TABLE 3. Simple Correlations of Cholesterol Profiles by HPLC Method With Clinical Parameters (n=62)

Clinical Parameters	Age	BMI	VFA	SFA	UA	IRI	PAI-1	TG
Total VLDL	0.100	0.208	0.508†	0.055	0.368‡	0.283§	0.171	0.943†
Large VLDL	0.013	0.245	0.405‡	0.120	0.242	0.185	0.224	0.930†
Medium VLDL	0.126	0.210	0.488†	0.066	0.385‡	0.254§	0.192	0.951†
Small VLDL	0.119	0.070	0.434†	−0.063	0.327‡	0.318§	0.002	0.531†
Total LDL	0.229	0.146	0.431‡	0.090	0.220	0.349‡	0.010	0.057
Large LDL	0.079	0.009	0.115	0.016	0.083	0.183	−0.121	−0.329‡
Medium LDL	0.219	0.144	0.386‡	0.098	0.190	0.311§	0.006	−0.014
Small LDL	0.243	0.202	0.571†	0.111	0.268§	0.380‡	0.122	0.406‡
Very small LDL	0.240	0.219	0.556†	0.105	0.292§	0.375‡	0.164	0.577†
Total HDL	−0.230	−0.281§	−0.528†	−0.180	−0.269§	−0.295§	0.091	−0.418†
Very large HDL	0.200	−0.191	−0.095	−0.232	−0.139	−0.149	−0.180	−0.151
Large HDL	0.057	−0.334‡	−0.426†	−0.212	−0.356‡	−0.385‡	−0.277	−0.400‡
Medium HDL	−0.329‡	−0.251§	−0.502†	−0.159	−0.182	−0.220	0.203	−0.378‡
Small HDL	−0.382‡	0.240	−0.022	0.145	0.178	0.241	0.617†	0.126
Very small HDL	−0.303§	0.153	−0.029	0.238	0.031	0.010	0.369‡	0.045
LDL size*	−0.071	−0.246	−0.389‡	−0.188	−0.158	−0.170	−0.325§	−0.577†
HDL size*	0.166	−0.371‡	−0.368‡	−0.236	−0.354‡	−0.393‡	−0.325§	−0.364‡

Values are Pearson correlation coefficients.
*Average particle diameters (nm) obtained from LDL and HDL peak time by HPLC.
†$P<0.001$; ‡$P<0.01$; §$P<0.05$.

($r=0.431$, $P<0.001$) between VFA and total LDL-C was obtained as presented in Table 3. There was no correlation between VFA and total LDL-C in the low LDL-C group but a significant positive correlation ($r=0.546$, $P<0.002$) in the high LDL-C group.

Scattered plots between VFA and LDL subclasses are presented in Figure 2. Large LDL-C showed no significant correlations with VFA in total population and the high LDL-C group but showed a significant negative correlation ($r=-0.446$, $P<0.02$) in the low LDL-C group. Small LDL-C and very small LDL-C showed significant positive correlations with VFA in total population and both subgroups, except for very small LDL-C in high LDL-C group.

TABLE 4. Partial Correlations of Cholesterol Profiles by HPLC Method With VFA (n=62)

Controlling Factor	TG	TC	HDL-C	LDL-C
Total VLDL	0.009	0.362‡	0.363‡	0.495†
Large VLDL	−0.301§	0.278§	0.253§	0.427†
Medium VLDL	−0.084	0.386‡	0.336‡	0.522†
Small VLDL	0.257§	0.227	0.344‡	0.324§
Total LDL	0.476†	0.016	0.317§	0.141
Large LDL	0.366‡	−0.259§	0.155	−0.356‡
Medium LDL	0.466†	−0.026	0.266§	−0.035
Small LDL	0.458†	0.346‡	0.403†	0.437†
Very small LDL	−0.357‡	0.348‡	0.389‡	0.424†
Total HDL	−0.397‡	−0.514	−0.044	−0.460
Very large HDL	−0.016	−0.126	0.074	−0.106
Large HDL	−0.274§	−0.348‡	−0.082	−0.337‡
Medium HDL	−0.383‡	−0.497†	−0.089	−0.445†
Small HDL	−0.107	−0.157	0.134	−0.061
Very small HDL	−0.063	−0.047	0.292§	0.044
LDL size*	−0.116	−0.313§	−0.221	−0.379‡
HDL size*	−0.220	−0.277§	−0.081	−0.278§

Values are Pearson partial correlation coefficients. TC, HDL-C, and LDL-C are the values obtained by enzymatic method, precipitation method, and Friedewald equation.
*Average particle diameters (nm) obtained from LDL and HDL peak time by HPLC.
†$P<0.001$; ‡$P<0.01$; §$P<0.05$.

Discussion

It is well known that measurement of lipoprotein subclasses other than LDL-C and HDL-C is very important for prediction of risk for CHD. Recently, the Adult Treatment Panel (ATP)-III claimed that one component of atherogenic dyslipidemia is small LDL particles[4] but did not recommend a routine clinical measurement of small LDL particles because of lack of standard and inexpensive methodologies. We offer in this study another alternative method superior to NMR and other methods, giving cholesterol levels for all lipoprotein subclasses simultaneously. Size exclusion HPLC has been used for decades in lipoprotein research applications[23,30] but only recently has become sufficiently robust for consideration as a routine method.

Analytical precision of HPLC was demonstrated for the first time to be acceptable in the determination of 20 component peaks for 3 VLDL, 4 LDL, and 5 HDL subclasses as shown in Table 2, which is well comparable to major lipoprotein quantification reported previously.[25,30,31]

Our HPLC and the traditional methods gave a good agreement in LDL-C and HDL-C values ($r>0.97$) for 62 men in this study. Because Friedewald equation and precipitation methods were used for determination of LDL-C and HDL-C in large-scale epidemiological studies, our HPLC method could be used as an alternative technique in clinical studies. We already compared this method with ultracentrifugation, and very high correlations were obtained.[23] Each of the ultracentrifugally isolated fractions consisted of several sub-

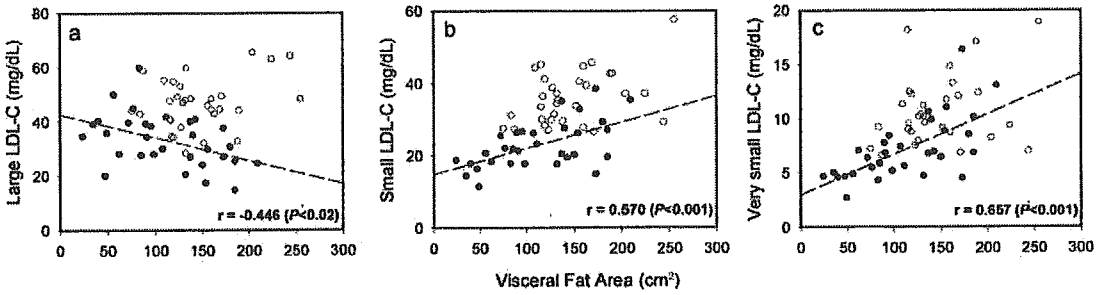

Figure 2. Scatter plots of VFA against (a) large LDL-C, (b) small LDL-C, and (c) very small LDL-C for high LDL-C group (○) and low LDL-C group (●). Dashed lines represent a linear regression for low LDL-C group. Correlation coefficients and *P*-values are also presented for low LDL-C group (n=31).

classes as follows: VLDL fraction, large VLDL (10% to 30%)+medium VLDL (45%)+small VLDL (10% to 25%); IDL fraction, small VLDL (40%)+large LDL (35%)+medium LDL (25%); LDL fraction, large LDL (10% to 30%)+medium LDL (35% to 40%)+small LDL (20% to 35%)+very small LDL (5% to 15%); HDL_2 fraction, very large HDL (5% to 10%)+large HDL (50%)+medium HDL (30% to 40%); and HDL_3 fraction, medium HDL (55%)+small HDL (35% to 40%).

LDL subclass profiles were compared between HPLC and an electrophoretic method using 3% polyacrylamide gels (Lipoprint LDL system)[16] on non–insulin-dependent diabetes mellitus patients (n=87), and LDL-score values determined by the Lipoprint system were positively correlated with small LDL-C (r=0.356, P<0.001) and very small LDL-C (r=0.604, P<0.0001), respectively (M. Okazaki et al, unpublished data, 2004).

Obesity is a major cause of atherosclerotic vascular disease in industrial countries. Obesity is a heterogeneous phenotype, and there is some confusion in the fat distribution literature regarding measurements and indices used to assess regional fat distribution. Subcutaneous skinfolds, skinfold ratio, circumferences, or circumference ratios have been used, and more recently CT has been used to distinguish between measurements of subcutaneous and visceral fat accumulation at any site of the body.[29] We examined the relationship of cholesterol profiles in major lipoproteins and their subclasses by HPLC to various clinical parameter in 62 men with a wide range of anthropometric values to cover a large spectrum of body fat variation (Table 1).

Recent advances in the biology of adipose tissue have revealed that adipose tissue is not simply an energy storage organ, but it also secretes a variety of molecules which affect the metabolism of the whole body.[32] It has been clarified that adipose tissue development and the extent of subsequent fat accumulation are closely associated with the occurrence of advancement of the metabolic syndrome. The presence of obesity increases the risk of thrombotic vascular diseases. Plasma PAI-1 levels were closely correlated with VFA but not with SFA in human subjects.[33] Moreover, visceral fat accumulation is well known to be associated with insulin resistance through the increase of serum free fatty acid followed by the increase of VLDL production by liver.[34,35] In the subjects of this study, there was a weak correlation between VFA and PAI-1 (r=0.261, P<0.05; data not shown) but a strong positive correlation between VFA and IRI (r=0.443, P<0.001; data not shown). As presented in Table 3, the degree of correlations of major lipoproteins and their subclasses with IRI and PAI-1 were different. The strong positive correlations of small and very small HDL with PAI-1 were observed, although positive correlations of small LDL and very small LDL and negative correlation of large HDL with IRI were obtained. Similar correlations were obtained between subclasses and UA, which is increased under overnutrition state in obesity.

Visceral obesity causes various metabolic abnormalities including the increase of serum TG, and there was a strong positive correlation between VFA and serum TG levels in this study (r=0.536, n=62, P<0.0001; data not shown). The increase of serum TG was the result of the increased VLDL as shown by very strong positive correlations of TG with total VLDL-C and all VLDL-C subclasses (Table 3). The increased TG in VLDL results in its flow into LDL and HDL by cholesterol ester transfer protein (in exchange with cholesterol ester), and TG is kept hydrolyzed in LDL and HDL,[36,37] which clearly were demonstrated in our data by the results of negative correlations of serum TG levels with LDL and HDL particle size and by the positive correlation with small and very small LDL-C and negative correlations with large and medium HDL-C. Among anthropometric values, VFA showed a strong positive correlation with total VLDL, all VLDL subclasses, total LDL, and LDL subclasses, except for large LDL, and a negative correlation with total HDL and large and medium HDL as presented in Table 3. These statistical correlations between lipoprotein subclasses and VFA may be the consequence of the increase of TG. But in this study, these significant correlations remained after adjustment for serum TG level (Table 4). Therefore, we did not think the increase of small LDL or decrease of HDL might simply be the consequence of high serum TG in this study. Moreover, the large VLDL and small VLDL were correlated positively and negatively with VFA, respectively, after adjustment for serum TG levels. In our study, the influence of TG increase in fasting state could be evaluated, but the contributions of TG increase in postprandial state to lipoprotein subclass distributions need to be examined in future studies.

As shown in Table 4, partial correlation analysis by controlling LDL-C showed a different contribution of visceral fat accumulation to the cholesterol levels in LDL subclasses. In this study, large LDL-C was negatively associated with visceral fat accumulation in low LDL-C groups as presented in Figure 2. Increased CHD risk associated with

reductions of HDL could be related to reciprocal increases of IDL or smaller, denser LDL, or related considerably to parallel reduction of a component within the larger LDL subclasses. Different contribution of visceral fat accumulation to large LDL-C level demonstrated only by a new subclass analysis with HPLC might reflect the difference of atherogenicity of LDL subclasses. Our new approach demonstrating the presence of unique LDL particles by component analysis will help to discover new lipoprotein particles contributing to atherosclerotic diseases.

Our HPLC method can provide the cholesterol levels of major lipoproteins and their subclasses in a small amount of whole serum or plasma (<10 μL) within 16 minutes; this technique might be very useful in a large-scale clinical study.

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Original Article

Blunted Reduction of Pulse Pressure during Nighttime Is Associated with Left Ventricular Hypertrophy in Elderly Hypertensive Patients

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Increased pulse pressure (PP) is recognized as a risk factor for cardiovascular disease, especially in elderly patients. However, blood pressure (BP) is known to have a circadian variation. Therefore, this study asked whether or not PP has a circadian variation and, if so, whether a circadian variation of PP has clinical importance. Ambulatory BP monitoring (every 30 min for 48 h) was performed in 255 patients with untreated essential hypertension (24 to 82 years old; mean: 52±12 years). Left ventricular mass index (LVMI) was estimated from M-mode echocardiography. PP was decreased during nighttime (10±11% reduction from daytime PP). Multivariate linear regression analysis showed that, among four variables—the degree of nighttime PP reduction, daytime PP, 48-h systolic BP, and nondipper hypertension—the degree of nighttime PP reduction had the strongest (inverse) correlation with LVMI in a subgroup of elderly patients (≥60 years old, n=67) (standardized regression coefficient=−0.32, p=0.02), whereas this association was not significant in the whole patient population unclassified by age. Furthermore, a blunted reduction of nighttime PP in combination with nondipper hypertension was an incremental risk for increase in LVMI in the elderly patients. In conclusion, PP is reduced during nighttime, but the degree of reduction varies among patients. The blunted reduction of nighttime PP is a risk for left ventricular hypertrophy, an established predictor of hypertension-induced cardiovascular events, and it may thus play a role in cardiovascular complications, especially in elderly patients with nondipper hypertension. (*Hypertens Res* 2004; 27: 573–579)

***Key Words*: blood pressure, hypertension, hypertrophy**

Introduction

Hypertension is the most prevalent cardiovascular disease and causes various cardiovascular complications. Recently, increased pulse pressure (PP) was shown to be a risk factor for cardiovascular disease, especially in elderly patients (*1*). The association between PP and cardiovascular diseases is thought to be independent of systolic and diastolic blood pressure (SBP and DBP, respectively).

Mean ambulatory PP correlates with organ damage more closely than does office PP (*2*). It is known that blood pressure (BP) rises during the day and decreases during nighttime (*3*), although the magnitude of fluctuation varies among

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Table 1. Clinical Characteristics of Study Patients

	<60 years (n=188)	≥60 years (n=67)
Sex (% men)	55	52
Age (years)	47±9	68±6##
BMI (kg/m²)	25±3	23±3##
Smoker (%)	44	35
Diabetes mellitus (%)	10	3
Total cholesterol (mg/dl)	208±41	208±41
HDL cholesterol (mg/dl)	56±16	57±15
Triglyceride (mg/dl)	152±95	124±63#
Serum creatinine (mg/dl)	0.68±0.19	0.74±0.21#
Hemoglobine A1c (%)	5.3±0.7	5.4±0.4
LVMI (g/m²)	121±33	132±35#
SV (ml)	71±16	68±19
Office pulse rate (bpm)	71±9	71±9
Office BP (mmHg)		
Systolic	160±18	160±16
Diastolic	100±10	92±9##
Pulse pressure	59±14	68±15##

Values represent % of total or mean±SD. # $p<0.05$, ## $p<0.01$ compared with <60 years old patients. BMI, body mass index; HDL, high-density lipoprotein; LVMI, left ventricular mass index; SV, stroke volume; BP, blood pressure.

individual cases. Previous studies (*4–6*) have demonstrated that hypertensive target organ damage—including left ventricular hypertrophy (LVH)—is frequent in nondipper hypertension, a decreased fall in nighttime BP. It is well-known that LVH is causally related to high BP and represents hypertensive target organ damage (*7*). This study, therefore, investigated a possible circadian change in PP and its relation to LVH in 255 patients with untreated essential hypertension.

Methods

Study Subjects

The study population consisted of 255 consecutive patients with essential hypertension. All of these patients were untreated for hypertension and visited Yamanashi University Hospital. Hypertension was defined according to the Sixth Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-VI) criteria (*7*). All patients fulfilled the following inclusion criteria: the averaged values of two or more BP measurements obtained on at least two separate occasions were ≥140 mmHg SBP or ≥90 mmHg DBP, with waking ambulatory BP measurements ≥135/85 mmHg or sleeping ambulatory BP measurements ≥120/75 mmHg. None of the patients had secondary hypertension, congestive heart failure, previous myocardial infarction, cardiomyopathy, valvular heart diseases, previous stroke, or serum creatinine concentration >1.5 mg/dl. Diabetes mellitus was diagnosed when the random glucose level was ≥200 mg/dl, or when the hemoglobin A1c was ≥6.5%, or when the patient was treated by dietary restrictions, oral hypoglycemics, or insulin. Smoking was defined as >10 cigarettes per day for >1 year. Patients' characteristics are shown in Table 1. Written informed consent was obtained from all patients prior to commencement of the study. The study protocol was approved by the Ethics Committee of the University of Yamanashi.

Ambulatory BP Measurements

SBP, DBP, PP, and heart rate (HR) during daily activities were measured every 30 min for 48 h by the oscillometric method, using a noninvasive ambulatory BP monitoring system (TM-2425; A&D, Tokyo, Japan) (*8*). The daytime and nighttime mean values of SBP, DBP, PP, and HR during the 48-h period were analyzed by reviewing the patients' diaries. We defined daytime as the period from the time the patients awoke to the time they went to sleep, and nighttime as the period during which they were sleeping. The daytime and nighttime SBP, DBP, PP, and HR were the averages of the respective values over the 2 days of monitoring. Dipper hypertension was defined by the presence of a fall (≥10%) in the mean nighttime SBP and DBP from the respective daytime values. Nondipper hypertension was defined by the absence of the fall (≥10%) in the mean nighttime SBP, and/or in the mean nighttime DBP. The percent reduction of nighttime PP from daytime PP was calculated as (daytime mean PP − nighttime mean PP) × 100 / (daytime mean PP).

Echocardiography

We performed echocardiography using a 2.5 MHz transducer with a Sonos-5500 echocardiographic unit (Hewlett Packard, Andover, USA). Left ventricular mass (LVM) and stroke volume (SV) were estimated from M-mode echocardiography (*8*). The LVM index (LVMI) was defined as LVM divided by body surface area (BSA). All echocardiographic studies were performed by physicians unaware of the patients' clinical data.

Statistical Analysis

Results are expressed as the mean±SD. The mean values and frequencies of continuous variables were compared between the 2 groups using the unpaired t-test and χ^2 analysis, respectively. Comparison between more than 3 groups was performed using one-way ANOVA. Analyses for the associations were performed using a linear regression technique. Nondipper hypertension, 48-h SBP, and daytime PP, which have traditionally been considered to be risk factors for LVH, were included as independent covariables in a multivariate linear regression analysis for the association between

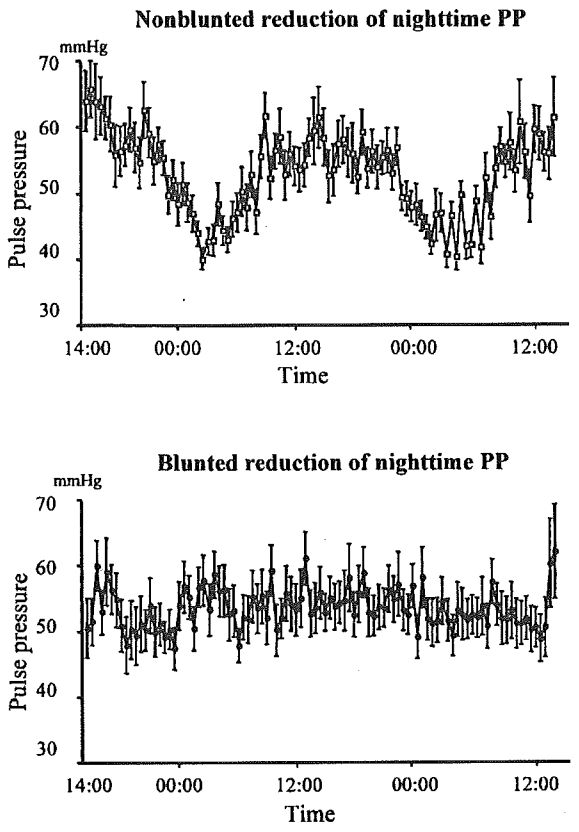

Fig. 1. *Circadian changes in pulse pressure during 48-h ambulatory blood pressure monitoring. The upper panel shows the circadian changes in pulse pressure from 20 representative patients with nonblunted reduction of nighttime pulse pressure. The lower panel shows the data from 20 representative patients with blunted reduction of nighttime pulse pressure. Data are shown as the mean±SD.*

LVMI as a dependent variable and percent reduction of nighttime PP as an independent variable. The circadian variation of BP was coded by the following dummy variables: 0 for dipper hypertension; or 1 for nondipper hypertension. A confidence level of $p<0.05$ was considered statistically significant. Analyses were partially assessed using StatView 5.0 (SAS Institute, Cary, USA).

Results

Circadian Changes of PP

In all patients, PP was not constant throughout either day or night, but, in a majority of patients, there was a circadian fluctuation of PP (*i.e.*, a reduction during nighttime), as shown in Fig. 1. The percent reduction of nighttime PP from daytime PP reached up to 40% with a mean of 10±11% of the daytime PP. The percent reduction of nighttime PP from daytime PP had a significant and inverse relation with 48-h SBP, nighttime SBP, nighttime DBP, 48-h PP, and nighttime PP, as shown in Table 2. It was significantly lower in those with nondipper hypertension than those with dipper hypertension (% reduction from daytime PP: 3±9% in nondippers [$n=114$] *vs.* 16±8% in dippers [$n=141$], respectively, $p<0.01$). The percent reduction of nighttime PP had no significant relation with serum levels of total cholesterol or high-density lipoprotein cholesterol, as shown in Table 2. The percent reduction of nighttime PP was comparable between patients with and without diabetes (data not shown).

Table 2. Relation of % Reduction of Nighttime Pulse Pressure to Clinical Parameters in the Whole Patient Population Unclassified by Age

		r	*p*
Age		−0.11	0.09
Body mass index		−0.04	0.52
Total cholesterol		−0.02	0.82
HDL cholesterol		0.06	0.72
Left ventricular mass index		−0.11	0.07
Stroke volume		−0.25	0.48
Office BP			
Systolic		−0.07	0.22
Diastolic		0.05	0.48
Pulse pressure		−0.12	0.049
Ambulatory BP			
Systolic	48 h mean	−0.18	0.003
	Daytime	0.05	0.44
	Nighttime	−0.55	<0.001
Diastolic	48 h mean	−0.03	0.57
	Daytime	0.07	0.28
	Nighttime	−0.24	<0.001
Pulse pressure	48 h mean	−0.25	<0.001
	Daytime	−0.04	0.93
	Nighttime	−0.63	<0.001

HDL, high-density lipoprotein; BP, blood pressure.

Association of Nighttime PP Reduction with LVMI

The percent reduction of nighttime PP from daytime PP had a significant and inverse relation to LVMI in a subgroup of elderly hypertensive patients (≥60 years), as shown in Fig. 2 and in Table 3, whereas this relation was not significant in either the whole patient population unclassified by age or a subgroup of non-elderly hypertensive patients (<60 years), as shown in Tables 2 and 3. Daytime PP and 48-h SBP also had a significant and positive relation with LVMI in the elderly patients ($r=0.33$, $p=0.004$ and $r=0.31$, $p=0.007$, respectively) in univariate linear regression analyses. Further, nondippers had a greater LVMI than dippers (139±36 g/m^2 *vs.* 121±31 g/m^2, respectively, $p=0.04$) in the elderly hypertensive patients. However, multivariate regression analy-

Table 3. Comparisons of Relation of % Reduction of Nighttime Pulse Pressure to Clinical Parameters between the Elderly Patients (≥ 60 Years) and the Non-Elderly Patients (< 60 Years)

		<60 years		≥60 years	
		r	*p*	*r*	*p*
Age		−0.01	0.87	−0.16	0.20
Body mass index		−0.06	0.41	−0.13	0.31
Total cholesterol		−0.04	0.58	0.08	0.52
HDL cholesterol		0.05	0.61	0.16	0.31
Left ventricular mass index		−0.01	0.86	−0.39	0.009
Stroke volume		−0.04	0.59	−0.10	0.40
Office BP					
Systolic		−0.04	0.61	−0.23	0.06
Diastolic		0.03	0.67	−0.16	0.43
Pulse pressure		−0.07	0.33	−0.20	0.11
Ambulatory BP					
Systolic	48 h mean	−0.15	0.04	−0.34	0.004
	Daytime	0.09	0.22	−0.16	0.20
	Nighttime	−0.54	<0.001	−0.58	<0.001
Diastolic	48 h mean	0.04	0.38	−0.14	0.25
	Daytime	−0.06	0.38	−0.06	0.61
	Nighttime	−0.25	<0.001	−0.26	0.03
Pulse pressure	48 h mean	−0.20	0.007	−0.36	0.003
	Daytime	0.07	0.37	−0.17	0.18
	Nighttime	−0.62	<0.001	−0.65	<0.001
Heart rate	48 h mean	0.09	0.22	0.08	0.51
	Daytime	0.14	0.06	0.09	0.47
	Nighttime	−0.03	0.64	0.12	0.92

HDL, high-density lipoprotein; BP, blood pressure.

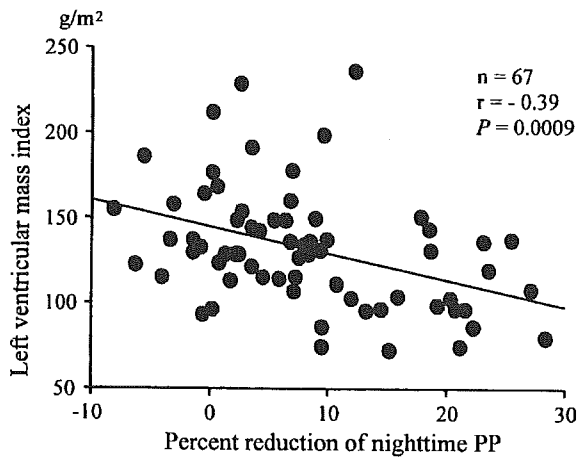

Fig. 2. *Correlation between the percent reduction of nighttime pulse pressure (PP) and left ventricular mass index in elderly hypertensive patients.*

sis showed that, among four variables—the percent reduction of nighttime PP, daytime PP, 48-h SBP, and nondipper hypertension—only the percent reduction of nighttime PP and daytime PP remained significantly correlated with LVMI in the elderly patients, as shown in Table 4. And the percent reduction of nighttime PP had the strongest (inverse) association with LVMI.

Table 4. Multivariate Linear Regression Analysis for Association of Left Ventricular Mass Index with Parameters Related to Blood Pressure in the Elderly Patients

	Standardized regression coefficients	*p*
% reduction of nighttime PP	−0.32	0.02
Daytime PP	0.39	0.03
48 h mean SBP	0.14	0.46
Nondipper hypertension	0.16	0.38

PP, pulse pressure; SBP, systolic blood pressure.

Blunted reduction of nighttime PP (<7% reduction of nighttime PP from daytime PP, with 7.0% corresponding to the median value of the % reduction of nighttime PP in the elderly patients) in combination with nondipper hypertension or higher daytime PP (>60 mmHg, corresponding to the median value of the daytime PP in the elderly patients) conferred an incremental risk of increase in LVMI in the elderly

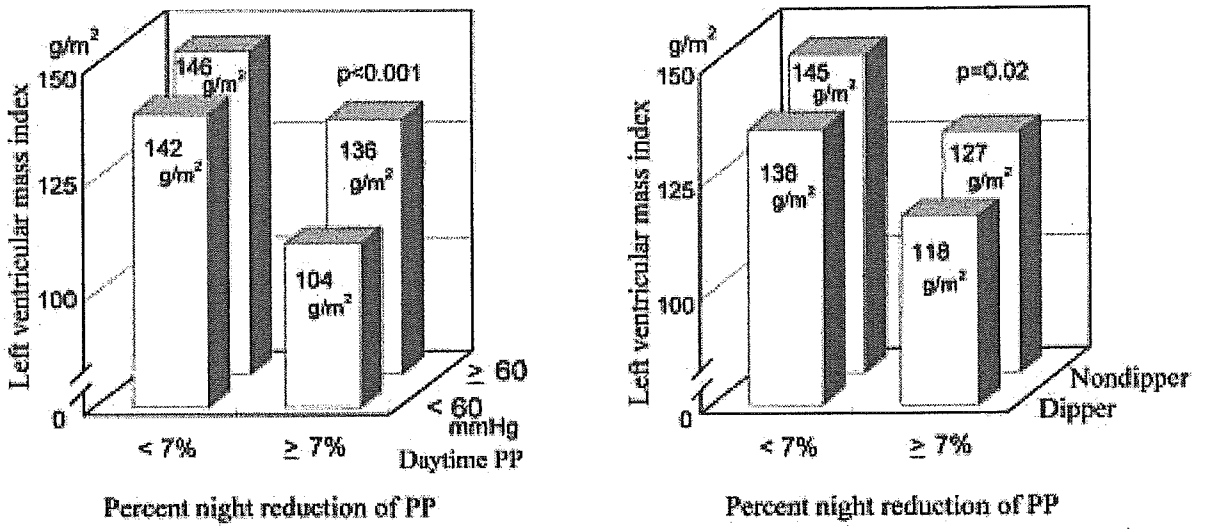

Fig. 3. *Incremental effects on left ventricular mass index with the combination of blunted reduction of nighttime pulse pressure (PP) and nondipper hypertension (right panel) or higher daytime PP (left panel). Blunted reduction of nighttime PP was defined as less than 7.0% reduction in nighttime PP with respect to daytime PP, with 7.0% corresponding to the median value in the elderly patients. Higher PP during daytime was defined as daytime PP exceeding 60 mmHg, which corresponds to the median value in the elderly patients. Statistical analyses were performed with ANOVA.*

Table 5. Comparisons of Variables of Amburatoly BP Measurments between Eldery Patients with and without Blunted Reduction of Nighttime PP

	% reduction of nighttime PP	
	≥7% (n=33)	<7% (n=34)
Systolic BP		
48 h mean	140±10	147±13#
Daytime	148±10	150±13
Nighttime	127±12	140±16##
Diastolic BP		
48 h mean	83±6	84±9
Daytime	86±6	88±8
Nighttime	75±7	78±10
PP		
48 h mean	58±9	62±8#
Daytime	61±9	63±8
Nighttime	52±9	62±9##

Values are mean±SD. # $p<0.05$, ## $p<0.01$ compared with ≥7% reduction of nighttime PP. BP, blood pressure; PP, pulse pressure.

patients, as shown in Fig. 3.

Comparisons of Ambulatory BP Measurements between Elderly Patients with and without Blunted Reduction of Nighttime PP

The elderly patients with <7% reduction of nighttime PP had higher 48-h SBP, nighttime SBP, 48-h PP, and nighttime PP, and similar levels of DBP parameters as compared with those with ≥7% reduction of nighttime PP, as shown in Table 5. The extent of decrease in SBP was greater than that of DBP, leading to a greater reduction in nighttime PP in the elderly patients with ≥7% reduction of nighttime PP. On the other hand, the extent of decrease in SBP was comparable to that of DBP, leading to a blunted reduction in nighttime PP in the elderly patients with <7% reduction of nighttime PP, as shown in Fig. 4. The elderly patients with <7% reduction of nighttime PP had a higher frequency of smoking, but not

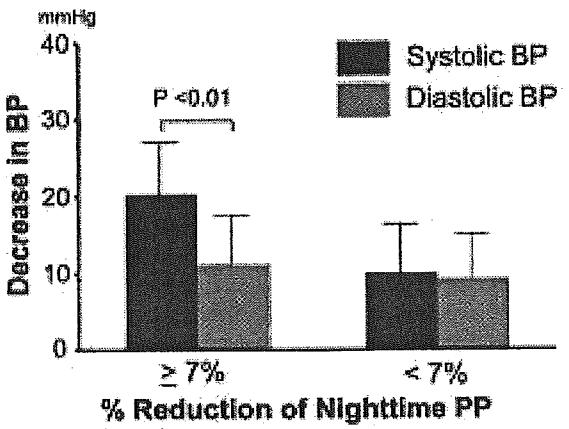

Fig. 4. *Comparisons of decreases in systolic and diastolic BP from daytime to nighttime in elderly patients.*

Table 6. Comparisons of Clinical Characteristics between Eldery Patients with and without Blunted Reduction of Nighttime PP

	% reduction of nighttime PP	
	≥7% (n=33)	<7% (n=34)
Sex (% men)	48	56
Age (years)	67±6	66±7
BMI (kg/m^2)	22±3	23±3
Smoker (%)	21	44$^{\#}$
Diabetes mellitus (%)	5	9
Total cholesterol (mg/dl)	209±32	207±48
HDL cholesterol (mg/dl)	60±17	55±14
Serum creatinine (mg/dl)	0.7±0.2	0.8±0.3
LVMI (g/m^2)	119±34	146±30$^{\#\#}$
SV (ml)	65±18	70±21

Values are mean±SD. $^{\#}p<0.05$, $^{\#\#}p<0.01$ compared with ≥7% reduction of nighttime PP. PP, pulse pressure; BMI, body mass index; HDL, high-density lipoprotein; LVMI, left ventricular mass index; SV, stroke volume.

of diabetes or dyslipidemia, as compared with those with ≥7% reduction of nighttime PP, as shown in Table 6.

Discussion

This study demonstrated that PP has a circadian variation, decreasing during nighttime in a majority of patients. However, the extent of nighttime reduction of PP varies among individuals. The present study further showed that the blunted reduction of nighttime PP is significantly associated with LVH in elderly hypertensive patients independently of daytime PP, 48-h SBP, and nondipper hypertension, and that the association of the blunted reduction of nighttime PP with LVH is the strongest among the covariates. Therefore, the blunted reduction of PP during nighttime might play a role in the pathogenesis of hypertension-induced cardiac damage in elderly patients. The present study also showed that nondipper hypertension is associated with a smaller reduction of nighttime PP. This was expected, because patients with nondipper hypertension are known to show a minimal decrease in BP during nightitme, which would lead to a blunted reduction of nighttime PP. In fact, this study also showed that a smaller reduction of nighttime SBP resulted in a blunted reduction of nighttime PP. Thus, the blunted reduction of nighttime PP may be intimately related to the pathogenesis of cardiovascular complications in nondipper hypertension. Furthermore, the present study demonstrated that the blunted reduction of nighttime PP confers an additional risk—in patients with nondipper hypertension—for LVH. Thus, the combination of a blunted reduction of nighttime PP and nondipper hypertension is a strong risk factor for LVH in elderly hypertensive patients.

Recently, increased PP was shown to be a risk factor for cardiovascular mortality in elderly patients (*1*). Stiffening of the central elastic arteries, which reflects biological aging of the arterial system, tends to raise SBP and lower DBP. The former, which causes a disproportionate increase in end-systolic stress, promotes the development of cardiac hypertrophy and requires a greater coronary blood flow. The latter reduces the pressure on which coronary flow is dependent, and together they increase the vulnerability of the heart to ischemia. This explains why an increase in PP is a major predictor of cardiovascular risk in elderly hypertensive patients. The blunted reduction of PP from day to night, which persistently increases PP, might cause further progression of hypertension-induced organ damage, thereby resulting in a higher incidence of cardiovascular events. In fact, the present study showed that, in patients with higher daytime PP, the blunted reduction of nighttime PP conferred an additional risk for LVH. Taken together, these results indicate that the combination of an increase in PP and the blunted reduction of nighttime PP could be a risk for cardiovascular complications in elderly hypertensive patients. It is, however, necessary to confirm the blunted reduction of nighttime PP as a new risk for cardiovascular disease in a prospective study with a large number of the patients. Previous studies (*9*, *10*) have shown that diabetes and dyslipidemia are associated with an increase in PP. The present study, however, showed that the elderly patients with a blunted reduction of nighttime PP had higher frequency of smoking, but not higher frequency of diabetes or dyslipidemia. Although the mechanism for the positive association between smoking and the blunted reduction of nighttime PP remains to be determined, this association may play a possible role in the pathogenesis of smoking-related cardiovascular diseases.

SBP increases with age, while DBP rises only until 50 years of age, after which it either becomes constant or even decreases slightly. In the Framingham Heart Study (*1*), increasing age entailed a shift from DBP to SBP and then to PP as the major predictor of cardiovascular risk. Thus, PP became superior to SBP as a predictor of cardiovascular diseases in elderly hypertensive patients (*1*). These features in elderly hypertension may explain why, in the present multivariate analysis, the percent reduction of nighttime PP, but not 48-h SBP, remained significantly associated with LVMI in elderly patients, and why the significant association was observed in the elderly hypertensive patients but not in the younger patients or the total population.

In conclusion, PP is decreased during nighttime in elderly hypertensive patients, but the extent of the decrease varies case-by-case. The blunted reduction of PP during nighttime is a risk factor for LVH and may play a role in cardiovascular complications in elderly patients.

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Circ J 2004; **68:** 923–927

Correlation of Plasma Concentrations of B-Type Natriuretic Peptide With Infarct Size Quantified by Tomographic Thallium-201 Myocardial Scintigraphy in Asymptomatic Patients With Previous Myocardial Infarction

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Background Secretion of A-type (atrial) and B-type (brain) natriuretic peptides (ANP and BNP) increases in relation to left ventricular (LV) dysfunction in patients with myocardial infarction (MI). However, it is unknown what determines the concentrations of ANP and BNP in asymptomatic MI patients with preserved LV function, so the aim of the present study was to examine if they are associated with MI size.

Methods and Results Plasma concentrations of ANP and BNP in the peripheral blood were measured in 88 asymptomatic (New York Heart Association class I) patients with previous MI. The infarct size was quantitatively calculated from rest thallium-201 myocardial single photon emission computed tomography. In multivariate linear regression analysis that included MI size, hemodynamic parameters, and age as covariables, only BNP concentrations had a significant association with MI size ($p=0.0001$). In contrast, ANP concentrations were not significantly correlated with MI size in either the univariate or multivariate analysis.

Conclusions BNP but not ANP concentrations increased in proportion to the scintigraphic MI size despite the lack of heart failure in asymptomatic patients with previous MI. Thus, the increase in plasma BNP concentrations reflects the MI size, an important determinant of prognosis, in asymptomatic patients with MI. (*Circ J* 2004; **68:** 923–927)

Key Words: Heart failure; Myocardial infarction; Myocardial scintigraphy; Natriuretic peptides

A-type (atrial) natriuretic peptide (ANP) and B-type (brain) natriuretic peptide (BNP) are hormones with a wide range of potent biological effects, including natriuresis, diuresis, vasodilatation, and inhibition of the renin–angiotensin–aldosterone and sympathetic nervous systems[1,2] We and others have shown that ANP is mainly synthesized and secreted from the atria in adult mammals[1,3–5] but it is also synthesized and secreted from the ventricles in patients with congestive heart failure[3–5] BNP is secreted mainly from the ventricles in normal adult humans as well as in patients with congestive heart failure, and the plasma concentrations of BNP are markedly increased in proportion to the severity of left ventricular (LV) dysfunction in patients with myocardial infarction (MI) or congestive heart failure[4,6–8] Plasma concentrations of ANP and BNP have been used to predict prognosis after MI[8,9]

It is widely accepted that asymptomatic post-MI patients have a high risk of progression to overt heart failure and death[10] so it is clinically important to prevent their transition to a stage of accelerated progression of LV dysfunction. BNP concentrations are used to assess prognosis and therapeutic effects in asymptomatic MI patients as well as symptomatic patients[8,11] and the expression of both ANP and BNP is increased in the localized myocardial infarct regions relative to the detrimental hemodynamic parameters in patients with MI[3,4,6,12] The increase in ANP and BNP concentrations precedes the development of symptoms in asymptomatic MI patients[4,8,11] but it is unknown what determines the plasma concentrations of ANP and BNP in asymptomatic MI patients with relatively preserved LV function.

Therefore, the present study was designed to examine whether the plasma concentrations of ANP and BNP serve as a clinical indicator of the extent of MI, an important determinant of mortality[13] For these purposes we examined the relationship of plasma concentrations of ANP and BNP with the infarct size quantitatively calculated with thallium-201 single photon emission computed tomography (SPECT), a noninvasive and accurate method of assessment during the chronic phase of MI[14]

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Table 1 Clinical and Hemodynamic Variables in Control Subjects and Patients With Myocardial Infarction

	Control (n=40)	*Asymptomatic patients (n=88)*	*Symptomatic patients (n=27)*
Age (years)	*60±13*	*64±10*	*71±7*
M/F	*16/24*	*62/26*	*22/5*
ANP (pg/ml)	*16.6±10.2*	*36.0±26.5*	*149.0±132.3*,†*
BNP (pg/ml)	*12.3±10.5*	*74.7±63.1**	*355.1±285.1*,†*
Location of MI (anterior/inferior/other)	–	*39/37/12*	*15/11/1*
Multi-vessel disease	–	*26 (29%)*	*10 (37%)*
Scintigraphic extent score (%)	–	*16.9±11.9*	*37.2±11.3†*
PCWP (mmHg)	*8.2±3.7*	*8.2±3.9*	*12.8±8.1*,†*
CI (L/min per m²)	*3.1±0.6*	*2.8±0.7*	*2.5±0.6**
LVEDP (mmHg)	*10.9±5.8*	*13.2±5.5*	*17.0±7.7*,†*
LVEF (%)	*74.3±8.4*	*58.8±12.5**	*37.0±17.5*,†*
LVEDVI (ml/m²)	*64.8±15.4*	*65.7±22.9*	*110.2±53.0*,†*
LVESVI (ml/m²)	*16.7±6.1*	*28.9±13.1**	*69.0±48.2*,†*

**p<0.05 vs control, †p<0.05 vs asymptomatic patients.*
ANP, A-type natriuretic peptide; BNP, B-type natriuretic peptide; PCWP, pulmonary capillary wedge pressure; CI, cardiac index; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; LVEDVI, left ventricular end-diastolic volume index; LVESVI, left ventricular end-systolic volume index; MI, myocardial infarction.

Methods

Study Patients

The study population consisted of a consecutive series of 115 patients with a first MI (84 men, 31 women; mean age, 63 years; range 32–83 years) admitted to Yamanashi University Hospital from January 2002 to December 2003. MI was diagnosed on the basis of chest pain persisting for at least 30 min, ST segment elevation of at least 0.1 mV in at least 2 contiguous leads, and elevation of serum creatine kinase-MB isozyme to more than twice the upper limit of the normal range. Patients' characteristics are shown in Table 1. This study was performed at 3–5 weeks after onset of the MI and was completed within 1 week. Based on the New York Heart Association (NYHA) classification of symptoms, 88 patients were categorized as class I, 17 as class II, and 10 as class III–IV. This study defined asymptomatic patients (n=88) as patients in NYHA class I and symptomatic patients (n=27) as the remaining patients in NYHA classes II–IV. Reperfusion therapies in the acute phase of MI were performed in 79 asymptomatic patients (percutaneous coronary intervention (PCI) in 77 and thrombolysis in 2) and 25 symptomatic patients (PCI in all).

This study also included 40 control subjects (16 men, 24 women; mean age, 60 years; range, 29–78 years). The control subjects had atypical chest pain and normal coronary angiograms, no perfusion abnormalities on thallium-201 scintigrams and normal left ventriculograms. None of the patients with MI or the control subjects had cardiomyopathy, valvular heart disease, congenital malformation of heart, renal failure, or intrinsic pulmonary disease.

This study protocol was in agreement with the guidelines of the ethical committee at our institution, and written informed consent was obtained from all patients.

Measurements of Plasma ANP and BNP Concentrations

Peripheral venous blood was taken just before cardiac catheterization for measurement of ANP and BNP concentrations. Blood samples, anticoagulated with EDTA, were centrifuged at 3,000 rpm at 4°C for 10 min. An aliquot of the plasma was stored at −80°C until analyzed. ANP and BNP concentrations were measured by an immunoradiometric assay (Shionogi Co, Osaka, Japan) as described previously[4,7]

Cardiac Catheterization

Cardiac catheterization, including right heart catheterization, left ventriculography and coronary angiography, was performed after overnight fasting in all control subjects and patients with MI. The Swan-Ganz catheter technique was used for right heart catheterization and pulmonary capillary wedge pressure (PCWP) and cardiac output were measured as in our previous studies[4,6] LV ejection fraction (LVEF), LV end-systolic and diastolic volume indexes (LVESVI and LVEDVI) were determined from the left ventriculograms by area–length methods using computer-assisted analysis (Cardio 2000, Fukuda-denshi Corporation, Tokyo, Japan).

Thallium-201 Myocardial Scintigraphy

Rest thallium-201 myocardial scintigraphy was performed within 1 week before or after cardiac catheterization in all patients with MI. A bolus of 111 MBq (3 mCi) of thallium-201 was injected intravenously at rest and the imaging was begun 15 min later. The SPECT system consisted of a large-field-of-view gamma camera with a high-resolution, parallel-hole collimator mounted on a gantry (GCA-9300A/DI, Toshiba Corporation, Tokyo, Japan)[15] Ninety projections taken every 4 degrees for 12 s each were obtained in a 360-degree arc around the long axis of the patient. The short-axis tomographic images encompassing the entire left ventricle were reconstructed at 6.4 mm intervals.

Quantitative Analysis of Defect Size on Scintigraphy

Computerized thallium-201 tomography was used to quantify the size of the perfusion defect[15] Circumferential profiles for each short-axis tomographic image were constructed from maximum-count values per pixel in each of 36 radii spaced at 10-degree intervals. Count values on each point in the profile were then normalized to the maximum counts in the profile of each image. The resulting profiles were arranged as a series of concentric circles forming a single 2-dimensional (D) polar map with the apex at the center and the base at the periphery. Then, extent polar maps were obtained by comparing normalized maximal count values per point on the generated 2-D polar map with the corresponding lower normal limits at 2.0 standard deviations below the mean derived from 20 (10 men, 10 women) normal subjects. The extent score for the

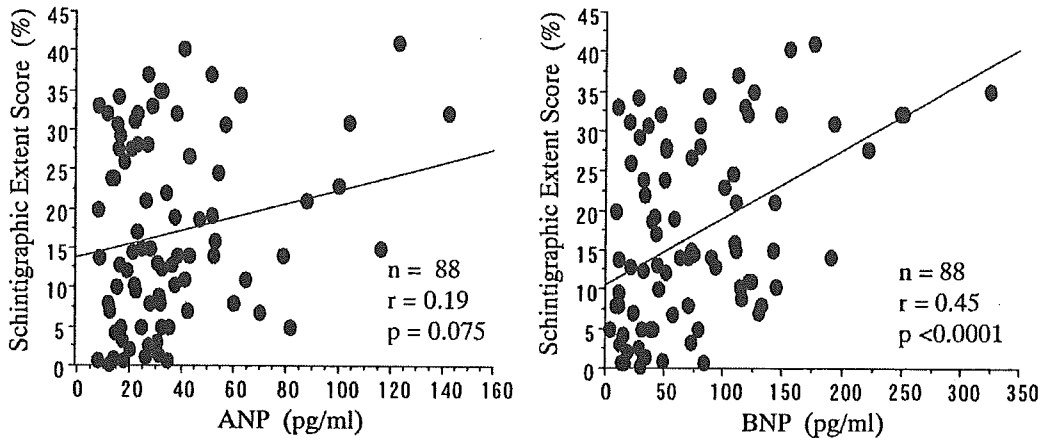

Fig 1. Correlation of the plasma concentrations of A-type and B-type natriuretic peptide (ANP and BNP) in the peripheral vein with the scintigraphic infarct size, expressed as the extent score in asymptomatic patients with a previous myocardial infarction.

Table 2 Correlation of Hemodynamic Parameters and Scintigraphic Extent Score With ANP and BNP Concentrations Using Univariate and Multivariate Linear Regression Analyses in Asymptomatic Patients With Myocardial Infarction (n=88)

	ANP				*BNP*			
	Univariate		*Multivariate*		*Univariate*		*Multivariate*	
	r	*p*	*Standardized regression coefficient*	*p*	*r*	*p*	*Standardized regression coefficient*	*p*
Age (years)	0.22	0.03	0.15	0.63	0.26	0.04	0.21	0.42
Scintigraphic extent score (%)	0.19	0.08	0.26	0.12	0.45	<0.0001	0.59	0.0001
PCWP (mmHg)	0.09	0.46	−0.06	0.84	0.29	0.02	−0.19	0.48
CI (L/min per m²)	0.05	0.70	0.001	1.00	0.05	0.68	−0.13	0.54
LVEDP (mmHg)	0.05	0.68	0.25	0.30	0.25	0.04	0.04	0.84
LVEF (%)	−0.14	0.25	−0.04	0.92	0.07	0.57	−0.20	0.43
LVEDVI (ml/m²)	0.18	0.14	0.16	0.63	0.15	0.21	0.26	0.40
LVESVI (ml/m²)	0.24	0.03	0.08	0.90	0.19	0.12	0.12	0.60

Abbreviations as in Table 1.

size of perfusion defect was defined by calculating the number of points falling below the corresponding lower normal limits and by expressing this number as a percent of the total LV points on the extent polar map.

Statistical Analysis

Results are expressed as mean±SD. Differences in the plasma concentrations of ANP and BNP and the hemodynamic measurements between the patients with MI and the control subjects were compared by one-way ANOVA and a post hoc testing with Sheffe's test. The correlation of ANP and BNP concentrations with the scintigraphic infarct size, hemodynamic parameters, and age was examined by linear regression analysis. Multivariate linear regression analysis was used to examine the relationship between the ANP or BNP and scintigraphic infarct size together with age and other hemodynamic parameters including PCWP, cardiac index (CI), LV end-diastolic pressure (LVEDP), LVEF, LVEDVI, and LVESVI. Statistical significance was defined as a p-value <0.05. Statistical analysis was performed with StatView 5.0 (SAS Institute, Cary, NC, USA).

Results

Plasma Concentrations of ANP and BNP (Table 1)

The BNP concentrations were increased in the asymptomatic patients (NYHA I) compared with control subjects, whereas the ANP concentrations did not differ between the 2 groups. The plasma concentrations of ANP and BNP were both higher in symptomatic patients with MI (NYHA II–IV) than in asymptomatic patients or control subjects.

Comparisons of Scintigraphic Infarct Size and Hemodynamic Parameters Among Symptomatic and Asymptomatic Patients and Controls (Table 1)

The thallium-201 scintigraphic extent scores ranged from 0% to 61% (mean 20±15%) and were significantly higher in the symptomatic patients than in the asymptomatic patients with MI. The asymptomatic patients had lower LVEF and higher LVESVI than controls, and other hemodynamic parameters, such as PCWP, CI, LVEDP, and LVEDVI, were comparable between the asymptomatic patients and controls.

Correlations Between Scintigraphic Scores of MI Size and ANP and BNP Concentrations

In the univariate linear regression analysis, BNP concentrations significantly correlated with the extent score in the asymptomatic patients (NYHA I) (Fig 1). Although the BNP concentrations had a non-Gaussian distribution, similar results were obtained when log-transformed values of the natriuretic peptides concentrations were statistically

analyzed (r=0.44, p<0.0001). Furthermore, in the asymptomatic patients, multivariate analysis showed that BNP concentrations had a significant association with the extent score only when extent score, PCWP, CI, LVEDP, LVEF, LVEDVI, LVESVI, and age were included as covariates (Table 2). Although ANP concentrations significantly correlated with the extent score in the entire patient population (symptomatic plus asymptomatic patients) in the multivariate linear regression analyses (standardized regression coefficient=0.23, p=0.03), they were not significantly correlated with the extent score in the asymptomatic patients in either the univariate or multivariate analysis (Fig 1, Table 2). Reperfusion therapies in the acute phase of MI and the extent of coronary artery disease had no influence on BNP concentrations in the asymptomatic patients (with reperfusion therapies 71±48 vs without reperfusion 78±82 pg/ml, p=NS; single-vessel disease 74±67 vs multivessel diseases 82±91 pg/ml, p=NS).

Discussion

The univariate statistical analysis conducted in the present study showed that BNP concentrations significantly correlated with MI size (extent score) in the asymptomatic patients. Furthermore, in multivariate linear regression analysis that included MI size, PCWP, CI, LVEDP, LVEF, LVEDVI, LVESVI, and age as covariates, only BNP concentrations had a significant association with MI size in the asymptomatic patients. These results suggest that BNP concentrations may directly reflect the MI size in asymptomatic post-MI patients and thus could serve as an important determinant of the prognosis in asymptomatic patients with preserved LV function, because the prognosis of MI is related to the extent of myocardial necrosis.[16,17]

Mechanisms of the Correlation Between Natriuretic Peptides Concentrations and MI Size

It has been reported that BNP concentrations in the anterior interventricular vein are higher in an anterior infarction than in an inferior infarction, suggesting that the secretion of BNP is significantly greater from the infarct region than from the non-infarct region in patients with MI.[6] Furthermore, it has been shown that the immunoreactivity for ANP and BNP is markedly increased in the area surrounding the infarct region.[18,19] The area surrounding a myocardial infarct is thought to suffer from a high level of regional wall stress, and regional wall stress has clearly been shown to be an important stimulus for secretion of ANP and BNP from the myocardium.[19,20] Thus, the correlation between the infarct size and the concentrations of these peptides may be explained by an increase in regional wall tension or stretch in viable cells both within and surrounding the infarct area.

The present study showed that asymptomatic patients had a positive correlation between infarct size and BNP concentrations, but not with ANP concentrations, which is related to the finding that the cardiac secretion of BNP is predominantly derived from the left ventricle regardless of the presence or absence of LV dysfunction, whereas ANP secretion is mainly from the atria when the magnitude of LV dysfunction is insignificant.[20] These results are consistent with previous reports[8,11,21] showing that BNP concentrations are a better predictor of prognosis after MI. Although we did not measure N-terminal pro-ANP concentrations, the increased clearance of ANP compared with BNP may possibly contribute to the disparity in the significance of the correlation between ANP and BNP concentrations?

Clinical Implications

Screening for high-risk asymptomatic MI patients is very important[21] because they may benefit from early treatment. An elevated BNP concentration in the absence of detrimental LV hemodynamic parameters does not represent a false-positive result, but might reflect a larger MI size in an asymptomatic patient with a prior MI. Therapeutic efforts should also be directed to preventing the progression of LV remodeling and dilation that occurs before symptomatic heart failure.[22]

Study Limitations

Serial measurement of serum concentrations of cardiac markers was widely used for estimating MI size in the pre-reperfusion era; however, coronary artery reperfusion dramatically changes the washout kinetics of the cardiac markers, thus limiting their usefulness as a measure of infarct size. Also, assessment of MI size using thallium-201 myocardial scintigraphy has several limitations, such as artifacts and showing the relative distribution, not the absolute distribution, of myocardial perfusion. Nevertheless, CT using the profile technique can provide quantitative data on MI size more readily than other noninvasive methods.[23] Although the BNP concentrations did not differ between those with single-vessel disease and those with multi-vessel disease in the present study, we did not estimate the extent of myocardial ischemia that potentially elevates the BNP concentration and could affect the present results.

Conclusions

In patients with a previous MI, the BNP but not the ANP concentrations were increased in proportion to the size of the MI, estimated by thallium-201 SPECT, despite the lack of symptoms of heart failure. Thus, the plasma BNP concentrations are a marker of MI size in asymptomatic patients with MI.

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Prognostic Value of Remnant-Like Lipoprotein Particle Levels in Patients With Coronary Artery Disease and Type II Diabetes Mellitus

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OBJECTIVES This study prospectively examined whether the levels of high remnant-like lipoprotein particles (RLP) cholesterol have a significant risk and influence prognosis in patients with coronary artery disease (CAD) and type II diabetes mellitus (DM).

BACKGROUND Several studies have shown that triglyceride-rich lipoproteins contribute to atherosclerotic complications in type II DM. However, it remains to be established which triglyceride-rich lipoproteins contribute to this risk.

METHODS Levels of RLP cholesterol in fasting serum were measured by an immunoseparation method in 240 type II DM patients with (n = 120) or without (n = 120) CAD. The patients with CAD were followed up for a period of ≤24 months until the occurrence of one of the following clinical coronary events: re-admission or coronary revascularization due to recurrent or refractory angina pectoris, nonfatal myocardial infarction, or cardiac death.

RESULTS Patients with CAD had higher RLP levels than patients without CAD. Multivariate logistic regression analysis showed that high RLP cholesterol levels (>4.7 mg cholesterol/dl, representing the 75th percentile of the distribution of RLP cholesterol levels in control subjects) were a significant risk factor for the presence of CAD, independent of traditional risk factors. Kaplan-Meier analysis demonstrated that higher RLP cholesterol levels in patients with CAD resulted in a significantly higher probability for the development of coronary events. Multivariate Cox hazards analysis showed that high RLP cholesterol levels in patients with CAD were a significant predictor of future coronary events, independent of other risk factors.

CONCLUSIONS Increased levels of RLP cholesterol are a significant and independent risk factor of CAD and predict future coronary events in patients with CAD and type II DM. (J Am Coll Cardiol 2004;43:2219–24) © 2004 by the American College of Cardiology Foundation

Several large, prospective cohort studies have demonstrated that diabetes mellitus (DM) is associated with an increased risk of coronary artery disease (CAD) (1,2). It is well known that CAD is a manifestation of macroangiopathy in type II DM. Diabetic macroangiopathy is also often associated with hyperglycemia and dyslipidemia (3). Although intensive diabetic therapies significantly delay the onset and slow the progression of microvascular complications, the frequency of major macrovascular events is almost comparable in patients receiving either intensive or conventional therapy (4). The majority of cases of type II DM have dyslipidemia characterized by increased triglyceride levels and decreased high-density lipoprotein (HDL) cholesterol levels (5). Several recent studies have provided evidence that hypertriglyceridemia and triglyceride-rich lipoproteins play a key role in the pathogenesis of diabetic macroangiopathy and that dyslipidemia is an important predictor of CAD mortality in patients with DM (6,7). However, it has yet to be established which specific lipoprotein fraction is responsible for this increased risk. Remnant lipoproteins, derived especially from very-low-density lipoproteins (VLDL), are considered to be atherogenic (8–10). Recently, a simple and reliable technique for measurement of remnant-like lipoprotein particles (RLP) cholesterol, using an immunoseparation method, has been developed (11,12). A cross-sectional study showed that RLP cholesterol levels were increased in patients with type II DM (13), although there is limited information on RLP cholesterol levels in patients with type II DM and CAD. In the present study, we prospectively examined whether RLP cholesterol levels had the potential to predict future coronary events in type II DM patients with CAD.

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Abbreviations and Acronyms

apo = apolipoprotein
CABG = coronary artery bypass graft surgery
CAD = coronary artery disease
DM = diabetes mellitus
HbA_{1c} = glycosylated hemoglobin
HDL = high-density lipoprotein
LDL = low-density lipoprotein
PCI = percutaneous coronary intervention
RLP = remnant-like lipoprotein particles
VLDL = very-low-density lipoprotein

METHODS

Study patients. This study at Kumamoto University Hospital involved consecutive enrollment of 120 patients with type II DM and CAD who underwent cardiac catheterization for chest pain or ischemic changes detected by electrocardiography. All patients had angiographic evidence of organic diameter stenosis of >70% of at least one major coronary artery (single-vessel disease, n = 32; two-vessel disease, n = 36; three-vessel disease, n = 52; left main coronary artery disease, n = 21). The diabetes entry criteria based on American Diabetes Association criteria (14) were type II diabetes, as indicated by a fasting plasma glucose concentration >7.8 mmol/l (126 mg/dl), or a 2-h plasma glucose concentration >11.0 mmol/l (200 mg/dl) after a 75-g oral glucose tolerance test or with glucose-lowering drug treatment.

This study also involved enrolling 120 type II DM patients without CAD who were age- and gender-matched to the patients with CAD. All of these control subjects underwent cardiac catheterization for atypical chest pain in the hospital during the same study period as the patients with CAD. These control subjects had angiographically normal coronary arteries (<10% stenosis) and a normal left ventriculogram and thereby formed a case-control study to evaluate whether RLP cholesterol levels as a risk factor differed between patients with and those without CAD. The baseline characteristics of the study patients are shown in Table 1. This study was conducted in agreement with guidelines approved by the Ethics Committee at our institution.

Measurement of lipoproteins. At the beginning of the study, venous blood was obtained from all patients after a 12-h overnight fast. All patients ate a standard Japanese meal (1,900 kcal/day, 25% fat, 59% carbohydrate, and 16% protein) the day before blood sampling. Serum was stored at 4°C and used for the assays within three days after sampling. The RLP was isolated by application of the fasting serum to an immunoaffinity-mixed gel that contained anti-apolipoprotein (apo) A-I and anti-apoB-100 monoclonal antibodies (Japan Immunoresearch Laboratories, Takasaki, Japan), according to the method described in a previous report (11). Levels of HDL cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides in fasting serum were measured as described previously (8,12).

Follow-up study. After laboratory samples and angiographic data were obtained, the 120 patients with type II DM and CAD were followed prospectively every month for ≤24 months in the hospital or by a visit until occurrence of a clinical coronary event. In parallel, the 120 type II DM patients without CAD were also followed prospectively. The clinical coronary events included re-admission or coronary revascularization due to recurrent or refractory angina pectoris, a nonfatal myocardial infarction, or cardiac death. The time to the first coronary event was evaluated prospectively. Coronary angiography was performed using standard techniques, and the angiograms were analyzed independently by two cardiologists (Drs. Honda and Koide) who had no knowledge of the patients' characteristics. All patients received standardized medical therapy. Coronary revascularization therapy (percutaneous coronary intervention [PCI] or coronary artery bypass graft surgery [CABG]) was defined as a clinical coronary event when progression of angiographic coronary stenosis was associated with recurrent and refractory angina pectoris and evidence of recurrent ischemic electrocardiographic (ECG) changes lasting >10 min, despite full medication. The need for

Table 1. Patient Characteristics

	With CAD (n = 120)	Without CAD (n = 120)	p Value
Age (yrs)	65.6 ± 8.4	65.6 ± 8.0	NS
Gender (male/female)	75/45	76/44	NS
Body mass index (kg/m^2)	24.2 ± 2.8	23.9 ± 3.6	NS
Cigarette smoker (%)	69 (58%)	55 (46%)	NS
Systemic hypertension (%)	64 (53%)	59 (49%)	NS
Total cholesterol (mg/dl)	202 ± 39	183 ± 34	<0.01
HDL cholesterol (mg/dl)	44 ± 14	50 ± 15	<0.01
LDL cholesterol (mg/dl)	128 ± 36	112 ± 30	<0.01
Triglycerides (mg/dl)	151 ± 71	123 ± 48	<0.01
Hemoglobin A_{1c} (%)	7.2 ± 1.5	6.5 ± 1.3	<0.01
RLP cholesterol (mg/dl)*	5.8 (3.1–6.2)	3.7 (2.5–4.7)	<0.01

*Expressed as the median value (interquartile range). Other data are presented as the mean value ± SD or number (%) of patients. These covariates were a risk of CAD in the univariate analysis.

CAD = coronary artery disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NS = not significant; RLP = remnant-like lipoprotein particles.

Table 2. Multivariate Logistic Regression Analysis: Variables Differing Between Patients With and Without Coronary Artery Disease

	OR	95% CI	p Value
High RLP cholesterol levels (>4.7 mg/dl)	2.2	1.2–6.4	<0.05
High hemoglobin A_{1c} levels (>7.0%)	2.2	1.1–5.3	<0.05
Low HDL cholesterol levels (<35 mg/dl)	1.7	0.7–3.5	NS
High LDL cholesterol levels (>130 mg/dl)	1.5	0.6–3.4	NS
Hypercholesterolemia (>220 mg/dl)	1.4	0.5–3.8	NS
Hypertriglyceridemia (>150 mg/dl)	0.7	0.4–1.5	NS

CI = confidence interval; OR = odds ratio; other abbreviations as in Table 1.

and timing of revascularization were decided by the attending physician and interventional cardiologists, independent of this prospective study. A diagnosis of myocardial infarction was made by symptomatic chest pain, the appearance of a new Q waves on the ECG, and a twofold elevation in creatine kinase relative to the upper limit of normal. Causes of death were obtained from hospital records. A blinded Clinical Events Committee, the members of which were blinded to the treatment assignment of the study patients, classified all end points.

Statistical analysis. The RLP cholesterol levels were not distributed normally; therefore, these data were analyzed using nonparametric statistical tests and are expressed as the median value and interquartile range. The Mann-Whitney *U* test was used to evaluate differences in RLP cholesterol levels between the two patient groups. The Kaplan-Meier log-rank test was used for survival analysis according to the levels of RLP cholesterol. The predictive value for coronary events during the follow-up period was assessed by Cox proportional hazards analysis. The multiple Cox analysis included only the covariates that predicted coronary events in the univariate analysis. The analyses included the following factors as categorical variables: high levels of RLP cholesterol (>4.7 mg/dl, corresponding to the 75th percentile in control subjects); a raised glycosylated hemoglobin (HbA_{1c}) level >7% (4); age ≥70 years; a family history of CAD; cigarette smoking, defined as smoking ≥10 cigarettes/day for ≥10 years; systemic hypertension (>140/90 mm Hg or use of antihypertensive medication); hypercholesterolemia (>220 mg/dl or use of cholesterol-lowering medications); low HDL cholesterol levels <35 mg/dl; high LDL cholesterol levels >130 mg/dl; hypertriglyceridemia >150 mg/dl; three-vessel disease; and a low left ventricular ejection fraction (<50%), measured at baseline left ventriculography. The mean value and frequency of continuous variables with a normal distribution were compared between the two groups by using the unpaired *t* test and chi-square analysis, respectively. Statistical significance was defined as $p < 0.05$. Analyses were assessed in part using StatView 5.0 for Macintosh (Tokyo, Japan).

RESULTS

Comparison of risk factors among study groups. Risk factor profiles in the study patients are shown in Table 1. The fasting serum levels of RLP cholesterol, total cholesterol, LDL cholesterol, triglycerides, and HbA_{1c} were significantly higher in patients with type II DM and CAD than in patients without CAD. The patients with CAD also had significantly lower HDL cholesterol levels than the control group without CAD. As shown in Table 2, a comparison of risk factors between the patients with type II DM patients with CAD and those without CAD, using multivariate logistic regression analysis, demonstrated that high RLP cholesterol and HbA_{1c} levels were independent risk factors for the presence of CAD.

RLP cholesterol as a predictor of coronary events in patients with type II DM and CAD. All of the patients with type II DM and CAD received standard medical therapy during the follow-up period, consisting of a combination of calcium channel blockers (78% of patients), beta-blockers (36%), nitrates (60%), angiotensin-converting enzyme inhibitors (52%), aspirin (96%), lipid-lowering drugs (33%), oral hypoglycemic agents (38%), and insulin therapy (21%). No patient was lost to follow-up. The patients were followed for a mean duration of 20.5 months (range 1 to 24). Patients with high RLP cholesterol levels (n = 52) had 27 coronary events during the follow-up period (10 PCIs; 6 CABGs; 7 cases of unstable angina pectoris, 2

Table 3. Comparison of Drugs Administered During the Follow-Up Period in Patients With and Without Coronary Events

	Patients With Coronary Events (n = 44)	Patients Without Coronary Events (n = 76)	p Value
Calcium channel blockers	31 (71%)	63 (83%)	NS
Beta-blockers	19 (43%)	24 (32%)	NS
Nitrates	29 (66%)	43 (57%)	NS
ACE inhibitors	24 (55%)	38 (50%)	NS
Aspirin	44 (100%)	71 (93%)	NS
HMG-CoA reductase inhibitors	14 (32%)	25 (33%)	NS
Fibrates	5 (11%)	6 (8%)	NS
Niacin	9 (20%)	19 (25%)	NS
Oral hypoglycemic agents	20 (46%)	25 (33%)	NS
Insulin therapy	8 (18%)	17 (22%)	NS

Data are presented as the number (%) of patients.
ACE = angiotensin-converting enzyme; HMG-CoA = 3-hydroxy-3-methyl-glutaryl-coenzyme A; NS = not significant.

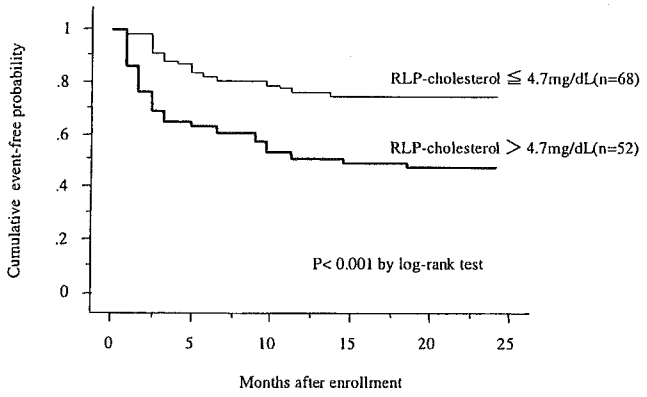

Figure 1. Kaplan-Meier curves comparing the probability of future coronary events in 120 patients with type II diabetes mellitus and coronary artery disease, according to remnant-like lipoprotein particles (RLP) cholesterol levels during a maximum follow-up period of 24 months after enrollment. The end points were either re-admission or coronary revascularization (percutaneous coronary intervention or coronary artery bypass graft surgery) due to recurrent and refractory angina pectoris, nonfatal myocardial infarction, or cardiac death. The time to the first coronary event was recorded. The cut-off level of RLP cholesterol (4.7 mg/dl) was set at the 75th percentile of the distribution of RLP cholesterol levels in age- and gender-matched control subjects. Fifty-two patients had levels of RLP cholesterol >4.7 mg/dl, whereas 68 patients had levels ≤4.7 mg/dl.

myocardial infarctions, and 2 cardiac deaths). In comparison, patients with low RLP cholesterol levels (n = 68) had 17 events (6 PCIs, 5 CABGs, 3 cases of unstable angina pectoris, 1 myocardial infarction, and 2 cardiac deaths; $p < 0.01$ for the frequency of coronary events between the 2 groups). There was no significant difference in the prevalence of each of the drugs used between patients with and those without coronary events during the follow-up period (Table 3). Kaplan-Meier analysis demonstrated that patients with type II DM and CAD with high RLP cholesterol levels had a significantly higher probability of developing coronary events ($p < 0.001$) (Fig. 1). The results of the univariate Cox analysis are summarized in Table 4 and show that high RLP cholesterol and HbA_{1c} levels and

Table 4. Univariate Cox Hazards Analysis of Risk of Future Coronary Events According to Fasting Levels of RLP Cholesterol in Patients With Type II Diabetes Mellitus and Coronary Artery Disease

	OR	95% CI	p Value
Age (≥70 yrs)	1.2	0.6–2.1	NS
Male gender	1.2	0.6–2.2	NS
Body mass index (>25 kg/m^2)	1.0	0.5–1.8	NS
Cigarette smoker	0.8	0.5–1.5	NS
Systemic hypertension	1.2	0.6–2.1	NS
Hypercholesterolemia (>220 mg/dl)	1.2	0.6–2.3	NS
Low HDL cholesterol levels (<35 mg/dl)	1.3	0.7–2.5	NS
High LDL cholesterol levels (>130 mg/dl)	1.0	0.6–1.8	NS
Hypertriglyceridemia (>150 mg/dl)	1.2	0.6–2.1	NS
High RLP cholesterol levels (>4.7 mg/dl)	2.6	1.4–4.8	<0.01
High hemoglobin A_{1c} levels (>7%)	2.0	1.1–3.8	<0.05
Three-vessel disease	1.8	1.0–3.3	<0.05
Low LVEF (<50%)	0.7	0.3–1.7	NS
Family history of CAD	1.3	0.6–2.5	NS

LVEF = left ventricular ejection fraction; other abbreviations as in Tables 1 and 2.

Table 5. Multivariate Cox Hazards Analysis of Risk of Future Coronary Events According to Fasting Levels of RLP Cholesterol in Patients With Type II Diabetes Mellitus and Coronary Artery Disease

	OR	95% CI	p Value
High RLP cholesterol levels (>4.7 mg/dl)	2.4	1.3–4.6	<0.01
High hemoglobin A_{1c} levels (>7%)	1.7	0.9–3.2	NS
Three-vessel disease	1.4	0.7–2.6	NS

Multivariate Cox hazards analysis included only the covariates that predicted coronary events in the univariate analysis (see Table 4).
Abbreviations as in Tables 1 and 2.

three-vessel disease were significant predictors of coronary events in type II DM patients with CAD. Multivariate Cox analysis revealed that high RLP cholesterol levels remained a significant predictor of coronary events, independent of traditional risk factors (Table 5).

In three patients with neither recurrent and refractory angina pectoris nor evidence of recurrent ischemic ECG changes, the revascularization therapies (2 PCIs and 1 CABG) were performed during the follow-up period. All of the three patients had ≤4.7 mg/dl of RLP cholesterol levels. High RLP cholesterol levels also represented a significant risk for future coronary events plus all revascularization therapies when these three cases were added into the Kaplan-Meier analysis ($p < 0.01$ by the log-rank test) and the Multivariate Cox hazards analysis (odds ratio 2.1, 95% confidence interval 1.1 to 3.8; $p < 0.01$).

In the 120 diabetic non-CAD patients with either high or normal RLP cholesterol levels, no clinical coronary event occurred during the same follow-up period as in the present diabetic CAD patients.

DISCUSSION

Microangiopathy and macroangiopathy are common complications of type II DM. Major risk factors for the progression of diabetic microangiopathy include poor glycemic control, a prolonged history of diabetes, and hypertension (15), whereas the main risk factors for macroangiopathy are aging, obesity, hyperlipidemia, hypertension, and smoking (16). Typically, the dyslipidemia associated with type II DM manifests as a moderate increase in plasma triglycerides and a decrease in HDL cholesterol, whereas total cholesterol and LDL cholesterol levels are normal or mildly elevated. Although the precise mechanism underlying hypertriglyceridemia in type II DM is not fully understood, it is caused partly by an increase in hepatic VLDL production and a delay in the clearance of triglyceride-rich lipoproteins (3,17). Among triglyceride-rich lipoproteins, remnant lipoproteins are believed to have a strong atherogenic effect. In the present study, multivariate logistic regression analysis showed that high RLP cholesterol and HbA_{1c} levels were risk factors for CAD in patients with type II DM. Furthermore, the prospective component of this study found that increased levels of RLP cholesterol predicted the development of clinical coronary events in

these patients, with this predictive potential being greater than that measured for high HbA_{1c} levels. These results indicate that high levels of RLP cholesterol have a crucial role in the pathogenesis of CAD in type II DM. It has been recently shown that diabetics without CAD have event rates that are nearly equal to that of nondiabetic patients with CAD (2). However, few clinical coronary events occurred in the present diabetic non-CAD patients during these two years. We need a larger scale study to examine possible role of high RLP levels in primary coronary events in diabetic non-CAD patients.

Proatherothrombogenic effects of RLP. It is well established that type II DM may be associated with enhanced thrombogenic and atherogenic states, which together trigger atherothrombotic complications. We showed recently that RLP, at concentrations similar to those found in the plasma of patients with CAD, upregulated the expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in cultured human endothelial cells (18). The induction of these adhesion molecules is responsible for monocyte recruitment into the arterial walls, an early step of atherosclerosis (19,20). In this earlier study, we also showed that RLP increased production of tissue factor that is essential for thrombotic events in endothelial cells (18). In addition, there is evidence that RLP enhances aggregation of platelets (21). High plasma levels of RLP may therefore have an important role in the development of atherosclerosis and thrombotic events by the combined effects of upregulation of endothelial-derived proatherothrombogenic molecules and enhanced platelet reactivity. These proatherothrombogenic effects of RLP may explain the association of high RLP cholesterol levels with the increased prevalence of future coronary events in type II DM patients with CAD, which we observed in the present study. Taken together, these results indicate that high levels of RLP cholesterol have a crucial role in the pathogenesis of CAD in patients with type II DM patients. Lipid-lowering drugs such as fibrates or statins, dietary intervention, and obesity reduction may decrease remnant lipoproteins levels, and therefore remnant lipoproteinemia represents a risk factor that should be a therapeutic target in patients with type II DM.

Assays of RLP cholesterol. Measurement of remnant lipoproteins has been difficult because of the heterogeneous nature of these macromolecules. Traditional methods using ultracentrifugation or agarose gel or low-concentration polyacrylamide gel electrophoresis are complex and time-consuming (22) and therefore are not applicable for clinical use. We have shown previously that RLP isolated from fasting plasma in patients with CAD by the immunochemical separation method used in the present study had beta or slow pre-beta mobility on agarose gel electrophoretograms, a particle size in the range between VLDL and intermediate-density lipoprotein on high-performance liquid chromatography, and enrichment in apoE on slab gel electrophoresis, all of which are properties characteristic of VLDL remnants (9,10,12). The immunoseparation method used in the present study has been shown by us and other investigators to be both simple and reliable and therefore useful for assessing and monitoring CAD risk.

Study limitations. The majority of the coronary events recorded during the follow-up period were soft end points, and accordingly, these were checked by an independent and blinded Clinical Events Committee. This process was required because the relatively small number of patients limited the statistical power of this study. A prospective trial incorporating lipid-lowering therapy in a large number of patients with homogeneous risk is required in order to more precisely assess the role of RLP in the pathogenesis of CAD associated with type II DM.

Conclusions. Increased levels of RLP are a significant and independent risk factor for CAD and predict future coronary events in patients with type II DM and CAD.

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