**). The mean age and the prevalence of alcohol drinkers were higher in the first quartile than the other quartiles, which might affect less PV change in the first quartile. Total cholesterol, LDL-cholesterol, non-HDL-cholesterol, apo B, and apo B/apo AI were higher in the third and fourth quartiles than the others.

Because we noticed a smaller percent change of PV in the fourth quartile than the others, we compared the percent change of PV between the combined data from the first to third quartiles and the fourth quartile in each lipid parameter (**Fig. 2**). There was a significant difference between the two groups by t test in all the lipid parameters, indicating that the fourth quartile had less plaque regression than the others.

Next, we compared the percent change of PV in diabetic patients. The baseline characteristics of diabetic patients according to the quartiles of follow-up LDL are shown in **Table 4**. There was no significant difference in age, sex, BMI, waist circumference, or the prevalence of hypertension, family history of coronary artery disease, smoking, and alcohol drinking in this cohort, while total cholesterol, LDL-cholesterol, non-HDL-cholesterol, LDL-C/HDL-C ratio, apo B, apo B/apo AI ratio were higher in the third and fourth quartiles than the others. When we performed the same analysis with the total cohort, a significant difference was found between the combined data from the first to third quartiles and the fourth quartile, except non-HDL-cholesterol (**Fig. 3**). Because further

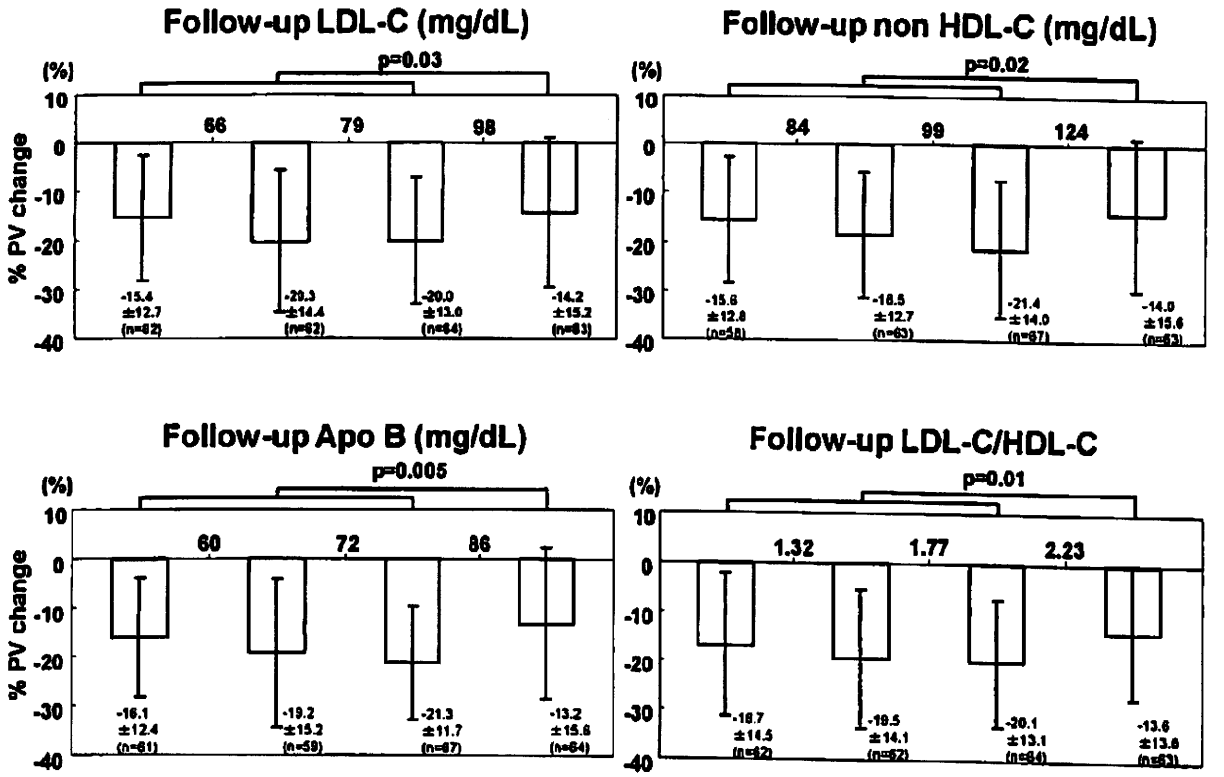

Fig. 2. Percent change of PVin each quartile of follow-up lipid parameters in the total cohort.

reduction of lipid profiles seemed to result in further reduction of PV in diabetic patients, we also compared the percent PV change after dividing them into 2 groups according to the median. As shown in **Fig. 4**, a significant difference was found between the two groups, except in non-HDL-cholesterol. However, the *p* value was smaller in LDL-cholesterol when we used the median as a cutoff value, while it was larger in apo B. Although we did not find significant differences in non-HDL-cholesterol, a *p* value of 0.028 was obtained when we used a cutoff of 100 mg/dL for non HDL-cholesterol.

Effect of HDL-Cholesterol Levels on Major Adverse Cardiovascular Events

To examine the effect of HDL-cholesterol levels on major adverse cardiovascular events (MACE), such as target lesion revascularization (TLR) and target vessel revascularization (TVR), we compared their incidence in the total cohort according to baseline and follow-up HDL-cholesterol levels (≥40 mg/dL or <40 mg/dL). As shown in **Table 5**, patients with lower HDL-cholesterol at baseline or follow-up showed a significantly higher incidence of TLR, but not of TVR or other vessel revascularization. The baseline characteristics of the two groups according to the levels of follow-up HDL-cholesterol levels are shown in **Table 6**. There was no significant difference in demographic characteristics between the two groups. As expected, HDL-cholesterol and apo AI were higher and the LDL-C/HDL-C ratio and apo B/AI were lower in patients with higher HDL-cholesterol. A similar finding was observed when we divided the patients according to baseline HDL-cholesterol levels (data not shown).

Discussion

In this post-hoc analysis of the JAPAN-ACS study we have shown that diabetic patients had more regression by targeting lower levels of LDL, non-HDL cholesterol, and LDL-C/HDL-C with intensive lipid-lowering therapy in Japanese; however, our data may indicate that the same target can be used for apo B in diabetic or non-diabetic ACS patients. We also found that patients with lower HDL-cholesterol had a higher

Table 4. Baseline characteristics of diabetic patients with quartiles of follow-up LDL-C

Characteristic	1st (n=18)	2nd (n=18)	3rd (n=19)	4th (n=18)	p value
Age (years)	64.2±10.8	62.0±9.7	60.6±10.8	64.2±11.3	0.7
Male (%)	83	94	84	67	0.2
BMI (kg/m^2)	24.4±3.6	25.9±3.1	24.8±3.8	24.4±4.4	0.6
Waist circumference (cm)	87.1±7.4	89.1±6.0	89.4±8.0	88.4±10.7	0.9
Hypertension (%)	67	78	74	67	0.8
Family history of CAD (%)	28	11	16	17	0.6
Smoking (%)	39	39	63	50	0.4
Alcohol drinker (%)	67	61	47	39	0.3
Culprit vessel (%)					
RCA	28	22	47	44	0.5
LAD	44	61	32	39	
LCx	28	17	21	17	
LMT	0	0	0	0	
BMS (%)	44	72	74	72	0.2
DES (%)	56	22	21	28	
Other than stent (POBA) (%)	0	6	5	0	
TC (mg/dL)	186.4±33.8	187.1±25.0	201.5±23.0	217.3±48.7	0.03
LDL-C (mg/dL)	115.9±30.0	123.5±21.3	138.7±17.0	149.1±44.3	0.006
TG (mg/dL)	112.0 (76.0, 153.0)	117.0 (77.0, 140.5)	127.0 (107.0, 173.0)	134.0 (76.3, 191.3)	0.6[a]
HDL-C (mg/dL)	48.6±9.7	42.7±6.3	41.3±13.3	45.9±12.1	0.2
non-HDL-C (mg/dL)	134.5±28.6	144.4±24.1	160.2±19.4	170.6±43.4	0.004
LDL-C/HDL-C	2.4±0.8	3.0±0.7	3.6±0.9	3.3±0.9	0.0004
Apo A-I (mg/dL)	123.6±21.3	107.4±16.3	106.4±26.5	109.1±23.6	0.09
Apo B (mg/dL)	92.4±20.9	96.9±18.5	111.8±14.9	115.7±27.3	0.003
Apo E (mg/dL)	4.3±1.2	4.3±1.0	4.2±1.0	4.4±1.0	0.95
Apo B/Apo A-I	0.76±0.20	0.92±0.23	1.10±0.26	1.09±0.26	0.0001

TG is expressed as median and interquartile range, [a]: Wilcoxon/Kruskal-Wallis test

risk for target lesion revascularization, and should be considered for additional therapy to prevent restenosis.

IVUS provides a precise evaluation of the vascular wall and has been shown to be the most sensitive and reliable technique for measuring coronary atherosclerosis progression and regression[15]. Several IVUS trials have shown that intensive lipid-lowering therapy is associated with a decrease of atherosclerosis progression or regression of plaque burden[6]. In the JAPAN-ACS we found much more regression of coronary atheroma after statin therapy than these studies in US[6, 7]. Consistent with our findings, Okazaki *et al.* also showed similar regression with 20 mg atorvastatin after ACS[8]. These data may indicate that Japanese patients are more susceptible to statin therapy in terms of atheroma regression; however, Takayama *et al.* have recently shown that rosuvastatin can induce significant regression of coronary PV (−5.1%) in Japanese patients with stable CAD[16], consistent with the findings by Nissen *et al.*[6, 7]. Taken together, the differences in regression rates between ours and those of Nissen *et al.* might be derived from the patient population; stable CAD and ACS patients. It is still difficult to investigate non-culprit coronary arteries by IVUS in Japan, which might also explain the difference between Japanese and US studies.

The National Cholesterol Education Program currently recommends an optional target LDL-cholesterol of <70 mg/dL for patients at high risk of cardiovascular events, including those with an ACS event[17], while the Japanese guideline recommends an LDL-cholesterol target <100 mg/dL for secondary prevention[18]. However, this study might provide a rationale for more aggressive lipid lowering, targeting LDL-cholesterol of <75 or 70 mg/dL in diabetic patients after ACS, while non-diabetic patients can be treated to reach LDL-cholesterol of <100 mg/dL. Our data also support non-HDL-cholesterol as an additional target

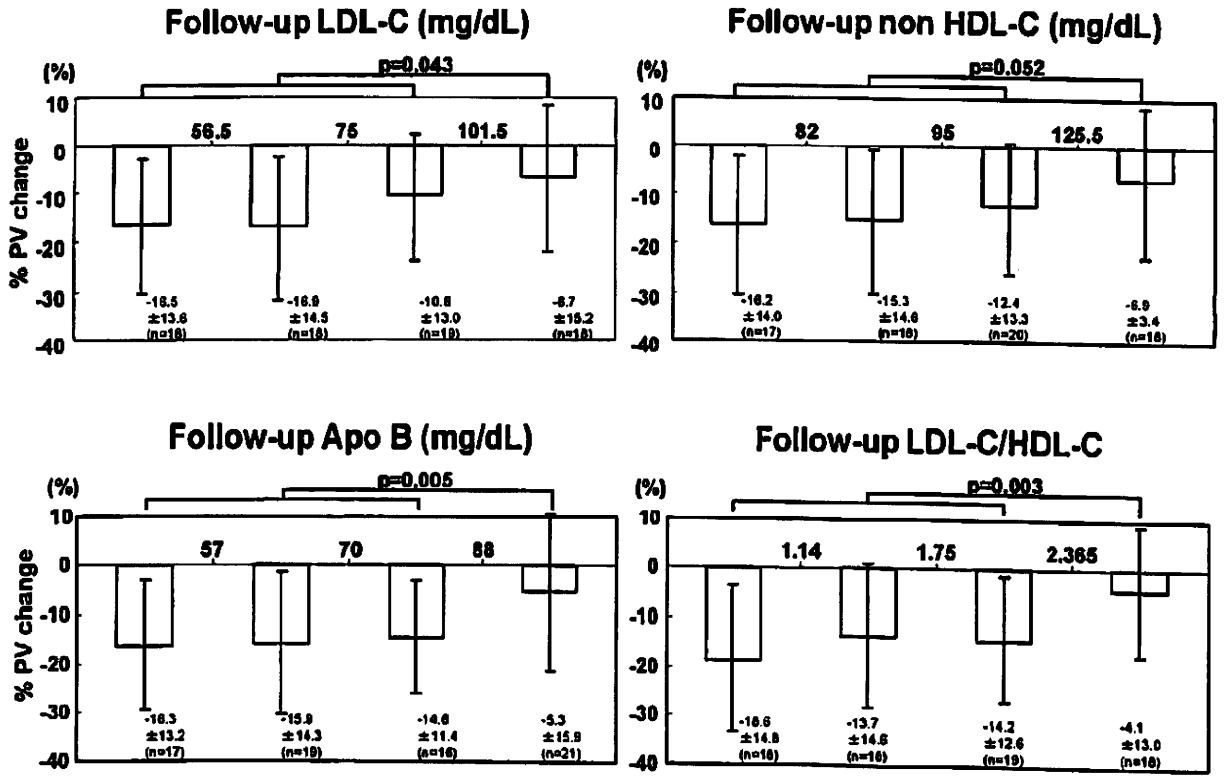

Fig. 3. Percent change of PV in each quartile of follow-up lipid parameters in diabetic patients.

for the management of ACS patients. Although the median cutoff did not result in a significant difference, a significant difference was observed when we used 100 mg/dL for the cutoff, which is consistent with the guidelines of the National Cholesterol Education Program for very high-risk patients. In terms of apo B, we obtained a smaller p value when we used a cutoff of 88 mg/dL than 70 mg/L in diabetic patients, which was almost the same in the total cohort. We showed less regression of coronary atheroma in diabetic patients after intensive statin treatment even though the mean LDL-cholesterol levels were almost the same in diabetic and non-diabetic patients. Considering that diabetic patients tend to have small dense LDL, the data on apo B might indicate that LDL particle number should be reduced to a certain level to obtain the maximum effects for plaque regression in diabetic patients. Further study is required to develop a rationale for aggressive lipid-lowering therapy in Japanese.

In this sub-analysis, we showed that low HDL-cholesterolemia <40 mg/dL was associated with increased TLR after ACS. As shown in **Table 6**, there was no demographic difference between the two groups except apo A1 and the ratio of LDL to HDL-cholesterol and the apo B to apo A1 ratio, indicating that low levels of HDL-cholesterol are a powerful predictor of major cardiovascular events even in patients treated with the maximum dose of statins. Previous studies have also shown that HDL-cholesterol levels during statin treatment are independently predictive of major cardiovascular events even in patients with LDL-cholesterol levels less than 70 mg/dL[11, 12]. Recently, Taylor *et al.* have shown that the use of extended-release niacin causes significant regression of carotid intima-media thickness when combined with a statin[19]; therefore, additional treatment might be required to raise HDL-cholesterol to prevent major cardiovascular events in patients with low HDL-cholesterolemia.

The current study has some limitations. The first is that LDL-cholesterol was determined by a direct method, not by a Friedwald equation because the equation could not be applied for blood samples from some patients. Recently, Nakamura *et al.* have shown that the direct measurement of LDL-cholesterol is still poor in terms of accuracy and stability[20]; however, even when we used the equation, we found similar

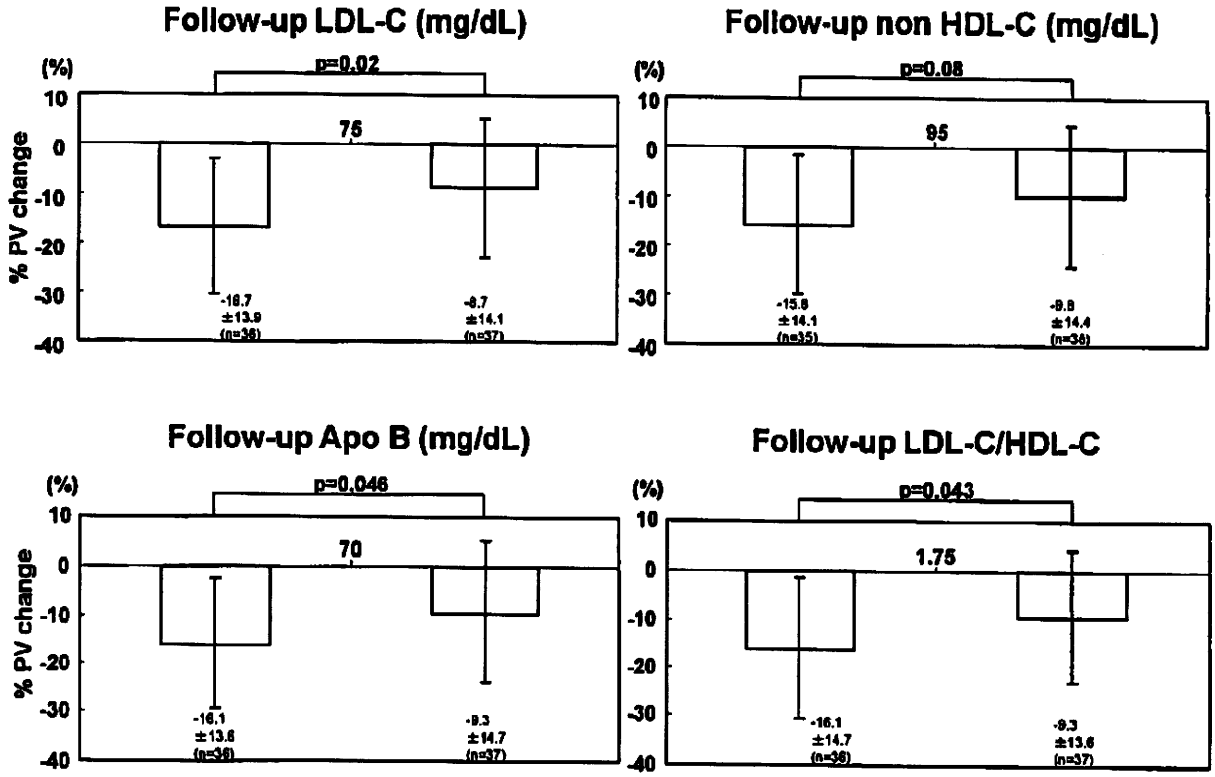

Fig. 4. Percent change of PV according to the median of follow-up lipid parameters in diabetic patients.

Table 5. Relationship between baseline and follow-up HDL-C levels with major adverse cardiovascular events (MACE)

baseline HDL-C	HDL-C <40 mg/dL (n=85)	HDL-C ≧40 mg/dL (n=164)	p value
MACE	21 (24.7)	32 (19.5)	0.9
TLR	16 (18.8)	13 (7.9)	0.01
TVR (non-TLR)	3 (3.5)	11 (6.7)	0.3
Other vessel revascularization	6 (7.1)	10 (6.1)	0.09
follow-up HDL-C	HDL-C <40 mg/dL (n=64)	HDL-C ≧40 mg/dL (n=187)	p value
MACE	15 (23.4)	38 (20.3)	0.6
TLR	12 (18.8)	17 (9.1)	0.046
TVR (non-TLR)	3 (4.7)	11 (5.9)	0.7
Other vessel revascularization	5 (7.8)	11 (5.9)	0.6

MACE: Major Adverse Cardiac Events
TLR: Target Lesion Revascularization
TVR: Target Vessel Revascularization

n (%)

results with this analysis (data not shown). The second is that this study lacked a control group receiving a placebo or less-intensive lipid-lowering therapy because the JAPAN-ACS study was designed to prove the non-inferiority of pitavastatin against atorvastatin. In this sub-analysis we combined the data on both statins; however, we deemed it ethically unacceptable to give a placebo to patients with ACS. The third is that the diagnosis of diabetes mellitus was made by the attending physicians, and no oral glucose tolerance

Table 6. Baseline characteristics of total cohort with HDL-C

Characteristic	follow-up HDL-C <40 mg/dL (n=64)	follow-up HDL-C ≧40 mg/dL (n=187)	p value
Age (years)	62.8 ± 10.6	62.2 ± 11.3	0.7
Male (%)	89	79	0.064
BMI (kg/m^2)	24.4 ± 3.4	24.4 ± 3.6	0.99
Waist circumference (cm)	88.4 ± 8.9	86.6 ± 9.1	0.2
Diabetes (%)	25	30	0.4
Hypertension (%)	61	63	0.8
Family history of CAD (%)	22	17	0.3
Smoking (%)	52	45	0.4
Alcohol drinker (%)	45	49	0.6
Culprit vessel (%)			
RCA	25	34	
LAD	61	52	0.4
LCx	14	13	
LMT	0	0	
BMS (%)	70	64	
DES (%)	28	33	0.6
Other than stent (POBA) (%)	2	3	
TC (mg/dL)	189.9 ± 28.6	200.0 ± 37.8	0.052
LDL-C (mg/dL)	130.9 ± 27.3	133.0 ± 33.9	0.6
TG (mg/dL)	119.0 (85.3, 155.5)	105.0 (74.0, 143.0)	0.09*
HDL-C (mg/dL)	37.5 ± 6.5	46.8 ± 9.6	<0.0001
non-HDL-C (mg/dL)	152.3 ± 27.7	152.5 ± 35.2	0.98
LDL-C/HDL-C	3.6 ± 0.9	2.9 ± 0.8	<0.0001
Apo A-I (mg/dL)	98.0 ± 14.8	115.4 ± 18.9	<0.001
Apo B (mg/dL)	105.2 ± 20.1	103.9 ± 24.3	0.7
Apo E (mg/dL)	3.9 ± 1.1	4.2 ± 1.2	0.057
Apo B/Apo A-I	1.09 ± 0.22	0.92 ± 0.24	<0.0001

TG is expressed as median and interquartile range, *: Wilcoxon/Kruskal-Wallis test

test was performed to confirm diabetes, which is why we did not analyze non-diabetic patients.

In conclusion, early intensive statin therapy in Japanese patients after ACS resulted in the marked regression of coronary PV in total and diabetic patients. Diabetic patients can obtain more benefit from intensive lipid-lowering therapy with lower target levels of LDL, non-HDL-cholesterol, and LDL-C/HDL-C in Japanese. These lipid profiles may be related to the coronary plaque burden in statin-treated patients. On the other hand, low HDL-cholesterol levels are related to major cardiovascular events; therefore, patients with lower HDL-C are recommended for more intensive and comprehensive management to prevent the recurrence of coronary events.

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Appendices

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Exercise Training Stimulates Ischemia-Induced Neovascularization via Phosphatidylinositol 3-Kinase/Akt-Dependent Hypoxia-Induced Factor-1α Reactivation in Mice of Advanced Age

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Background—Exercise stimulates the vascular response in pathological conditions, including ischemia; however, the molecular mechanisms by which exercise improves the impaired hypoxia-induced factor (HIF)-1α–mediated response to hypoxia associated with aging are poorly understood. Here, we report that swimming training (ST) modulates the vascular response to ischemia in aged (24-month-old) mice.

Methods and Results—Aged wild-type mice (MMP-$2^{+/+}$) that maintained ST (swimming 1 h/d) from day 1 after surgery were randomly assigned to 4 groups that were treated with either vehicle, LY294002, or deferoxamine for 14 days. Mice that were maintained in a sedentary condition served as controls. ST increased blood flow, capillary density, and levels of p-Akt, HIF-1α, vascular endothelial growth factor, Fit-1, and matrix metalloproteinase-2 (MMP-2) in MMP-$2^{+/+}$ mice. ST also increased the numbers of circulating endothelial progenitor cells and their function associated with activation of HIF-1α. All of these effects were diminished by LY294002, an inhibitor of phosphatidylinositol 3-kinase; enhanced by deferoxamine, an HIF-1α stabilizer; and impaired by knockout of MMP-2. Finally, bone marrow transplantation confirmed that ST enhanced endothelial progenitor cell homing to ischemic sites in aged mice.

Conclusions—ST can improve neovascularization in response to hypoxia via a phosphatidylinositol 3-kinase–dependent mechanism that is mediated by the HIF-1α/vascular endothelial growth factor/MMP-2 pathway in advanced age. (***Circulation.*** **2010;122:707-716.)**

Key Words: exercise ■ angiogenesis, physiological ■ phosphatidylinositol 3-kinase ■ hypoxia-inducible factor 1, α subunit ■ aging ■ neovascularization, physiological

Aging is associated with a decreased ability to form new blood vessels in response to ischemia, which results in higher rates of cardiovascular complications and diminished capacity for tissue regeneration.[1] There is therefore considerable interest in understanding the mechanisms of angiogenesis in advanced age. Accumulating evidence suggests that the process of new blood vessel formation is associated with extracellular matrix remodeling, mainly involving the matrix metalloproteinase (MMP) family.[2,3] In particular, aging reduces MMP-2 expression in vitro and in vivo.[4,5] Genetic and pharmacological intervention studies have demonstrated in several animal models that MMP-2 plays an important role in angiogenesis and vasculogenesis.[5,6] Recently, a few studies have shown that knee-extension exercise activates MMP expression in human skeletal muscle.[7] On the basis of these findings and past reports that exercise increases coronary vascularization by promoting vascular growth and remodeling in response to stress,[8] we hypothesize that the activation of MMP-2 might represent a crucial mediator by which exercise triggers protective vascular action.

Clinical Perspective on p 716

Administration of bone marrow (BM)–derived or peripheral blood–derived endothelial progenitor cells (EPCs) has improved postischemic neovascularization in various experimental and clinical trials[9,10]; however, several recent randomized clinical

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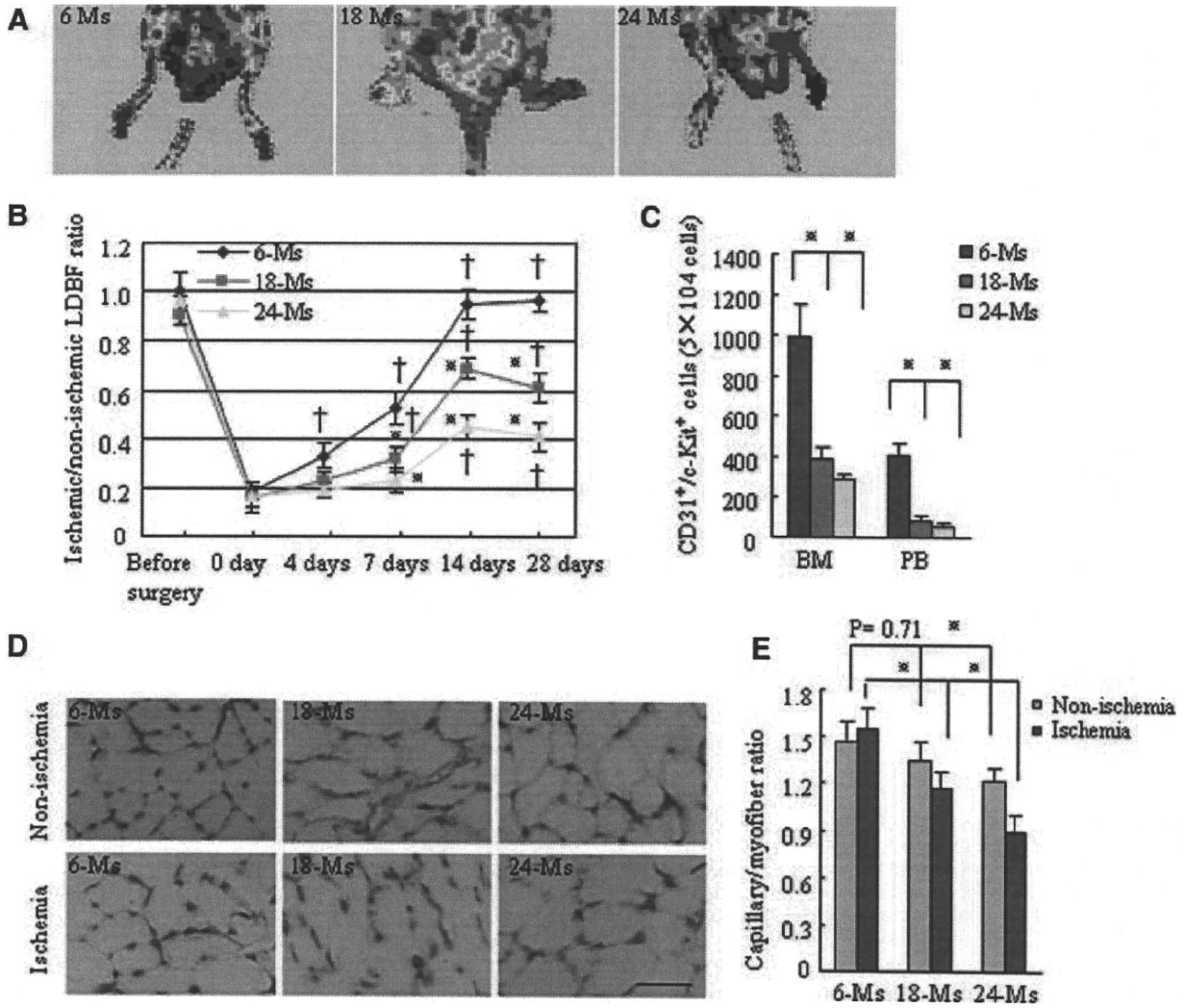

Figure 1. Aging reduces vessel density and blood flow in ischemic tissues of MMP-$2^{+/+}$ mice. A, A low perfusion signal (dark blue) was observed in the ischemic hindlimbs of aged MMP-$2^{+/+}$ mice (18 and 24 months old) with laser Doppler perfusion imaging, whereas a high signal (red) was detected in young (6-month-old) MMP-$2^{+/+}$ mice. B, The ratio of ischemic-to-normal laser Doppler blood flow (LDBF) in aged MMP-$2^{+/+}$ mice (n=10 per group; †$P<0.05$ vs each group at day 0, *$P<0.05$ vs the corresponding 6-month-old mice at days 7 to 28 after ischemia; 2-way repeated-measures ANOVA and Bonferroni post hoc tests). C, Quantitative analysis of the numbers of EPCs in BM and peripheral blood (PB) of WT mice (n=10 per group; *$P<0.05$, Tukey post hoc test). D, Immunohistostaining showed the capillaries in the thigh adductor muscle at postoperative day 28. Scale bar=100 μm. E, Quantitative analysis of capillary density in 3 groups of mice (n=8 per group; *$P<0.05$, paired Student *t* test). Ms indicates months.

trials of stem and progenitor cell treatment for ischemic diseases have been disappointing in subjects of advanced age.[11] Impaired angiogenesis in advanced age might be due to an intrinsic decline in the regenerative capacity of vascular progenitors or a decline in a proregenerative niche.[12] On the other hand, physical training increases circulating EPCs in patients with ischemic syndromes.[13] Further work is necessary to determine whether exercise improves EPC mobilization and function in individuals of advanced age, as well as to determine the mechanisms underlying these processes.

Exercise promotes ischemic angiogenesis by increasing vascular endothelial growth factor (VEGF) in plasma or ischemic tissue in humans and animals[14,15]; however, angiogenic growth factors and related transcriptional factor hypoxia-induced factor-1α (HIF-1α) activity decreased in aged cell lines and animals.[16,17] In the present study, we investigate the effects of swimming training (ST) on angiogenic mechanisms and HIF-1α function in a mouse model of limb ischemia at advanced age. We evaluated whether ST was able (1) to enhance HIF-1α transcriptional activity through activation of the insulin-like growth factor (IGF)-1–mediated phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway and attenuation of the prolyl hydroxylases (PHDs) degradation system, (2) to stimulate reactivation of VEGF and MMP-2 expression in ischemic tissue and BM-derived EPCs, (3) to improve EPC mobilization and homing to the vasculature, and (4) to enhance neovascularization in response to hypoxia.

Methods

An expanded Methods section is available in the online-only Data Supplement.

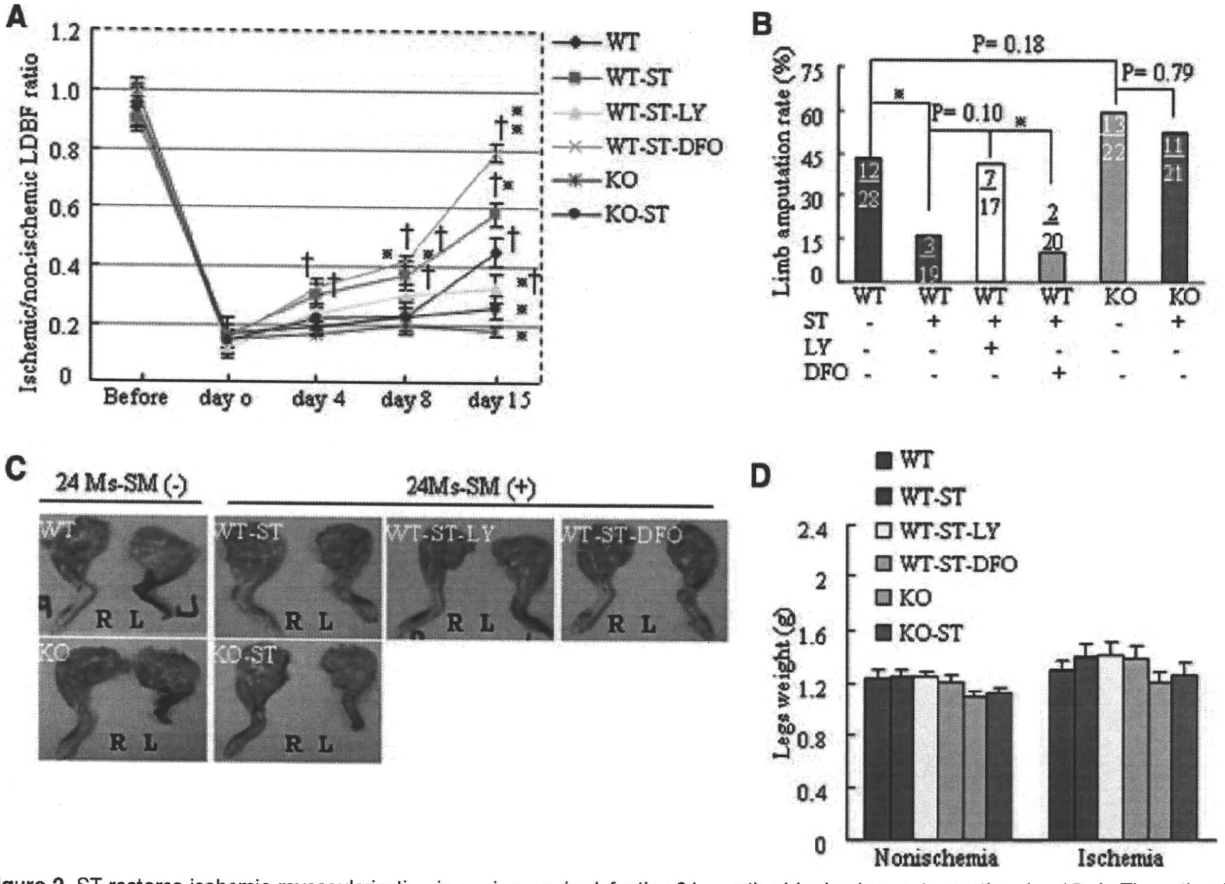

Figure 2. ST restores ischemic revascularization in angiogenesis-defective 24-month-old mice by postoperative day 15. A, The ratio of ischemic-to-normal laser Doppler blood flow (LDBF) in aged MMP-$2^{+/+}$ mice (n=8 per group; †$P<0.05$ vs corresponding day 0, *$P<0.05$ vs corresponding MMP-$2^{+/+}$ mice during ischemia; 2-way repeated-measures ANOVA and Bonferroni post hoc tests). B, Quantitative analysis of foot amputation in 6 groups (*$P<0.05$, χ^2 test). Upper number indicates number of amputations; lower number, number of animals. C, Photographs of typical hindlimbs of the 6 groups of mice. R indicates right (nonischemic); L, left (ischemic). D, Weights of ischemic and nonischemic legs of mice (paired Student *t* test). Ms indicates months.

Mouse Model of Revascularization Without or With Exercise

Studies of wild-type (WT; MMP-$2^{+/+}$; Chubu Kagaku Shizai Co., Ltd. Nagoya, Japan) and MMP-2 knockout (KO, MMP$^{-/-}$, gifted by S. Itohara RIKEN Brain Science, Institute, Wako, Saitama, Japan)[5] mice in a C57/BL6 background were approved by the Animal Studies Committee of Nagoya University. Male young (6 months) and aged (18 and 24 months) mice of both genotypes were subjected to unilateral hindlimb ischemic surgery and ST programs.

Statistical Analysis

Data are expressed as mean±standard error of the mean (SEM). Student *t* tests (for comparison between 2 groups) or 1-way ANOVA (for comparison of 3 or more groups) followed by Tukey post hoc tests were used for statistical analyses. The nonparametric Kruskal-Wallis test (Tukey-type multiple comparison) was used ANOVA for the gene expression data. Blood flow data were subjected to 2-way repeated-measures ANOVA and Bonferroni post hoc tests. The comparative incidence of limb amputation was evaluated by the χ^2 test. SPSS software version 17.0 (SPSS Inc, Chicago, Ill) was used. A value of $P<0.05$ was considered statistically significant.

Results

Aging Reduces HIF-1α–Induced Growth Factors and Impairs Neovascularization in Response to Hypoxia

Serial laser Doppler blood flow measurements showed that aged WT mice (18 to 24 months old) had lower ratios of ischemic-to-nonischemic blood flow (Figure 1A and 1B) than young (6-month-old) WT mice. The ratio decreased further from 18 to 24 months of age. The numbers of CD31$^+$/c-Kit$^+$ progenitor cells in both BM and peripheral blood also decreased markedly in an age-dependent manner (Figure 1C), which suggests a vasculogenesis-specific impairment with age. The capillary density of nonischemic and ischemic muscle also correlated with age (Figure 1D and 1E).

ST Restores Ischemic Neovascularization in Mice of Advanced Age (24 Months)

On day 15 after the induction of ischemia, aged WT mice exposed to ST (WT-ST mice) had markedly higher blood perfusion than WT mice (Figure 2A), which suggests that ST stimulated neovascularization in response to hypoxia. This was further supported by data from longer-term ST (29 days; online-only Data Supplement Figure I). ST also increased capillary density (Figure 3A). HIF-1α, which is regulated by the PI3K signaling pathway, is less stable and active in aged animals during ischemia.[18,19] We hypothesized that ST protects against HIF-1α destabilization by activating the PI3K signaling pathway; the increased stability of HIF-1α would then increase ischemic neovascularization. We tested this hypothesis by treating aged WT mice with LY2940029 (LY), an inhibitor of PI3K,