31. Witko-Sarsat V, Friedlander M, Nguyen Khoa T, et al: Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. J Immunol 161(5):2524-2532, 1998

32. Buzello M, Tornig J, Faulhaber J, Ehmke H, Ritz E, Amann K: The apolipoprotein e knockout mouse: a model documenting accelerated atherogenesis in uremia. J Am Soc Nephrol 14(2):311-316, 2003

33. Bro S, Bentzon JF, Falk E, Andersen CB, Olgaard K, Nielsen LB: Chronic renal failure accelerates atherogenesis in apolipoprotein E-deficient mice. J Am Soc Nephrol 14(10):2466-2474, 2003

34. Bro S, Moeller F, Andersen CB, Olgaard K, Nielsen LB: Increased expression of adhesion molecules in uremic atherosclerosis in apolipoprotein-E-deficient mice. J Am Soc Nephrol 15(6):1495-1503, 2004

35. Massy ZA, Ivanovski O, Nguyen-Khoa T, et al: Uremia accelerates both atherosclerosis and arterial calcification in apolipoprotein E knockout mice. J Am Soc Nephrol 16(1):109-116, 2005

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ORIGINAL ARTICLE

Risk factors for coronary atherosclerosis in a general Japanese population: The Hisayama study

Yutaka Nakashima[a,d,*], Yutaka Kiyohara[b], Yasufumi Doi[c], Michiaki Kubo[c], Mitsuo Iida[c], Katsuo Sueishi[a]

[a]*Pathophysiological and Experimental Pathology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*
[b]*Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*
[c]*Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*
[d]*Division of Pathology, Japanese Red Cross Fukuoka Hospital, 3-1-1 Ogusu, Minami-ku, Fukuoka 815-8555, Japan*

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Abstract

To investigate risk factors for coronary atherosclerosis in men and women in the recent general Japanese population, we examined coronary arteries obtained from subjects autopsied in the Hisayama cohort study (autopsy rate: 78.7%). The subjects were over 40 years of age and consisted of 125 men and 108 women. They underwent an antemortem medical examination in 1988 and were subject to autopsy at death during an 8-year follow-up period. Atherosclerosis was globally assessed by examining 14 specimens taken from wide areas of epicardial coronary arteries and classified into 6 grades. The frequency of more severe grades of coronary atherosclerosis increased with age in both genders and was greater in men than in women of the same age. Multiple regression analysis revealed that age, systolic blood pressure, serum total cholesterol, and hemoglobin A_{1C} were significant risk factors for men. Age, systolic blood pressure, and waist to hip ratio were risk factors for women. Smoking was not significantly correlated with the grade of coronary atherosclerosis in either gender. Thus, aging, hypertension, hypercholesterolemia, obesity, and glucose intolerance are risk factors for coronary atherosclerosis in recent Japanese populations, and the significance of the metabolic risk factors is different between men and women.

Keywords: Risk factors; Coronary atherosclerosis; Epidemiology; Autopsy; Japanese

Introduction

The mortality and morbidity of cardiovascular diseases have changed over the last several decades in Japan. The most striking change is the decrease in the incidence of stroke, and declining blood pressure in the general population has contributed greatly to this trend [15,37,39]. Although a reduced risk of hypertension was

*Corresponding author at: Division of Pathology, Japanese Red Cross Fukuoka Hospital, 3-1-1 Ogusu, Minami-ku, Fukuoka 815-8555, Japan.
E-mail address: y-nakashima@fukuoka-med.jrc.or.jp (Y. Nakashima).

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expected to diminish the morbidity and mortality of coronary heart disease (CHD), this change is minimal [15,39]. This is thought to be the result of mixed effects of other risk factors, such as hyperlipidemia, obesity and glucose intolerance, because the prevalences of these risk factors have rapidly increased in Japanese society during the same time period through Westernization of the Japanese lifestyle [7,15,39]. However, it is not well understood how changes in the risk factors actually affect the development of CHD events and coronary atherosclerosis. From 1961 to 1984, we conducted investigations on risk factors for CHD [14] and coronary atherosclerosis [24] in the Hisayama cohort study, using CHD patients and autopsy results, respectively. In the present study, we examined coronary arteries obtained from more recently autopsied subjects, aiming to clarify the risk factors for coronary atherosclerosis in men and women in the recent general Japanese population and to compare the results with those of the previous studies. The risk factors for coronary atherosclerosis are sometimes regarded as the same as those for CHD. However, they should be distinguished, because coronary atherosclerosis is not the only causative factor for CHD, and CHD is not necessarily developed in all patients with severe coronary atherosclerosis but developed often in patients with moderate atherosclerosis [16].

The method most frequently used in assessing coronary atherosclerosis is to measure surface involvement (SI), in which the proximal portion of the artery is longitudinally opened, and the area affected by atherosclerosis is measured. While this method certainly works well for young and middle-aged subjects [1,8,9,17,34,35], it is ineffective for older subjects like those in the present study. First, calcification is often present in the proximal portion of coronary arteries and prevents longitudinal opening. Second, older subjects tend to have higher SI scores for the proximal portion, so that the discriminatory value of the method is reduced. Therefore, we employed a novel method in the present study in which atherosclerosis was globally assessed by examining histologic specimens taken from wide areas of epicardial coronary arteries.

Material and methods

Hisayama study

A prospective population survey of cardiovascular disease and its risk factors has been conducted in Hisayama, a suburban community adjacent to Fukuoka, since 1961. The population over 40 years of age on January 1, 1988, included 3558 subjects. Full details of the sampling procedures, the methods of baseline examination and subsequent follow-up have been previously described [15,37,38]. From June 29 to November 10, 1988, 2742 men and women over 40 years of age underwent a medical examination (participation rate, 80.9%). Six subjects who died before the follow-up period and 12 subjects who had already had a history of myocardial infarction were excluded from the study, leaving 2724 subjects.

Subjects of the present study

During the follow-up period from December 1, 1988, to July 31, 1996, 310 subjects died, of whom 244 were autopsied (autopsy rate: 78.7%). Eleven subjects were excluded because whole lengths of coronary arteries were not available for pathologic examinations. Thus, 233 subjects (125 men and 108 women) were investigated in the present study. The study was approved by the Ethics Committee of the Department of Pathology, Kyushu University, and informed consent was given by the families of the autopsied subjects. The intervals between the antemortem examination and the autopsy ranged from 1 month to 8 years. The mean ages at the antemortem examination and death are shown in Table 1. Eighteen subjects were affected by CHD during the follow-up period, i.e., 14 subjects experienced myocardial infarction, and 4 subjects were victims of sudden death with severe chest pain or precordial oppression. Sixty six and 18 subjects were treated with anti-hypertensive and anti-diabetic drugs, respectively.

Risk factors

The variables that were selected as possible predictors for coronary atherosclerosis included age at the antemortem examination, systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), waist to hip ratio (WHR), total serum cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), hemoglobin A_{1C} (HbA_{1C}), smoking habits, and daily alcohol intake. The mean values of the factors are shown in Table 1. Blood pressures were measured 3 times in a sitting position and averaged. The concentrations of TC and HDL-C were measured enzymatically. HbA_{1C} levels were measured by high-pressure liquid chromatography (TBA-80S, Toshiba Inc., Tokyo, Japan). Non-smokers at the time of the antemortem examination were classified as 0 and ex-smokers and current smokers as 1. Non-drinkers and ex-drinkers were classified as 0 and current drinkers as 1.

Coronary arteries

In all subjects, the heart was immersion-fixed with 10% formalin. As illustrated in Fig. 1, 14 specimens of

Table 1. Characteristics of the subjects.

Variable	Men			Women		
	n	Mean ± SD	Range	*n*	Mean ± SD	Range
Age at exam. (years)	125	72.56 ± 10.75	(44.00–94.00)	108	74.41 ± 11.01	(46.00–96.00)
Age at death (years)	125	76.95 ± 10.35	(49.00–94.00)	108	78.84 ± 11.09	(53.00–98.00)
SBP (mmHg)	125	143.96 ± 23.03	(103.00–237.00)	108	148.35 ± 25.85	(96.00–213.00)
DBP (mmHg)	125	77.55 ± 10.75	(41.00–104.00)	108	75.05 ± 11.76	(47.00–111.00)
BMI (kg/m^2)	125	21.15 ± 2.76	(14.67–27.46)	106	21.24 ± 3.66	(13.33–34.04)
WHR	122	0.92 ± 0.06	(0.80–1.08)	103	0.91 ± 0.09	(0.70–1.12)
TC (mg/dL)	125	187.04 ± 48.64	(75.00–354.00)	108	201.76 ± 38.84	(123.00–311.00)
HDL-C (mg/dL)	125	46.50 ± 12.48	(20.00–84.00)	108	47.06 ± 13.03	(25.00–82.00)
HbA_{1C} (%)	125	5.77 ± 0.96	(4.00–11.10)	108	5.68 ± 0.80	(4.20–8.90)

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WHR, waist to hip ratio; TC, serum total cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA_{1C}, hemoglobin A_{1C}.

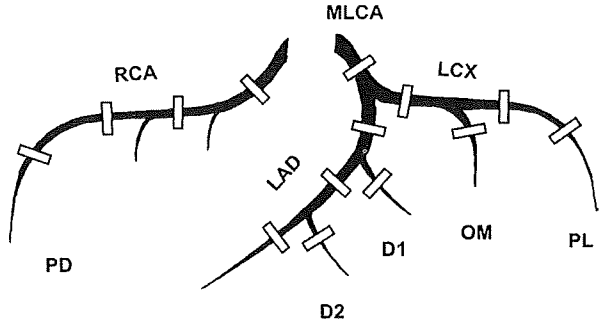

Fig. 1. Examination of 14 sites (gray bars) from epicardial coronary arteries. D1: first diagonal branch, D2: second diagonal branch, LAD: left anterior descending artery, LCX: left circumflex artery, MLCA: main left coronary artery, OM: obtuse marginal branch, PD: posterior descending branch, PL: posterolateral branch, RCA: right coronary artery.

epicardial coronary arteries were taken from each heart for histologic examinations; 4 specimens from the right coronary artery (RCA) system (proximal, mid and distal portions of RCA and posterior descending branch), 1 from the main left coronary artery (MLCA), 5 from the left anterior descending artery (LAD) system (proximal, mid and distal portions of LAD and first and second diagonal branches), and 4 from the left circumflex artery (LCX) system (proximal and distal portions of LCX and obtuse marginal and posterolateral branches). The arteries were cut *in situ* at 3–4 mm intervals, and the most narrowed sites were taken. In cases of anatomical variations, specimens were obtained from other arteries distributing blood to the corresponding cardiac areas such that the total number was 14. Specimens with severe calcification were decalcified.

Histologic classification

Histologic sections were stained with hematoxylin and eosin, elastica van Gieson, and Masson's trichrome stains. Atherosclerotic lesions were classified into two categories, i.e., preatheromas and advanced lesions, according to the classification of the American Heart Association [31]. Preatheromas included foam cell lesions (type II) and intermediate lesions (type III). Advanced lesions included atheromas (type IV) and fibroatheromas (type V).

Grading of coronary atherosclerosis

As shown in Table 2, the global state of coronary atherosclerosis in each subject was classified into 6 grades by assessing the histology of intimal lesions and luminal stenosis in 14 specimens. When there was neither preatheroma nor advanced lesion, the subjects were classified as grade 0. If the number of preatheromas was 1–4, and no advanced lesions were present, the subjects were classified as grade 1. In grade 2, subjects who fulfilled either of the following criteria were included. In criterion A, the number of preatheromas was 5 or more, and no advanced lesions were present. In criterion B, the number of advanced lesions was 1 or 2, and the total number of lesions (preatheromas + advanced lesions) was less than 5. For example, a subject with 12 preatheromas and no advanced lesions and a subject with no preatheromas and only 1 advanced lesion were classified in this grade. In grade 3, subjects who had 1–4 advanced lesions with variable numbers of preatheromas were included, and those who fulfilled criterion B in grade 2 were excluded. Subjects were classified as grade 4 when the number of advanced lesions was 5–8. For grade 5, subjects who fulfilled either of the following criteria were included. In criterion A, the number of advanced lesions was the same as that for grade 4 and, in addition, the luminal stenosis was over 80% in at least 2 of 3 (RCA, LAD, and LCX) arterial systems. In criterion B, the number of the advanced lesions was 9 or more. For example, a subject having 6 advanced lesions with severe stenosis in RCA and LAD

Table 2. Grading of coronary atherosclerosis.

Grade		Number of lesions in 14 sites		Luminal stenosis
		Preatheroma	Advanced lesion	
0		0	0	
1		$1 \leq < 5$	0	
2	A	$5 \leq$	0	
	B	(preatheroma + advanced lesion: < 5)	$1 \leq < 3$	
3		[a]	$1 \leq < 5$ (except subjects who fulfill the criterion B in grade 2)	
4		[a]	$5 \leq < 9$	
5	A	[a]	$5 \leq < 9$ and	Over 80% stenosis in at least 2 of 3 major arterial systems
	B	[a]	$9 \leq$	

[a]In grades 3–5, the number of preatheroma was not concerned in grading.

and a subject having 11 advanced lesions were classified in this grade. Luminal stenosis was measured as previously described [24] with a computer-assisted morphometric analyzer (MacScope, Fukui, Japan). To investigate the effect of SBP and DBP on LAD and RCA systems, atherosclerosis of LAD and RCA was classified into 6 grades, respectively, using a similar grading method as described above.

Statistical analysis

An SAS computer package (SAS Institute, Cary. N.C., USA) was used for regression analysis and stepwise multiple regression analysis. A Mann–Whitney non-parametric test was used to compare the grade of atherosclerosis in subjects with CHD with those with non-CHD. The age-adjusted SBP and DPB values were calculated by the covariance method to investigate their effects on atherosclerosis in LAD and RCA. A $p<0.05$ value was considered to be statistically significant.

Results

Frequency of each grade of coronary atherosclerosis

The number of subjects who were classified in grades 0, 1, 2, 3, 4, and 5 were 20, 32, 47, 62, 45, and 27, respectively. The mean (m) of the grade for all subjects was 2.70. The grade of the subjects affected by CHD during the follow-up period ($m = 3.83$, $n = 18$) was significantly higher than that for non-CHD subjects ($m = 2.60$, $n = 215$, $p<0.001$). The number of subjects treated with anti-hypertensive drugs was greater in the higher grades than in lower grades (3, 6, 6, 17, 20, and 14 in grades 0, 1, 2, 3, 4, and 5, respectively), and so was the number of subjects treated with anti-diabetic drugs (1, 0, 3, 4, 5 and 5 in grades 0, 1, 2, 3, 4, and 5, respectively).

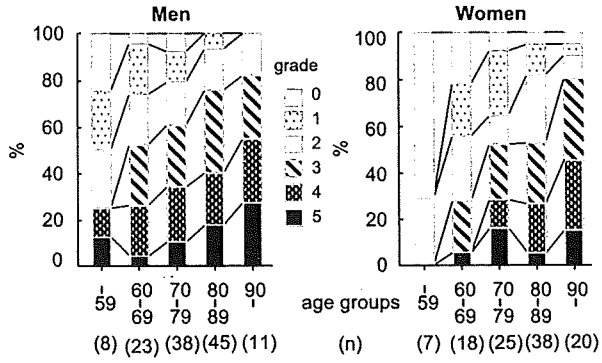

Fig. 2. Frequency (%) of coronary atherosclerosis graded as grades 0–5 in five age groups. n: number of subjects.

Correlation of coronary atherosclerosis with age and gender

Fig. 2 shows the correlation between the grade of coronary atherosclerosis and age at death. Subjects were divided into 5 groups according to their age (less than 60 years of age, 60–69, 70–79, 80–89, and 90 or more). In the youngest group, all subjects were over 50 years of age, except one 49-year-old man. The ordinate represents the frequency of grades 0–5 expressed as a percentage. As shown in this figure, the percentages of higher grades of coronary atherosclerosis increased with age in both genders, and were greater in men than in women of the same age. Generally, the proportion of

each grade in men was similar to that for women in the age group one decade older.

Age-adjusted regression analysis of risk factors

As shown in Table 3, age-adjusted regression analysis revealed significant correlations between the grade of coronary atherosclerosis and SBP, BMI, WHR, TC, and HbA_{1C} for men, and SBP, BMI, and WHR for women. In addition, a negative correlation was found for HDL-C for women.

Multivariate analysis

As shown in Table 4, stepwise multiple regression analysis revealed age, SBP, TC, and HbA_{1C} as risk factors for coronary atherosclerosis in men, and age, SBP, and WHR as risk factors in women.

Effect of blood pressure on atherosclerosis in LAD and RCA

SBP was a significant risk factor for atherosclerosis in LAD ($p = 0.0063$) and RCA ($p = 0.0391$) in men, and for atherosclerosis in RCA ($p = 0.0003$) but not in LAD ($p = 0.2790$) in women. DPB was not a significant risk factor for atherosclerosis either in LAD or in RCA in men and women.

Discussion

The Hisayama study

Although there are numerous reports examining risk factors for CHD, there are fewer studies on risk factors for coronary atherosclerosis, and the factors investigated are limited [1,2,8,9,10,19,24,28,30,32,34]. It is likely that the major reason for this paucity of information is the difficulty of obtaining sufficient data on risk factors and of precisely assessing the grade of coronary atherosclerosis in the same individuals. This is especially true when general populations are targeted because autopsy is required for the assessment of atherosclerosis. The Hisayama study is a prospective cohort study of the general Japanese population, having favorable prerequisite conditions. The autopsy ratio is high (about 80%), and data on risk factors were obtained from records of antemortem examinations performed by the same examiners in the same period.

Table 3. Age-adjusted regression analysis of risk factors for coronary atherosclerosis.

Risk factor	Men ($n = 125$)			Women ($n = 108$)		
	β[a]	F	R^2[b]	β[a]	F	R^2[b]
SBP	0.014	7.40*	0.0509	0.017	11.04**	0.0789
DBP	0.012	1.15	0.0083	0.014	1.51	0.0117
BMI	0.098	4.71*	0.0330	0.106	8.69**	0.0646
WHR	5.895	8.56**	0.0594	4.168	7.71**	0.0607
TC	0.287	10.11**	0.0680	0.188	2.06	0.0159
HDL-C	−0.327	0.81	0.0059	−0.779	4.09*	0.0311
HbA_{1C}	0.332	7.84*	0.0537	0.216	1.71	0.0133
Smoking	0.406	1.54	0.0111	0.125	0.14	0.0011
Drinking	−0.164	0.43	0.0031	−0.614	2.51	0.0193

[a] β: regression coefficient.
[b] R^2: coefficient of determination.
* $p < 0.05$.
** $p < 0.01$.

Table 4. Stepwise multiple regression analysis of risk factors for coronary atherosclerosis.

Risk factor	Men ($n = 125$)			Women ($n = 108$)		
	β[a]	F	R^2[b]	β[a]	F	R^2[b]
Age	0.041	14.90**	0.1151	0.042	12.48**	0.0827
SBP	0.013	6.97**	0.0444	0.015	8.54**	0.1586
WHR				3.193	4.59*	0.0339
TC	0.224	6.11**	0.0730			
HbA_{1C}	0.275	5.37*	0.0337			
Sum			0.2662			0.2752

[a] β: regression coefficient.
[b] R^2: coefficient of determination.
* $p < 0.05$.
** $p < 0.01$.

Global assessment of coronary atherosclerosis

In the present study, coronary atherosclerosis was globally assessed by examining wide areas of epicardial coronary arteries. Consequently, the number of subjects was largest in grade 3 and gradually decreased in both higher and lower grades. This result indicates that the grading method functioned well in the present study, and suggests that it can also be applied to other studies aiming at similar age groups. However, the grade of atherosclerosis can be variable among races and people who live in different geographic conditions [32,35]. Therefore, a small probing study will be necessary to see whether this method gives a proper distribution of subjects when applied to other situations.

Age and gender

Many studies have revealed that age is a significant risk factor for coronary atherosclerosis [9,10,19,34,35]. However, most of the data were obtained from young to middle-aged subjects, and subjects over 70 years of age have seldom been surveyed. The present study demonstrated that atherosclerosis continued to progress even in

the elderly in both genders. Examining coronary stenosis in similar age groups of Hisayama-autopsied subjects, Okumiya et al. [24] reached the same conclusion.

Coronary atherosclerosis in women is milder than in men [21,35] and this is particularly true in premenopausal women [19,34]. However, we are unaware of any detailed reports on coronary atherosclerosis in postmenopausal women. The results of the present study suggest that the progression of coronary atherosclerosis in women is about 10 years behind that found in men in middle-aged and elderly people. This is consistent with clinical observations that the incidence and mortality rate of CHD in women is lower than that for men at the same age even in the postmenopausal period [11,36].

Blood pressure

A positive association between blood pressure and coronary atherosclerosis has been reported by many authors [1,8–10,30,34]. Investigating Hisayama autopsy subjects who died between 1971 and 1981, Okumiya et al. [24] also reached the same conclusion. At the time of this study, blood pressure began to be managed rigorously in Hisayama, and the prevalence of hypertension was significantly decreased in the examination in 1988, compared to those in the 1960s and 1970s when the old WHO criteria were used [7]. Therefore, we expected some modification in the correlation between blood pressure and coronary atherosclerosis before the present study started. However, the analyses revealed that blood pressure remained a strong risk for coronary atherosclerosis in both genders even in the newer subjects. This result may be explained by the fact that the prevalence of hypertension in 1988 was not different from that of the 1960s and 1970s when new WHO criteria were used [15]. The average blood pressures of the present subjects, in particular those for SBP, were high in the light of the new WHO criteria, as shown in Table 1.

Hemodynamics is different between the left coronary artery (LCA) and RCA systems. A major difference is that blood flow in LCA is dominant in diastole, while RCA has a relatively systolic predominance [5]. This difference suggests that LCA is more affected by DBP than SBP and RCA by SBP. However, the present study showed that SBP, but not DBP, was a risk factor for atherosclerosis both in LCA and in RCA. The difference in the blood flow pattern between LCA and RCA may not be large enough to create the different effects of SBP and DBP on coronary atherosclerosis.

TC and HDL-C

TC has been demonstrated as a strong risk factor for coronary atherosclerosis in western countries [1,8,30]. As shown in the present study and other domestic studies performed in the past [24,34], TC is also a risk factor for Japanese people. Regrettably, the risk of TC may become more significant in the future, because the level of TC has rapidly increased in the Japanese population recently [39]. The present study also demonstrated a correlation of TC and coronary atherosclerosis in men but not in women. Although no data on gender differences are available in other pathologic studies, the same trend is demonstrated for CHD. Some epidemiologic studies, including Kiyohara's Hisayama study, demonstrated that the association between TC and CHD was significant for men but not for women [12,14,29], and men had higher rates of CHD than women with the same TC values [3]. Thus, TC has a stronger association with coronary atherosclerosis and CHD in men than in women.

A negative correlation between HDL-C and coronary atherosclerosis has been reported in the Oslo study and the PDAY (Pathobiological Determinants of Atherosclerosis in Youth) study [8,19]. In the present study, however, HDL-C showed a weak association with the grade of coronary atherosclerosis only in women in the age-adjusted regression analysis, and no significance was found in the multivariate analysis in either gender. This difference between the present study and other studies may be related to the difference in the age of the subjects and the grade of atherosclerosis. Compared to the present study, the age groups examined in the Oslo and PDAY studies are much younger [8,19]. In addition, a significant association between HDL-C and atherosclerosis was detected only in early preatheromatous lesions but not in advanced lesions in the PDAY study [19]. However, advanced lesions were observed in many subjects in the present study.

HbA_{1C} and glucose intolerance

A positive correlation between glucose intolerance and coronary atherosclerosis has been reported in only a few epidemiological studies [10,18]. On the other hand, a positive association with glucose intolerance and CHD has been reported in many epidemiologic studies. However, Kiyohara's Hisayama study, in which the subjects were followed from 1961 to 1984, did not reveal a significant correlation between glucose intolerance and the development of CHD events [14]. Interestingly, a subsequent study of Hisayama subjects, in which the same cohort as that used in the present study was followed from 1988 to 1993, demonstrated a positive association [6]. The difference between the two studies is probably explained by the difference in the morbidity of diabetes mellitus at the times of the two investigations. Along with the rapid increase in diabetic patients among Japanese populations, the prevalence of glucose

intolerance in Hisayama was significantly increased at the examination in 1988 compared to those conducted in the 1960s and 1970s [7,15]. This trend is also likely to have played a role in leading to the positive association between HbA_{1C} and coronary atherosclerosis in men in the present study. The reason why there was no significant association between HbA_{1C} and coronary atherosclerosis in women is not well understood, but a similar gender difference in the relationship between HbA_{1C} and CHD has been reported in the recent EPIC (European Prospective Investigation into Cancer) study in Norfolk, in which the risk of CHD among men had already been significantly increased in those with an HbA_{1C} concentration of 5.0–5.4% compared to those with an HbA_{1C} concentration of less than 5.0%, but the coronary risk in women was significantly increased only at an HbA_{1C} concentration of 6% or greater [13].

WHR and obesity

A positive correlation between obesity and coronary atherosclerosis has been reported in Okumiya's Hisayama study and the PDAY studies, in which BMI was identified as a risk factor, but no investigation was done for WHR [17–19,24]. However, epidemiologic studies have demonstrated that indices of abdominal obesity, such as WHR and waist circumference, are more strongly correlated with CHD than BMI [4,27]. This is consistent with the result of multiple regression analysis in the present study, revealing the correlation of coronary atherosclerosis with WHR but not with BMI. As for gender difference, the epidemiologic studies performed in western countries show a correlation between WHR and CHD both in men and in women [4,27], but the correlation between WHR and coronary atherosclerosis was significant only in women in the present study. This difference may result from the difference in the baseline value of WHR between Japanese and Western populations. The mean WHR was generally smaller in men and larger in women in the present study (Table 1) than in Western studies. For example, the mean value of WHR is 0.94 for men and 0.79 for women in the EPIC study [25]. Abdominal obesity is closely related to metabolic syndrome. Many epidemiological studies, including Ninomiya's Hisayama study, demonstrated that metabolic syndrome is a risk factor for CHD [22]. However, the effects of metabolic syndrome on coronary atherosclerosis have not been thoroughly investigated yet and should be elucidated in the future.

Smoking

Evidence from many epidemiologic studies has definitively identified smoking as a strong risk factor for CHD [12,14,40]. On the other hand, there is no clear agreement on the correlation between smoking and coronary atherosclerosis. Some studies have shown a significant correlation between the two [1,19,28], but others, like the present study, have not [8,10,24,30]. The reason for this discrepancy between the clinical and autopsy findings is not thoroughly understood. However, factors other than atherogenesis, such as vasospasm [33], plaque erosion [2], and increased coagulability [20], must be considered when the correlation between smoking and CHD is evaluated. Particularly, vasospasm can be a very important risk factor for Japanese people, because Japanese people are more sensitive to vasospastic agents than Caucasians [26]. Examining angiographic data of Japanese CHD patients, Nobuyoshi et al. [23] found that smoking was strongly related to coronary vasospasm, but not to coronary stenosis. Thus, these results suggest that smoking is not significantly correlated with coronary atherosclerosis at least in Japanese people.

Conclusions

The present study revealed that aging, hypertension, hypercholesterolemia, obesity, and glucose intolerance are risk factors for coronary atherosclerosis in recent Japanese populations. As they are well-known risk factors for CHD as well, the results suggest that these factors may play a part in the development of CHD by affecting coronary atherosclerosis. However, smoking, another strong risk factor for CHD, was not found to be a risk for coronary atherosclerosis in the present study. Mechanism(s) other than atherosclerosis must be considered in evaluating the correlation between smoking and CHD. Finally, the significance of the metabolic risk factors for coronary atherosclerosis was different between men and women. The interaction of the various risk factors and underlying mechanisms for the observed gender differences should be elucidated in the future.

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References

[1] G.S. Berenson, S.R. Srinivasan, W. Bao, W.P. Newman III, R.E. Tracy, W.A. Wattigney, Association between multiple cardiovascular risk factors and atherosclerosis in

children and young adults. The Bogalusa Heart Study, N. Engl. J. Med. 338 (1998) 1650–1656.
[2] A.P. Burke, A. Farb, J. Pestaner, G.T. Malcom, A. Zieske, R. Kutys, J. Smialek, R. Virmani, Traditional risk factors and the incidence of sudden coronary death with and without coronary thrombosis in blacks, Circulation 105 (2002) 419–424.
[3] T.L. Bush, L.P. Fried, E. Barrett-Connor, Cholesterol, lipoproteins, and coronary heart disease in women, Clin. Chem. 34 (1988) B60–B70.
[4] D. Canoy, S.M. Boekholdt, N. Wareham, R. Luben, A. Welch, S. Bingham, I. Buchan, N. Day, K.T. Khaw, Body fat distribution and risk of coronary heart disease in men and women in the European prospective investigation into cancer and nutrition in Norfolk cohort. A population-based prospective study, Circulation 116 (2007) 2933–2943.
[5] Y.S. Chatzizisis, G.D. Giannoglou, G.E. Parcharidis, G.E. Louridas, Is left coronary system more susceptible to atherosclerosis than right? A pathophysiological insight, Int. J. Cardiol. 116 (2007) 7–13.
[6] M. Fujishima, Y. Kiyohara, I. Kato, T. Ohmura, H. Iwamoto, K. Nakayama, S. Ohmori, T. Yoshitake, Diabetes and cardiovascular disease in a prospective population survey in Japan: The Hisayama Study, Diabetes 45 (Suppl. 3) (1996) S14–S16.
[7] M. Fujishima, Y. Kiyohara, K. Ueda, Y. Hasuo, I. Kato, H. Iwamoto, Smoking as cardiovascular risk factor in low cholesterol population: the Hisayama Study, Clin. Exp. Hypertens. 14 (1992) 99–108.
[8] I. Holme, S.C. Enger, A. Helgeland, I. Hjermann, P. Leren, P.G. Lund-Larsen, L.A. Solberg, J.P. Strong, Risk factors and raised atherosclerotic lesions in coronary and cerebral arteries, statistical analysis from the Oslo study, Arteriosclerosis 1 (1981) 250–256.
[9] M. Imakita, C. Yutani, J.P. Strong, I. Sakurai, A. Sumiyoshi, T. Watanabe, M. Mitsumata, Y. Kusumi, S. Katayama, M. Mano, S. Baba, T. Mannami, J. Masuda, K. Sueishi, K. Tanaka, Second nation-wide study of atherosclerosis in infants, children and young adults in Japan, Atherosclerosis 155 (2001) 487–497.
[10] A.R. Kagan, N.H. Sternby, K. Uemura, R. Vaněček, A.M. Vihert, A.M. Lifšic, E.E. Matova, Z. Záhoř, V.S. Ždanov, Atherosclerosis of the aorta and coronary arteries in five towns, Bull. WHO 53 (1976) 485–645.
[11] W.B. Kannel, M.C. Hjortland, P.M. McNamara, T. Gordon, Menopause and risk of cardiovascular disease: the Framingham study, Ann. Intern. Med. 85 (1976) 447–452.
[12] H. Kawano, H. Soejima, S. Kojima, A. Kitagawa, H. Ogawa, Japanese Acute Coronary Syndrome Study (JACSS) Investigators, Sex differences of risk factors for acute myocardial infarction in Japanese patients, Circ. J. 70 (2006) 513–517.
[13] K.T. Khaw, N. Wareham, S. Bingham, R. Luben, A. Welch, N. Day, Association of hemoglobin A1c with cardiovascular disease and mortality in adults: The European Prospective Investigation into Cancer in Norfolk, Ann. Intern. Med. 141 (2004) 413–420.
[14] Y. Kiyohara, K. Ueda, M. Fujishima, Smoking and cardiovascular disease in the general population in Japan, J. Hypertens. 8 (Suppl.) (1990) S9–S15.
[15] M. Kubo, Y. Kiyohara, I. Kato, Y. Tanizaki, H. Arima, K. Tanaka, H. Nakamura, K. Okubo, M. Iida, Trends in the incidence, mortality, and survival rate of cardiovascular disease in a Japanese community: the Hisayama study, Stroke 34 (2003) 2349–2354.
[16] W.C. Little, M. Constantinescu, R.J. Applegate, M.A. Kutcher, M.T. Burrows, F.R. Kahl, W.P. Santamore, Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease?, Circulation 78 (1988) 1157–1166.
[17] H.C. McGill Jr., C.A. McMahan, E.E. Herderick, A.W. Zieske, G.T. Malcom, R.E. Tracy, J.P. Strong for the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group, Obesity accelerates the progression of coronary atherosclerosis in young men, Circulation 105 (2002) 2712–2718.
[18] H.C. McGill Jr., C.A. McMahan, G.T. Malcom, M.C. Oalmann, J.P. Strong, the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group, Relation of glycohemoglobin and adiposity to atherosclerosis in youth, Arterioscler. Thromb. Vasc. Biol. 15 (1995) 431–440.
[19] H.C. McGill Jr., C.A. McMahan, A.W. Zieske, R.E. Tracy, G.T. Malcom, E.E. Herderick, J.P. Strong for the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group, Association of coronary heart disease risk factors with microscopic qualities of coronary atherosclerosis in youth, Circulation 102 (2000) 374–379.
[20] G.J. Miller, K.A. Bauer, J.A. Cooper, R.D. Rosenberg, Activation of the coagulant pathway in cigarette smokers, Thromb. Haemost. 79 (1998) 549–553.
[21] S.J. Nicholls, K. Wolski, I. Sipahi, P. Schoenhagen, T. Crowe, S.R. Kapadia, S.L. Hazen, E.M. Tuzcu, S.E. Nissen, Rate of progression of coronary atherosclerotic plaque in women, J. Am. Coll. Cardiol. 49 (2007) 1546–1551.
[22] T. Ninomiya, M. Kubo, Y. Doi, K. Yonemoto, Y. Tanizaki, M. Rahman, H. Arima, K. Tsuryuya, M. Iida, Y. Kiyohara, Impact of metabolic syndrome on the development of cardiovascular disease in a general Japanese population. The Hisayama study, Stroke 38 (2007) 2063–2069.
[23] M. Nobuyoshi, M. Abe, H. Nosaka, T. Kimura, H. Yokoi, N. Hamasaki, T. Shindo, K. Kimura, T. Nakamura, Y. Nakagawa, N. Shiode, A. Sakamoto, H. Kakura, Y. Iwasaki, K. Kim, S. Kitaguchi, Statistical analysis of clinical risk factors for coronary artery spasm: identification of the most important determinant, Am. Heart J. 124 (1992) 32–38.
[24] N. Okumiya, K. Tanaka, K. Ueda, T. Omae, Coronary atherosclerosis and antecedent risk factors: pathologic and epidemiologic study in Hisayama, Japan, Am. J. Cardiol. 56 (1985) 62–66.
[25] T. Pischon, P.H. Lahmann, H. Boeing, C. Friedenreich, T. Norat, A. Tjønneland, J. Halkjaer, K. Overvad, F.

Clavel-Chapelon, M.C. Boutron-Ruault, G. Guernec, M.M. Bergmann, J. Linseisen, N. Becker, A. Trichopoulou, D. Trichopoulos, S. Sieri, D. Palli, R. Tumino, P. Vineis, S. Panico, P.H. Peeters, H.B. Bueno-de-Mesquita, H.C. Boshuizen, B. van Guelpen, R. Palmqvist, G. Berglund, C.A. Gonzalez, M. Dorronsoro, A. Barricarte, C. Navarro, C. Martinez, J.R. Quirós, A. Roddam, N. Allen, S. Bingham, K.T. Khaw, P. Ferrari, R. Kaaks, N. Slimani, E. Riboli, Body size and risk of colon and rectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC), J. Natl. Cancer Inst. 98 (2006) 920–931.

[26] C. Pristipino, J.F. Beltrame, M.L. Finocchiaro, R. Hattori, M. Fujita, R. Mongiardo, D. Cianflone, T. Sanna, S. Sasayama, A. Maseri, Major racial differences in coronary constrictor response between Japanese and Caucasians with recent myocardial infarction, Circulation 101 (2000) 1102–1108.

[27] K.M. Rexrode, V.J. Carey, C.H. Hennekens, E.E. Walters, G.A. Colditz, M.J. Stampfer, W.C. Willett, J.E. Manson, Abdominal adiposity and coronary heart disease in women, JAMA 280 (1998) 1843–1848.

[28] G.G. Rhoads, W.C. Blackwelder, G.N. Stemmermann, T. Hayashi, A. Kagan, Coronary risk factors and autopsy findings in Japanese-American men, Lab. Invest. 38 (1978) 304–311.

[29] L.A. Simons, Interrelations of lipids and lipoproteins with coronary artery disease mortality in 19 countries, Am. J. Cardiol. 57 (1986) 5G–10G.

[30] P.D. Sorlie, M.R. Garcia-Palmieri, M.I. Castillo-Staab, R. Costas Jr, M.C. Oalmann, R. Havlik, The relation of antemortem factors to atherosclerosis at autopsy. The Puerto Rico Heart Health Program, Am. J. Pathol. 103 (1981) 345–352.

[31] H.C. Stary, A.B. Chandler, R.E. Dinsmore, V. Fuster, S. Glagov, W. Insull Jr., M.E. Rosenfeld, C.J. Schwartz, W.D. Wagner, R.W. Wissler, A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association, Circulation 92 (1995) 1355–1374.

[32] N.H. Sternby, J.E. Fernandez-Britto, P. Nordet, Pathobiological determinants of atherosclerosis in youth (PBDAY Study), 1986–96, Bull. WHO 77 (1999) 250–257.

[33] M. Sugiishi, F. Takatsu, Cigarette smoking is a major risk factor for coronary spasm, Circulation 87 (1993) 76–79.

[34] K. Tanaka, J. Masuda, T. Imamura, K. Sueishi, T. Nakashima, I. Sakurai, T. Shozawa, Y. Hosoda, Y. Yoshida, Y. Nishiyama, C. Yutani, S. Hatano, A nationwide study of atherosclerosis in infants, children and young adults in Japan, Atherosclerosis 72 (1988) 143–156.

[35] C. Tejada, J.P. Strong, M.R. Montenegro, C. Restrepo, L.A. Solberg, Distribution of coronary and aortic atherosclerosis by geographic location, race, and sex, Lab. Invest. 18 (1968) 509–526.

[36] H. Tunstall-Pedoe, Myth and paradox of coronary risk and the menopause, Lancet 351 (1998) 1425–1427.

[37] K. Ueda, T. Omae, Y. Hirota, M. Takeshita, S. Katsuki, K. Tanaka, M. Enjoji, Decreasing trend in incidence and mortality from stroke in Hisayama residents, Japan, Stroke 12 (1981) 154–160.

[38] K. Ueda, T. Omae, Y. Hasuo, Y. Kiyohara, Y. Toshiro, I. Kato, J. Wada, H. Kawano, E. Kajiwara, M. Fujishima, Prevalence and long-term prognosis of mild hypertensives and hypertensives in a Japanese community, Hisayama, J. Hypertens. 6 (1988) 981–989.

[39] H. Ueshima, Explanation for the Japanese paradox: prevention of increase in coronary heart disease and reduction in stroke, J. Atheroscler. Thromb 14 (2007) 278–286.

[40] H. Ueshima, S.R. Choudhury, A. Okayama, T. Hayakawa, Y. Kita, T. Kadowaki, T. Okamura, M. Minowa, O. Iimura, Cigarette smoking as a risk factor for stroke death in Japan: NIPPON DATA80, Stroke 35 (2004) 1836–1841.

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Overexpression of heme oxygenase-1 in coronary atherosclerosis of Japanese autopsies with diabetes mellitus: Hisayama study

Jingyu Song [a,c,1], Shinji Sumiyoshi [a,1], Yutaka Nakashima [d], Yasufumi Doi [b], Mitsuo Iida [b], Yutaka Kiyohara [b], Katsuo Sueishi [a,*]

[a] *Pathophysiological and Experimental Pathology, Department of Pathology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*

[b] *Department of Medicine, Graduate School of Medical Sciences, Kyushu University, Japan*

[c] *Department of Pathology, Yanbian University College of Basic Medicine, Japan*

[d] *Division of Pathology, Fukuoka Red Cross Hospital, Japan*

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Abstract

Few studies regarding the topographical expression of heme oxygenase-1 (HO-1) and its pathophysiological role in human coronary atherosclerotic lesions, particularly in relation to type 2 diabetes mellitus (DM) and intimal angiogenesis, have been reported. HO-1 expression was immunohistochemically examined in 312 tissue blocks of coronary arteries obtained from 53 Japanese autopsy cases in Hisayama cohort study that included 19 diabetic subjects and 34 age- and sex-matched non-diabetic subjects (56–93 years old, mean ± S.D.: 73 ± 10). The HO-1 was ubiquitously distributed in atherosclerotic intima, and was mainly expressed by macrophages and endothelial cells, and partly by smooth muscle cells. The prevalence of HO-1 expression increased as the lesion type (as classified by the American Heart Association (AHA) Committee) and stenotic grade progressed ($p < 0.0001$), and was significantly higher in diabetic than in non-diabetic subjects ($p < 0.01$). This HO-1 overexpression was associated with greater CD-68-positive macrophage infiltration ($p = 0.005$). Interestingly, the distribution of HO-1-positive cells was accentuated in coronary atherosclerotic lesions with intimal microvessels in diabetic subjects ($p < 0.05$), particularly those with hypercholesterolemia ($p < 0.05$), and was preferentially distributed in the shoulder region of atherosclerotic lesion type IV in the AHA classification ($p < 0.01$). In conclusion, HO-1 expression was distributed in overall human coronary atherosclerotic lesions, particularly in diabetic subjects, indicating that HO-1 expression is intimately associated with atherogenesis and may play an important role as an adaptive molecule in the inflammatory-repair process. The association of HO-1 overexpression with a greater extent of intraplaque angiogenesis suggests a multi-faceted role for HO-1 in modulating the progression of atherosclerosis.

Keywords: Heme oxygenase-1; Human coronary artery; Atherosclerosis; Diabetes mellitus; Immunohistochemistry

1. Introduction

Recent emerging evidence supports the hypothesis that atherosclerosis is a chronic inflammatory disease evoked and enhanced by multifactorial etiologies such as dyslipidemia, hypertension, diabetes mellitus (DM), smoking, adiposity and others [1]. All these risk factors are related to oxidative stress. DM in particular has become a worldwide epidemic with its global incidence and prevalence rapidly increasing in both developing and developed countries [2]. Atherosclerosis is the major macro-vascular complication of DM, and coronary heart disease is highly prevalent as a major cause of morbidity and mortality in diabetics [3].

Heme oxygenase (HO) is an initial and rate-limiting enzyme in the oxidative degradation of heme to equimolar quantities of bilirubin as an antioxidant, carbon monoxide (CO) as a vasodilator, and free iron that is promptly sequestered into ferritin [4]. Among HO isoforms, HO-1 can be transcriptionally induced by a variety of

* Corresponding author. Tel.: +81 92 642 6060; fax: +81 92 642 5965.
E-mail address: sueishi@patho1.med.kyushu-u.ac.jp (K. Sueishi).
[1] These authors contributed equally to this work.

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pathophysiological conditions or substances in the cardiovascular system [4,5]. Furthermore, HO-1 has been assumed to have powerful cytoprotective effects on the vascular wall, particularly on endothelial cells (ECs), against oxidation stresses and inflammatory stimuli, mainly by decreasing reactive oxygen stresses [5]. Recent data obtained from animal models of atherosclerosis genetically or pharmacologically manipulated to suppress or overexpress HO-1 confirm that HO-1 is responsible for being atheroprotective [5,6]. In fact, HO-1 gene promoter polymorphism has been reported to relate to the susceptibility to cardiovascular disorders [7].

In addition, HO-1 has recently been assumed to intimately participate in angiogenesis in physiologic and pathologic conditions mainly via vascular endothelial growth factor (VEGF) function [8,9]. Newly formed blood vessels that are ubiquitously distributed in human atherosclerotic plaque [10–12] are assumed to play an important role not only in atherosclerotic plaque progression but also in the destabilization leading to plaque rupture [13–15]. Few studies regarding the relationship between HO-1 expression and plaque angiogenesis have been reported, and the pathophysiological role of HO-1 in human atherosclerotic lesions in diabetics remains unknown.

Wang et al. [16] reported that HO-1 was expressed mainly by macrophages and ECs, and partly by smooth muscle cells (SMCs) in atherosclerotic lesions, particularly fibrofatty lesions, in the human aorta. To our knowledge, however, little is known about the topographical expression of HO-1 and its pathophysiological role in the atherogenesis of human coronary arteries.

The purpose of the present study is to clarify the pathophysiological role of HO-1 in atherogenesis, particularly in diabetics. Thus, we morphometrically examined the relationship between HO-1 expression and macrophage infiltration and intimal neovascularization as morphological phenotypes of the inflammation-repair process in atherosclerotic lesions.

2. Materials and methods

2.1. Subjects and light microscopic examination

Since 1961, we have been conducting a long-term prospective cohort study of cardiovascular disease in the town of Hisayama, a suburb of Fukuoka City in Southern Japan. In 1988, a screening survey of 2742 residents over 40 years of age for the present study was performed in the town [17]. A total of 2742 residents over 40 years of age consented to participate in this survey examination (attendance rate: 80.9%). During the follow-up period from 1988 to July 31, 1996, 310 subjects died and 244 were autopsied (autopsy rate: 78.7%). Among them, 19 subjects with type 2 DM, i.e. with a HbA1c serum level more than 6.0%, and 34 age- and gender-matched NDM controls (56–93 years old, mean ± S.D.: 73 ± 10), who were autopsied within 16 h after death and in whom the histopathology of coronary arteries could be appropriately examined, were randomly selected for this study. Hypercholesterolemia corresponded to a serum level ≥200 mg/dl. Hypertension was defined as blood pressure ≥140/90 mmHg and/or current use of antihypertensie agents. Metabolic syndrome (MetS) was defined by using the criteria recommended in the National Cholesterol Education Program's adult Panel III guidelines [18] with a modification, abdominal obesity was assessed as a waist circumference >90 cm in men and >80 cm in women according to the International Obesity Task Force central obesity criteria for Asia [19]. The study was approved by the ethics committee of the Department of Pathology, Kyushu University, and was performed in accordance with the ethics standards laid down by the 5th revised Declaration of Helsinki, 2000.

The mean age, gender and variables that were selected as possible predictors for coronary atherosclerosis included serum levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and glycohemoglobin (HbA1c), systolic and diastolic blood pressure

Table 1
Mean values or frequencies of cardiovascular risk factors in subjects examined

	Diabetes (n = 19)	Non-diabetes (n = 34)	p
Male (%)	73.7	64.7	0.50
Age (year)	69.8 (65.3–74.3)	74.4 (71.1–77.7)	0.12
Total cholesterol (mg/dl)	195.8 (174.1–217.5)	192.9 (176.5–209.3)	0.83
High-density lipoprotein cholesterol (mg/dl)	47.9 (42.6–53.2)	48.7 (44.2–53.2)	0.83
Triglycerides (mg/dl)	146.6 (103.3–189.9)	115.6 (94.4–136.8)	0.16
HbA1c (%)	7.0 (6.2–7.8)	5.4 (5.2–5.6)	<0.001
Systolic blood pressure (mmHg)	130.0 (124.6–135.4)	127.6 (121.9–133.2)	0.59
Diastolic blood pressure (mmHg)	72.5 (68.0–77.0)	72.8 (69.4–76.2)	0.92
Body mass index (kg/m^2)	22.2 (21.3–23.1)	20.9 (19.9–21.9)	0.08
Waist-to-hip ratio	0.94 (0.90–0.98)	0.90 (0.88–0.92)	0.08
Metabolic syndrome (%)	26.3	9.4	<0.001
Smokers (%)	63.2	61.8	0.92
Drinkers (%)	52.6	41.2	0.42
Sudden death (%)	5.3	8.8	0.64

All values except percentages are expressed as means and 95% confidence interval. Sudden death: death 1 h after clinical onset of symptoms.

(BP), body mass index (BMI), waist-to-hip ratio (WHR), the frequency of MetS, and smoking and drinking habits. These are shown in Table 1.

2.2. Histopathological examination

In all subjects, the heart was fixed with 10% formalin. Six specimens of coronary arteries were taken from each heart for histopathological examination. According to the American Heart Association (AHA) classification of coronary sections, these included segments 1, 2, 6, 7, 11 and 12. All specimens embedded in paraffin were cut into 3-μm-thick serial sections. Specimens with severe calcification were decalcified with acetic acid, and, therefore, the histological evaluation of the extent of calcium was based on the visualization of the calcified matrix and not on calcium per se. Histological sections were stained with hematoxylin and eosin (HE), Elastica-van Gieson (EVG) and Masson's trichrome (MT). The atherosclerotic lesion type of each specimen was carefully classified in accordance with the definitions proposed by the Committee on Vascular Lesions of the Council on Arteriosclerosis of AHA [20]. The atherosclerotic lesions were again classified into two categories, with lesion types I through III classified as early lesions and lesion types IV through VI classified as advanced lesions.

2.3. Immunohistochemistry

An anti-human HO-1 rabbit polyclonal antibody against recombinant N-terminal 15 amino-peptide of HO-1 (HC3001) and this recombinant HO-1 amino-peptide were purchased from Biomol International LP (PA, USA). The cell species-specific antibodies used were as follows: CD68 (KP-1, Dako A/S, Glostrup, Denmark) for the monocytes/macrophages, α-smooth muscle actin (α-SMA) for SMCs and CD34 (Novocastra, Newcastle upon Tyne, UK) mainly for the ECs.

An immunohistochemical examination was performed according to the standard two-step technique using polymeric conjugates as secondary antibodies (ChemMate EnVision, DAKO A/S) as previously reported [21]. In brief, the sections were deparaffinized, boiled in 10 mM citrate buffer (pH 6.0) in a pressure vessel to unmask the antigens, and then incubated with 3% skimmed milk-PBS solution to minimize the nonspecific binding of the primary antibody. The sections were incubated with the primary antibodies overnight at 4 °C in a moisture chamber, then incubated with the appropriate polymeric conjugate. To inhibit any endogenous peroxidase activity, the sections were incubated with % (wt/vol) H_2O_2–methanol solution. The visualization of a positive reaction was developed using a peroxidase substrate solution containing 3, 3′-diaminobenzidine tetrahydrochloride, and the sections were then lightly counterstained with hematoxylin.

To confirm the immunohistochemical specificity of each reaction, non-immune rabbit and mouse isotype IgGs were used as the negative controls instead of the respective primary antibody. Human liver and spleen tissue sections were used as the positive controls for HO-1 immunohistochemistry. In addition, the specificity of the anti-human HO-1 antibody was further confirmed with an antibody absorption experiment; namely, the primary anti-HO-1 antibody solution was incubated with a fivefold excess of recombinant human HO-1 at the molar ratio for 1 hr at room temperature, then centrifuged and the supernatant was used for immunohistochemical examination.

2.4. Morphometric study

By using an Olympus high-image color-camera OHD-200 and Scion Image Soft, the luminal stenosis of each coronary artery section was calculated as reported previously [11]. According to the Scion Image program instructions, the necrotic core size and calcified matrix area of the intima in each section were also measured, and the area percentage of each parameter per intimal area was then calculated.

The number of CD68-, α-SMA- or HO-1-positive cells in the intima at high-power field (HPF, 400×) were counted in more than 2 areas where the positive cells were mostly distributed, and was recorded as the number of positive cells. The immunohistochemically positive cell numbers/HPF for respective cell species were compared with those in matched serial sections separately stained with other immunohistochemical stains. The total number of intimal blood vessels, which were lined with CD34-positive ECs, was also counted under HPF [11]. To analyze topographical differences in the HO-1-positive cell distribution, the AHA-Type IV lesion (atheormatous plaque) was classified into the following four area categories depending on its location: plaque shoulder (S), fibrous cap (Fc), deep region of necrotic core (D), and other (O) [21]. The above-mentioned comparison was also made among these categories.

2.5. Statistical analysis

The results are presented as means ± S.D. unless otherwise stated. The data were statistically analyzed by means table ANOVA with Fisher's post hoc test and the chi-square test, and a comparison of the non-paired non-parametric data among more than three groups was made using the Kruskal–Wallis rank test followed by Mann–Whitney's *U*-test. The correlation between the HO-1-positive cell number/HPF and the intimal microvessel number was analyzed using Spearman's correlation analysis and j-statistic analysis. A value of $p < 0.05$ was considered statistically significant. Tests were performed using Statview software (SAS Institute, Cary, NC, USA).

3. Results

3.1. Clinical characteristics of subjects examined in DM and NDM groups

The clinical characteristics of the subjects in the diabetic and nondiabetic (DM and NDM, respectively) groups are shown in Table 1. The mean serum level of HbA1c and the frequency of MetS were statistically higher in the DM group than the NDM group, but other possible risk factors for cardiovascular disease were not, including serum levels of TC and HDL-C, systolic and diastolic BP, BMI, WHR current smokers and drinkers, and the incidence of sudden death.

3.2. Histopathology of coronary arteries

The mean heart weight was not statistically different between the DM and NDM groups (data not shown). Out of 318 coronary arterial specimens obtained from 53 subjects, 6 specimens were avoided due to marked artificial changes, and 312 coronary arterial specimens available for analysis were classified according to the AHA classification. Forty percent were classified as diffuse intimal thickening (DIT), 5% as type I, 10% as type II, 21% as type III, 4% as type IV, 18% as type V, and 2% as type VI (Supplementary data Fig. I). Therefore, the major histologic types consisting of about three-fourths in this study were DIT and early atherosclerotic lesions of types I–III.

To characterize the histopathological differences of atherosclerotic plaque in DM and NDM, the area percentages of necrotic core size, luminal stenosis and calcified matrix area were morphometrically assessed, and the occurrence of complex lesions such as thrombus formation, plaque rupture and intraplaque hemorrhage were counted (Supplementary data Table I). Necrotic core was apparent in 42 specimens (17 in DM and 25 in NDM), and 53 calcification foci (23 in DM and 30 in NDM) were noticed in atherosclerotic intimas. The area percentages of the necrotic core size and calcified matrix area were not statistically different between the two groups. As the coronary atherosclerotic lesions progressed from type I to type VI, the luminal stenosis correlatively progressed ($P < 0.0001$, data not shown), but was not significantly different between the DM and NDM groups. In addition, the mean percentage of luminal stenosis was higher in both the early (lesion types I–III) and advanced lesions (types IV–VI) in DM than it was in NDM, but this difference was not statistically significant. Among six sections with type VI lesions, thrombus formation without atheroma rupture was associated with DM in two sections; two sections showed atheroma rupture, one with DM and one with NDM; and two sections were complicated with intraplaque hemorrhage around the calcified area, one each in DM and NDM. However, the frequency of occurrence of these complicated lesions also shows no statistical significance between DM and NDM (Supplementary data Table I).

3.3. Immunohistochemical expression of HO-1 and HO-1-positive cell species in coronary artery

To characterize the topographical distribution of HO-1 in DIT and atherosclerotic plaques, all 312 blocks were immunohistochemically examined, and HO-1-positive cell numbers/HPF were calculated in each tissue type. Furthermore, to define the HO-1-positive cell species, the sequential sections of each block were immunohistochemically examined with CD68, α-SMA and CD34 for macrophages, SMCs and ECs, respectively. The expression of HO-1 was ubiquitously distributed in atherosclerotic lesions, but was extremely scarce in DIT, where HO-1 was expressed only by luminal ECs. The number of HO-1-positive cells/HPF increased as the atherosclerotic lesions progressed (data not shown, $p < 0.0001$ by Kruskal–Wallis rank test). In atherosclerotic lesions, HO-1 was mainly expressed by macrophages/monocytes (Figs. 1B and C, and 2B and C), ECs not only of the luminal surface (Fig. 1B and F) but also intimal newly formed blood vessels (Fig. 2B and D), and partly by SMCs, particularly in the fibrous cap of lesions types IV–VI (Fig. 1B, D, F and G). HO-1 reactivity was apparent not only in foamy macrophages around the atheroma (Fig. 1B and C) and within fatty streaks but also in non-foamy macrophages infiltrating around intimal microvessels (Fig. 2B and C). Interestingly, the macrophages scattered around the microvessels and ECs of these newly formed blood vessels were frequently and simultaneously positive for HO-1 (Fig. 2B and D). Lymphocytes and plasmacytes showed no apparent positive reaction to HO-1.

3.4. Topographical distribution of HO-1-positive cells, macrophages and SMCs in coronary atherosclerotic lesions with DM and NDM

To characterize the topographical distribution of HO-1-positive cells in coronary atherosclerotic lesions of the DM and NDM groups, all specimens except those with DIT were analyzed for the density of HO-1-positive cells as well as macrophages and SMCs. The mean of each positive cell density of six arterial specimens examined per patient was compared among the groups with atherosclerotic risk factors, including DM, hypercholesterolemia, hypertension and smoking. The mean number of HO-1-positive cells and CD-68-positive cells in coronary atherosclerotic lesions was significantly higher in DM than NDM (Table 2). Furthermore, in patients additionally with hypercholesterolemia and current smoking, the density of HO-1-positive cells and macrophages was also higher in DM than in NDM ($p < 0.05$). From these findings, DM seemed to correlate strongly with HO-1 expression and macrophage infiltration.

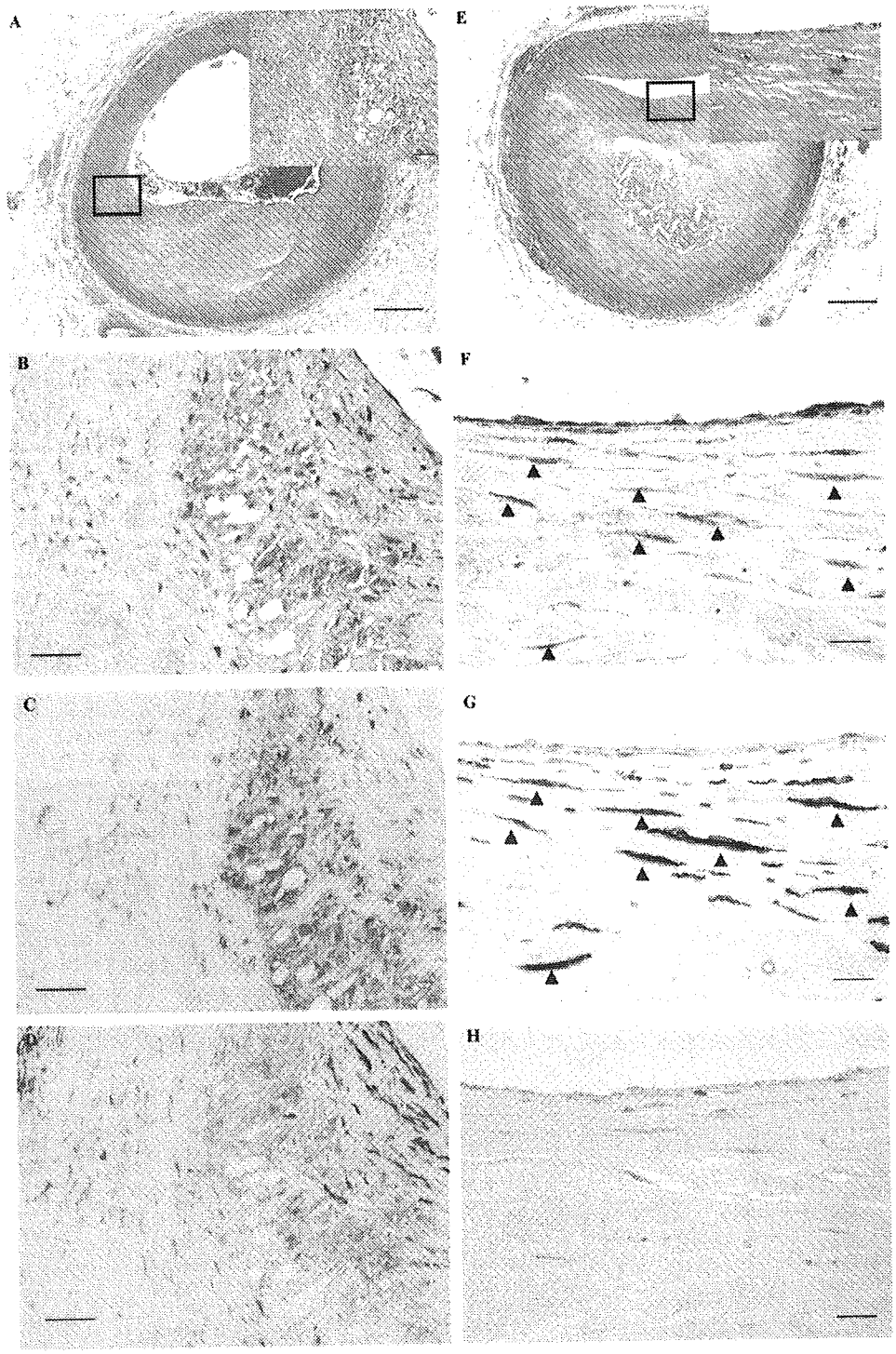

Fig. 1. Immunohistochemical expression of HO-1 in macrophages, endothelial cells and smooth muscle cells in atherosclerotic lesions, Serial sections were examined with HE staining (A and E) and immunohistochemically for HO-1 (B and F), CD68 (C and H), and (-SMA (D and G). The boxed square areas shown in the inserts, indicated in A and E, are demonstrated for the immunohistochemistry of B–D and F–H, respectively. HO-1 is apparently expressed in the macrophages/monocytes (B and C), SMCs (F and G) and luminal ECs of the coronary artery (B and F). The arrowheads in F and G indicate the cells to be positive for HO-1 (F) and (-SMA (G), but negative for CD68 (H). Scale bars represent 500 μm in A and E, 50 μm in B through D, and 20 μm in F through H.

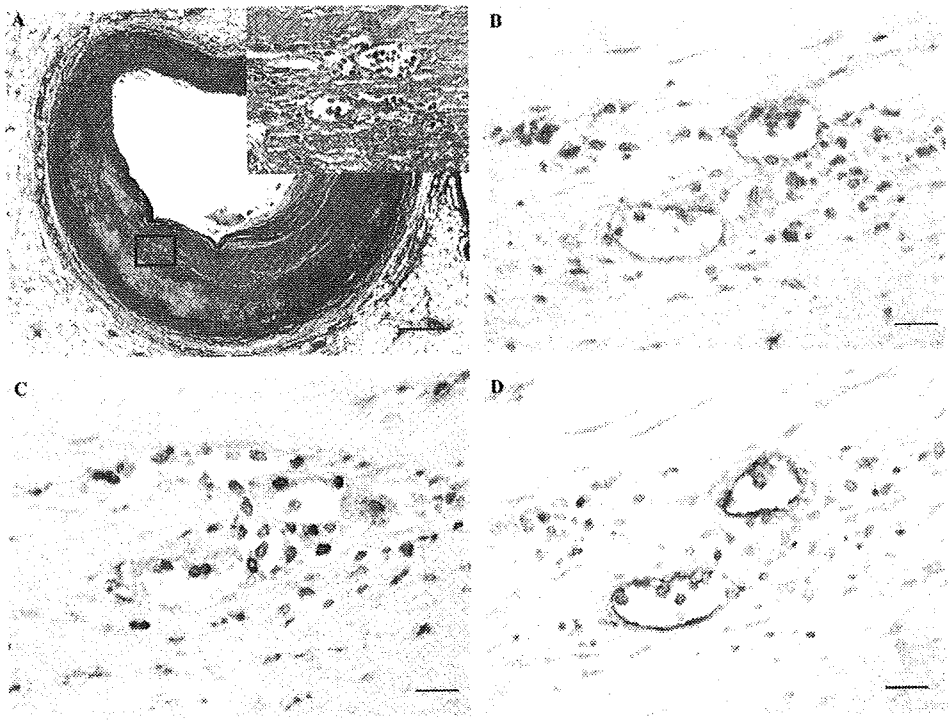

Fig. 2. Immunohistochemical expression of HO-1 in endothelial cells in atherosclerotic lesions, Serial sections were examined with Masson's trichrome (E), and immunohistochemically for HO-1 (B), CD68 (C) and CD34 (D). The boxed square area shown in the insert, indicated in A, demonstrates the immunohistochemistry of B–D. HO-1 is expressed by endothelial cells of newly formed microvessels (B and D), and macrophages/monocytes (B and C) within and around microvesels in atherosclerotic intima. Scale bars represent 500 μm in A and 50 μm in B through D.

3.5. HO-1 expression and intimal neovascularization in atherosclerotic lesions of DM and NDM

As HO-1-positive macrophages frequently infiltrated around newly formed blood vessels in atherosclerotic intimas (Fig. 2B and C), we next examined next the status of vascular density in atherosclerotic intimas in DM and NDM and its correlation with HO-1 expression and atherosclerosis risk factors including hypercholesterolemia. As shown in Table 2, the intimal vascular density per patient was significantly higher in DM than in NDM ($p < 0.05$). With the addition of hypercholesterolemia, the intimal vascular density was more accentuated in DM than NDM ($p < 0.05$). This intimal vascular density, however, was not affected by current smoking. For further confirming evidence regarding the role of HO-1 in intimal angiogenesis, we examined the correlation between the HO-1-positive cell number and the newly formed microvessel number in all subjects examined. Among

Table 2
Immunohistochemical characteristics of atherosclerotic lesions in diabetes and non-diabetes

	Diabetes	Non-diabetes	*p*
HO-1-positive cells ($n = 48$)	27.0 ± 11.3 (17)	19.2 ± 6.0 (31)	0.008
Hypercholesterolemia ($n = 23$)	26.9 ± 12.1 (7)	19.0 ± 6.1 (16)	0.045
Hypertension ($n = 16$)	22.1 ± 8.3 (4)	20.4 ± 8.6 (12)	0.705
Smoking ($n = 28$)	27.3 ± 12.5 (11)	18.9 ± 8.2 (17)	0.037
CD68-positive cells ($n = 48$)	25.2 ± 10.1 (17)	17.6 ± 7.5 (31)	0.005
Hypercholesterolemia ($n = 23$)	25.7 ± 11.8 (7)	17.9 ± 5.5 (16)	0.035
Hypertension ($n = 16$)	21.4 ± 7.9 (4)	18.0 ± 9.9 (12)	0.463
Smoking ($n = 28$)	25.5 ± 11.4 (11)	18.0 ± 8.1 (17)	0.042
Intimal microvessels ($n = 43$)	32.0 ± 20.8 (15)	21.6 ± 12.3 (28)	0.044
Hypercholesterolemia ($n = 23$)	41.0 ± 23.5 (8)	23.5 ± 12.9 (15)	0.030
Smoking ($n = 23$)	34.3 ± 23.0 (10)	20.0 ± 12.4 (13)	0.115

HO-1- and CD68-positive cell numbers were calculated from the mean of the 6 arterial specimens per patient. *n* in parentheses represents the number of patients examined in each group. Each value is expressed as mean ± S.D. Intimal microvessels: the number of microvessels lined with CD34-positive endothelial cells.

these patients the HO-1-positive cell number was well correlated with the microvessel number ($R=0.675$, $p<0.01$). This relationship was also statistically significant in both the DM ($R=0.762$, $p<0.01$) and NDM groups ($R=0.564$, $p<0.01$), but the correlation between the DM and NDM groups was not statistically significant.

3.6. Topographical characteristics of HO-1 expression in atheromatous plaque (lesion type IV) in DM and NDM

Fourteen tissue specimens of AHA-type IV lesion (5 of DM and 9 of NDM) were examined to confirm whether there were any differences in the degree of HO-1 expression among the topographically different areas of atheromatous plaque as described in Section 2. The number of HO-1-positive cells showed a significant variance ($p<0.0001$, Kruskal–Wallis rank test) among the four location areas of AHA-type IV lesions of DM compared with NDM, and the HO-1-positive cell density was significantly higher in the S and Fc regions of DM than in those of NDM ($p<0.01$ and $p<0.05$, respectively), but not in the D and O regions (Supplementary data Fig. II).

4. Discussion

Clinical accumulating evidence indicates that DM increases the incidence of and accelerates atherosclerotic diseases [3]. In the present study, to analyze the pathophysiological role of HO-1 in coronary atherogenesis of diabetic subjects, HO-1 expression was immunohistochemically examined in the coronary arteries of 53 Japanese autopsied patients composed of 19 type 2 diabetics and 34 age- and sex-matched nondiabetic subjects, all of whom had been surveyed in the "Hisayama cohort study" [18,19]. We directly clarified the following key observations: (1) The HO-1 was ubiquitous in human coronary atherosclerotic lesions and was largely expressed by macrophages and ECs of both coronary arteries and intimal newly formed microvessels, and partly by SMCs in atherosclerotic intimas. (2) The extent of HO-1 expression and macrophage infiltration increased as the lesion type and stenotic grade progressed, and was significantly higher not only in early lesions but also in advanced lesions in the DM group compared with the NDM group. (3) Interestingly, the distribution of HO-1-positive cells was accentuated in coronary atherosclerotic lesions with newly formed microvessels in the DM group. These findings indicate that HO-1 expression is intimately associated with human coronary atherogenesis including intimal angiogenesis. Though HO-1 could play an anti-inflammatory role in atherosclerotic lesions, the possible participation of HO-1 in the intimal angiogenesis of atherosclerosis may also be responsible for the progression of atherosclerosis and plaque instability, particularly in diabetic subjects.

Few reports studying the detailed pathological characteristics of HO-1 expression in human atherosclerotic lesions are available [17]. To our best knowledge, this is the first report indicating that HO-1 expression is ubiquitously distributed in human coronary atherosclerotic lesions and is significantly enhanced in diabetic compared with nondiabetic subjects. The inflammatory process evoked via oxidative stress has been thought to intimately participate in the development and progression of atherosclerosis [1], and the oxidative stress induces antioxidatives including HO-1, which has been well established to function antiatherogenically in animal models of atherosclerosis [5,6]. It is, however, unclear whether atherogenic stimuli such as DM, dyslipidemia, hypertension and others can equally promote HO-1 expression in human atherosclerosis. The present study demonstrates that HO-1 expression is significantly enhanced in diabetic compared with nondiabetic subjects, with a greater macrophage content in atherosclerotic lesions. This enhancement of HO-1 expression is additionally upregulated by hypercholesterolemia and smoking, but not by hypertension. These findings support the contention that human atherogenesis is really multifactorial and suggest that DM, hypercholesterolemia and smoking among various types of atherogenic risk factors contribute greatly to HO-1 expression in human coronary atherosclerotic lesions. Recent studies indicate that the stress-responsive elements-Bach1-Nrf2 signal pathway [22] and PPARα and PPARγ/their ligands [23] would transcriptionally regulate HO-1 expression in vascular SMCs and ECs. These transcriptional pathways have been well known to contribute widely to the regulation of the inflammatory-proliferative process in the vasculature, evoked by several atherogenic stimuli including advanced glycation end products overexpressed in DM [24]. In addition, the heterogeneous expression of HO-1 may relate to the severity of human atherosclerosis and the incidence of such atherosclerotic diseases. In fact, a longer GT repeat in human HO-1 promoter has been suggested to result in the decrease of HO-1 transcriptional activity [7,23]. Together with these findings, the cumulative evidence favors therapeutic exploitation using an HO-1 induction strategy [6,25,26].

A few studies comparing the pathologic characteristics of human atherosclerotic lesions in diabetic and nondiabetic patients have been reported [27,28]. A diabetic or prediabetic state as indicated by elevated serum glycohemoglobin levels promotes coronary and aortic atherosclerosis not only in youths but also in adults. Burke et al. [28] reported that macrophage infiltration and necrotic core size play a greater role in the progression of atherosclerosis in diabetic adults who die suddenly. The current study indicates that diabetes is not associated with larger necrotic core size than in nondiabetic subjects, partly due to the small number of subjects examined. In addition, the fact that the major histologic types of more than about three-fourths of the Japanese subjects examined in this study consisted mainly of DIT and early atherosclerotic lesions of types I–III (Supplementary data Fig. I) may be relevant to the larger population of early atherosclerotic lesions and fibrous plaque in Japanese youths [29]. Macrophage infiltration was significantly fre-

quent in diabetic subjects, particularly those with advanced atherosclerotic lesions, in comparison to nondiabetic subjects, and was further accentuated by the co-existence of hypercholesterolemia and smoking. Furthermore, in our study the frequency of MetS was higher in diabetic subjects. The Hisayama cohort study suggested that MetS is a significant risk factor for the development of cardiovascular disease and found a possibility that the increased risk of MetS for cardiovascular disease resulted from the influence of diabetes [30]. These findings suggest that the association of DM with atherosclerosis in the Japanese population is particularly significant for the prevention programs highlighted in the recent Hisayama study [30].

Angiogenesis is an essential process not only physiologically in organ development and tissue regeneration but also pathologically in inflammation and cancer. Newly formed microvessels are ubiquitously distributed in atherosclerotic plaque [11–13], and this angiogenic process has been recently assumed to participate intimately in atherosclerotic progression [14] and in the occurrence of atherothrombosis [15,16]. Recent studies suggest that HO-1 is also involved in physiologic and pathologic angiogenesis [9,10], essentially via the functions of VEGF-A and gas mediators such as NO and CO. Bussolati et al. [9] proposed that HO-1 would play a bifunctional role during an inflammation-repair process, namely, anti-inflammatory action inhibiting leukocytic infiltration and the promotion of VEGF-driven noninflammatory angiogenesis, resulting in the facilitation of a sequential transition from active inflammation to noninflammatory tissue repair. However, the pathological function of HO-1 has not been fully clarified in the chronic inflammatory process including atherogenesis. The current study clearly demonstrated that the extent of newly formed microvessels is well correlated with the degree of HO-1 expression in atherosclerotic plaque, and that hypercholesterolemia in addition to DM accentuates the incidence of intimal angiogenesis. Furthermore, the topographical localization of HO-1 expressed by macrophages and ECs is closely distributed within and around intimal microvessels (Fig. 2). Devesa et al. [9] reported that upregulated HO-1 sustained chronic inflammation in an animal model of chronic arthritis by enhancing inducible nitric oxide synthase expression and VEGF-related angiogenesis. Together with these findings, angiogenesis may play bifunctional and reverse roles in pathological states, partly resulting in the outcome of angiogenic diseases including atherosclerosis and cancer. Though it remains undetermined whether the modulation of HO-1 expression accelerates or suppresses atherogenesis in an animal model of atherosclerosis associated with intimal angiogenesis similar to human lesions, further studies will be necessary to clarify the pathophysiological role of HO-1 in human atherogenesis and to assess the therapeutic effect of HO-1 on atherosclerosis-related disease.

In conclusion, our data demonstrate that HO-1 is ubiquitously upregulated in human coronary atherosclerotic lesions, particularly in diabetics, and that the extent of HO-1 expression is well correlated with the degrees of macrophage infiltration and angiogenesis in atherosclerotic plaque. Thus, HO-1 may participate intimately in the inflammatory-repair process during atherogenesis. Although it has not been determined how HO-1 function activates intraplaque angiogenesis, our findings suggest that HO-1 plays diverse roles in the progression of human coronary atherosclerosis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2008.05.057.

References

[1] Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
[2] Qiao Q, Hu G, Tuomilehto J, et al. DECODA Study Group. Age- and sex-specific prevalence of diabetes and impaired glucose regulation in 11 Asian cohorts. Diabetes Care 2003;26:1770–80.
[3] Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA 2002;287:2570–625.
[4] Ryter SW, Alam J, Choi AMK. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiol Rev 2006;86:583–650.
[5] Morita T. Heme oxygenase and atherosclerosis. Arterioscler Thromb Vasc Biol 2005;25:1786–95.
[6] Orozco LD, Kapturczak MH, Barajas B, et al. Heme oxygenase-1 expression in macrophages plays a beneficial role in atherosclerosis. Circ Res 2007;100:1703–11.
[7] Kaneda H, Ohno M, Taguchi J, et al. Heme oxygenase-1 gene promoter polymorphism is associated with coronary artery disease in Japanese patients with coronary risk factors. Arterioscler Thromb Vasc Biol 2002;22:1680–5.
[8] Bussolati B, Ahmed A, Pemberton H, et al. Bifunctional role for VEGF-induced heme oxygenas-1 in vivo: induction of angiogenesis and inhibition of leukocytic infiltration. Blood 2004;103:761–6.
[9] Devesa I, Ferárndiz L, Guillén I, et al. Potential role of heme oxygenase-1 in the progression of rat adjuvant arthritis. Lab Invest 2005;85:34–44.
[10] Kumamoto M, Nakashima Y, Sueishi K. Intimal neovascularization in human coronary atherosclerosis: its origin and pathophysiological significance. Human Pathol 1995;26:450–6.
[11] Chen Y-X, Nakashima Y, Tanaka K, et al. Immunohistochemical expression of vascular endothelial growth factor/vascular permeability factor in atherosclerotic intimas of human coronary arteries. Arterioscler Thromb Vasc Biol 1999;19:131–9.
[12] Nakano T, Nakashima Y, Yonemitsu Y, et al. Angiogenesis and lymphangiogenesis and expression of lymphangiogenic factors in the

atherosclerotic intima of human coronary arteries. Human Pathol 2005;36:330–40.
[13] Ohtani K, Egashira K, Hiasa K, et al. Blockade of vascular endothelial growth factor suppresses experimental restenosis after intraluminal injury by inhibiting recruitment of monocyte lineage cells. Circulation 2004;110:2444–52.
[14] Moreno PR, Purushothaman KR, Fuster V, et al. Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: implications for plaque vulnerability. Circulation 2004;110:2032–8.
[15] Virmani R, Kolodgie FD, Burke AP, et al. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. Arterioscl Thromb Vasc Biol 2005;25:2054–61.
[16] Wang L-J, Lee T-S, Lee F-Y, et al. Expression of heme oxygenase-1 in atherosclerotic lesions. Am J Pathol 1998;152:711–20.
[17] Kubo M, Hata J, Ninomiya T, et al. A nonsynonymous SNP in *PRKCH* (protein kinase Cη) increases the risk of cerebral infarction. Nat Genet 2007;39:212–7.
[18] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–97.
[19] World Health Organization/International Association for the Study of Obesity/International Obesity Task Force. The Asia-Pacific perspective: redifining obesity and its treatment. Available at: http://www.diabetes.com.au/pdf/obesity_report.pdf.
[20] Stary HC, Chandler AB, Dinsmore RE, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 1995;92:1355–74.
[21] Sumiyoshi S, Nakashima Y, Chen Y-X, et al. Interleukin-10 expression is positively correlated with oxidized LDL deposition and inversely with T-lymphocyte infiltration in atherosclerotic intimas of human coronary arteries. Pathol Res Pract 2006;202:141–50.
[22] Sun J, Brand M, Zenke Y, et al. Heme regulates the dynamic exchange of Bach 1 and NF-E2-related factors in the Maf transcription factor network. Proc Natl Acad Sci USA 2003;101:1461–6.
[23] Kränke G, Kadl A, Ikonomu E, et al. Expression of heme oxygenase-1 in human vascular cells is regulated by peroxisome proliferator-activated receptors. Arterioscl Thromb Vasc Biol 2007;27:1276–82.
[24] Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. Nat Med 2004;10:355–61.
[25] Wu BJ, Kathir K, Witting PW, et al. Antioxidants protect from atherosclerosis by a heme oxygenase-1 pathway that is independent of free radical scavenging. J Exp Med 2006;203:1117–27.
[26] Ali F, Hamdulay SS, Kinderlerer AR, et al. Statin-mediated cytoprotection of human vascular endothelial cells: a role for Kruppel-like factor 2-dependent induction of heme oxygenase-1. J Thromb Haemost 2007:2537–46.
[27] McGill HC, McMahan CA, Malcom GT, et al. Relation of glycohemoglobin and adiposity to atherosclerosis in youth. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Arterioscler Thromb Vasc Biol 1995;15:431–40.
[28] Burke AP, Kolodgie FD, Zieske A, et al. Morphologic findings of coronary atherosclerotic plaques in diabetics: a postmortem study. Arteriocler Thromb Vasc Biol 2004;24:1266–71.
[29] Kisanuki Y, Asada Y, Sato Y, et al. Coronary atherosclerosis in youths in Kyushu island, Japan: histological findings and stenosis. J Atheroscler Thromb 2000;6:55–9.
[30] Ninomiya T, Kubo M, Doi Y, et al. Impact of metabolic syndrome on the development of cardiovascular disease in Japanese population: the Hisayama study. Stroke 2007;38:2063–9.

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Uric Acid and Left Ventricular Hypertrophy

Shigeyuki Saitoh, MD

In this issue of the Journal, Mitsuhashi et al report that levels of uric acid (UA) were positively associated with electrocardiographically diagnosed left ventricular hypertrophy (LVH) in healthy Japanese men! The result was independent of body mass index, hypertension, diabetes, hyperlipidemia and age; similar results were obtained in both the normal and high blood pressure (BP) subgroups. Theirs was an epidemiological study, but the reported serum concentrations of UA add important information to the assessment of risk factors and preventing cardiovascular disease, especially heart failure.

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The relationship between UA and cardiovascular disease has been known since the first half of the 20th century and several studies have identified an association between increased UA and cardiovascular risk in the general population. The positive association between serum UA and hypertension was also observed over a century ago. Although elevated UA levels have been predictive of hypertension in epidemiological studies, the relationship between UA and BP is confounded by numerous factors, including age, diabetes, obesity, alcohol use, and sodium intake or volume status. Recent findings in animal models have helped elucidate possible mechanisms whereby UA may lead to hypertension, and have spurred a renewed interest in discerning a causal role for elevated UA in hypertension.

On the other hand, the presence of hypertensive organ damage signals a condition of increased risk for cardiovascular and renal morbidity and mortality. Thus, the search for LVH, atherosclerosis and microalbuminuria as hypertensive organ damage, which likely reflect both the severity of BP load and other nonhemodynamic risk factors, is currently recommended as part of global risk assessment. Mitsuhashi et al show new findings of a relationship between UA and LVH in Japanese, regardless of the presence of hypertension!

There are already reports of the relation between UA and LVH in Japanese with hypertension. For example, Kurata et al reported that serum UA levels correlated positively with left ventricular (LV) mass and indexed LV mass (LVMI) in male hypertensive patients, but not in female hypertensive patients in a cross-sectional study[2] Iwashima et al also demonstrated that UA is independently associated with LVMI and suggested that the combination of hyperuricemia and LVH is an independent and powerful predictor of cardiovascular disease[3]

With the exception of specific genetic defects in purine metabolism, increased UA is generally associated with important risk factors for atherosclerosis, such as hypertension, abdominal obesity, insulin resistance, metabolic syndrome and heart failure. Many studies have also clearly shown an association between increased UA concentrations and oxidative stress, endothelial dysfunction and inflammation. At the very least, an increased UA level is an independent marker of cardiovascular disease and a risk factor in cardiovascular diseases and hypertension. The question is whether UA is the cause of these risk factors or a morbid vascular change.

Because of being an epidemiological study, the results of Mitsuhashi et al's investigation do not suggest whether an elevation of the serum UA level is the cause or result of LVH! A consideration of the mechanism of UA production and metabolism offers insight into the relationship between UA level and cardiovascular change. Primarily, the association between UA and LVH might relate to an association of UA with other risk factors, especially renal dysfunction, oxidative stress, BP, and obesity. UA is excreted primarily by the kidney, so decreased renal perfusion could lead to increased serum UA and activation of the renin–angiotensin system; angiotensin II is essential for the development of LVH by myocardial remodeling[4] It is well known that angiotensin II induces hypertrophy and hyperplasia of myocytes and vascular smooth muscle cells, as well as influencing the expression of fibrogenic cytokine, and possibly inducing perivascular and interstitial fibrosis[5]

Secondly, UA levels may reflect xanthine oxidase pathway activity, which has the potential to contribute to the progression of LV dysfunction by interfering with myocardial efficiency[6] and myofilament calcium sensitivity[7] UA is a metabolic byproduct of purine metabolism and its serum level may increase because of increased generation, decreased excretion, or a combination of these mechanisms. UA is produced in the terminal step of purine metabolism catalyzed by xanthine oxidase (XO). XO pathway activity also results in the production of superoxide. XO is inhibited by allopurinol, which inhibited progression of cardiac hypertrophy in an animal model of hypertension without changing BP[8]

Furthermore, there are several possible contributors to increased UA production in cardiac disease, especially heart failure, including increased abundance and activity of XO, increased conversion of xanthine dehydrogenase to XO, or increased XO substrate resulting from enhanced ATP breakdown to adenosine and hypoxanthine under such conditions. XO activity participates in both mechano-energetic uncoupling and vascular dysfunction in the failing circulation. Mechano-energetic uncoupling is the process whereby cardiac energy consumption remains the same or increases

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Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan
Mailing address: Shigeyuki Saitoh, MD, Second Department of Internal Medicine, Sapporo Medical University, S-1, W-16, Chuo-ku, Sapporo 060-8543, Japan. E-mail: ssaitoh@sapmed.ac.jp