Table 5
Changes in concentrations of serum insulin, plasma glucose, serum leptin, plasma LPL protein mass, plasma PAI-1

		Time after fat loading (h)				p value of ANOVA
		0	2	4	6	
Insulin (pmol/l)	DAG	45.0±13.2 (0.0)	46.8±6.6 (1.8±9.0)	36.0±7.8 (−9.0±9.0)	25.2±4.8 (−19.8±11.4)	p=0.936
	TAG	47.4±9.0 (0.0)	54.6±11.4 (7.2±6.0)	33.0±4.8 (−14.4±7.2)	31.2±9.6 (−16.2±7.8)	
Glucose (mmol/l)	DAG	8.3±0.9 (0.0)	7.8±0.8 (−0.4±0.3)*	6.8±0.8 (−1.5±0.3)*	6.1±0.6 (−2.2±0.4)	p=0.037
	TAG	8.8±0.8 (0.0)	7.5±0.7 (−1.3±0.2)	6.2±0.5 (−2.6±0.3)	5.9±0.4 (−2.9±0.6)	
Leptin (μg/l)	DAG	7.7±2.0 (0.0)	6.8±1.8 (−0.9±0.3)	6.4±1.8 (−1.4±0.3)	6.3±1.7 (−1.4±0.5)	p=0.741
	TAG	7.3±1.8 (0.0)	6.5±1.6 (−0.8±0.2)	5.9±1.4 (−1.4±0.4)	6.1±1.5 (−1.3±0.4)	
Preheparin LPL protein mass (g/l)	DAG	0.52±0.07 (0.00)	0.48±0.0 5 (−0.04±0.04)	0.45±0.05 (−0.07±0.04)	0.40±0.04 (−0.11±0.05)	p=0.460
	TAG	0.54±0.09 (0.00)	0.51±0.07 (−0.03±0.03)	0.49±0.07 (−0.06±0.03)	0.48±0.07 (−0.06±0.02)	
PAI-1 (μg/l)	DAG	17±3 (0)	16±3 (−1±4)	14±3 (−3±4)	9±1 (−8±3)	p=0.554
	TAG	15±3 (0)	17±3 (2±2)	14±2 (−1±2)	11±2 (−5±2)	
T-ketone bodies (μmol/l)	DAG	153±63 (0)	145±31 (−8±53)	443±74 (290±67)	506±108 (353±113)	p=0.074
	TAG	127±43 (0)	230±53 (102±69)	443±111 (315±118)	507±118 (380±112)	

Values are Mean±S.E. Mean±S.E. changes from baseline are shown in parentheses (Δ). P values are calculated by repeated-measures two-way ANOVA (Δ). DAG: diacylglycerol; TAG: triacylglycerol; LPL: lipoprotein lipase; T-ketone bodies: total ketone bodies.
* Significantly different from TAG ingesition at the same time points by paired t-test: $p<0.05$.

in comparison with those during DAG loading ($p<0.05$). The concentration of total ketone bodies in the serum was increased over time following either fat loading, but no significant difference was observed between the values for TAG and DAG loading. Plasma LPL protein mass, serum leptin, and plasma PAI-1 decreased over time after either fat loading, and there were no significant differences between these respective values in the TAG and DAG groups.

4. Discussion

The effects of DAG loading on postprandial changes in serum lipids and lipid parameters in diabetic patients were examined. Although the subject number of this study was small, the findings again showed the suppressed postprandial increases in serum TAG, RLP-TAG, and RLP-C in the DAG intake when compared with TAG intake in diabetic subjects as previously reported in healthy volunteers [15].

Increased fasting RLP-C concentrations have been reported in individuals with impaired glucose tolerance and in subjects with type 2 diabetes [1]. Ai et al. [28] reported that postprandial TAG and lipids in the RLP were also significantly increased in type 2 diabetics as compared with those in healthy subjects. In a recent report from the Framingham Study, RLP-C and RLP-TAG levels were shown to be higher in diabetic males and females, and these increases in RLP lipids were counted as a risk factor for CHD [29]. In the subanalysis of the Veterans Affairs HDL Intervention Trial (VA-HIT), a randomized controlled trial, Elam et al. [30] also reported that the incidence of CHD events was correlated positively to prepran-dial RLP-TAG and RLP-C levels. Furthermore, Mero et al. [31] suggested that postprandial changes in small remnant number might contribute to the severity of CAD in type 2 diabetes. These data suggest that the suppression of the postprandial increase in RLP lipids, achieved in our preliminary study by DAG loading, may be helpful to construct a nutritional therapeutic strategy, for reducing CHD risk, in diabetic patients.

The physicochemical mechanisms of the DAG oil-induced suppression of the postprandial increase in lipids have not yet been fully elucidated. During the processes of digestion, dietary TAG molecules are hydrolyzed to yield two free fatty acids and 2-monoacylglycerol (2-MAG) by intestinal lipases and absorbed into enterocytes. Triacylglycerol is then reassimilated again mainly via 2-MAG pathway in enterocytes [32]. On the other hand, 1,3-DAG, a major constituent of DAG, is hydrolyzed to glycerol and free fatty acids through 1-(or 3-)MAG during digestion. A portion of the 1-(or 3-)MAG enters enterocytes in the same manner as 2-MAG. However, resynthesis of TAG from 1-(or 3-)MAG will take

palace via the phosphatidic acid pathway, a slow turnover pathway, instead of the 2-MAG pathway. Hence, the lymphatic secretion of CM-TAG after the ingestion of DAG oil might be slower and possibly lower than that after the ingestion of naturally occurring TAG oil. These properties of 1,3-DAG may be beneficial in improving postprandial hyperlipidemia. Other metabolic characteristics of 1,3-DAG were discussed elsewhere recently [33].

As shown in Table 4, the increase in CM-TAG with DAG loading was slightly lower, and the time required for CM-TAG to reach the maximum level following DAG loading was longer as compared with TAG loading, although significant differences between the two groups were not detected. Eventually, however, these phenomena may lead to the significant suppression of the postprandial increase in serum TAG and RLP lipids after DAG loading.

Another aim of this experiment was to investigate the adverse effects of DAG intake especially on the formation of ketone bodies in diabetes. It is known that most diabetic patients have an abnormal ketone body metabolism. It was reported that higher activities of enzymes involved in the beta-oxidation pathway were detected with DAG feeding as compared with TAG feeding in the rat liver [34]. Increased fat oxidation was also detected in an experiment with 12 women who ingested DAG as compared with TAG oil [35]. Retarded TAG resynthesis in the enterocyte with DAG feeding, as described above, can result in an increased fatty acid concentration in enterocytes. Fatty acids unutilized for de novo TAG resynthesis in enterocytes may be utilized as energy. The retarded glucose reduction observed in our study during DAG loading as compared with TAG loading (Table 5) is conceivably due to the utilization of fatty acids instead of glucose as an energy source. This retarded glucose reduction during the DAG loading was also reported in healthy subjects by Taguchi et al. [36]. Thus, an increase in ketone bodies in the circulation following oral DAG loading in diabetics was our concern. However, this study showed no significant difference in the concentration of serum ketone bodies between the TAG and DAG groups for the moderately controlled diabetic patients with HbA1c levels <8%. Although we evaluated postprandial responses of single loading of DAG oil by the comparison with TAG oil using fat emulsion as a test food, oils in a typical diet should be evaluated as the next step with much larger population of diabetic subjects.

In summary, DAG ingestion in contrast to TAG ingestion suppressed postprandial increases in serum TAG, RLP-C, and RLP-TAG in six diabetics with moderate glycemic control. In addition, no significant differences were observed in postprandial changes in serum levels of insulin, free fatty acid, and ketone bodies between the DAG and TAG loading.

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Remnant lipoproteinemia is a risk factor for endothelial vasomotor dysfunction and coronary artery disease in metabolic syndrome

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Abstract

This study aimed to determine whether elevated levels of remnant lipoprotein, an atherogenic triglyceride-rich lipoprotein, might be associated with coronary artery disease (CAD) and endothelial vasomotor dysfunction in metabolic syndrome. The fasting serum levels of remnant lipoproteins (remnant-like lipoprotein particles cholesterol; RLP-C) were measured by an immunoseparation method in 210 patients with metabolic syndrome meeting ATP III criteria. Flow-mediated endothelium-dependent dilatation (FMD) in the brachial artery during reactive hyperemia was examined by high-resolution ultrasound technique. This study found that elevated RLP-C levels were a significant and independent risk factor for impaired FMD and angiographically proven coronary artery disease (CAD). Treatment with bezafibrate ($n=20$) or atorvastatin ($n=20$) for 4 weeks significantly reduced RLP-C levels, with a concomitant improvement in FMD. The % reduction in RLP-C levels from baseline after the treatment was independently correlated with the magnitude of improvement in FMD after adjustment for the % changes in levels of triglyceride, hsCRP, and IL-6, and HOMA index. Thus, elevated levels of RLP-C are a risk factor for CAD and endothelial vasomotor dysfunction, a predictor of coronary events, in metabolic syndrome. Measurement of RLP-C is useful for assessment of CAD risk and therapeutic effects in metabolic syndrome.

Keywords: Remnant lipoproteins; Coronary artery disease; Endothelium-dependent vasodilation; Atherosclerosis; Metabolic syndrome

1. Introduction

Metabolic syndrome is a clustering of atherosclerotic metabolic abnormalities characterized by insulin resistance, visceral adiposity, high triglyceride, and low high-density lipoprotein (HDL). This syndrome is highly prevalent (40% of the population in USA >60 years of age) [1,2] and strongly associated with cardiovascular diseases (CVD) [3,4]. Therefore, it is important to target prevention strategies for patients with metabolic syndrome. However, multiple metabolic disorders contribute to the pathogenesis of this syndrome [1,5]. Furthermore, these metabolic disorders are intimately linked with each other, and thus the primary pathogenesis of this syndrome is difficult to determine in each patient. It also remains to be determined which metabolic disorders should be primary the therapeutic targets to prevent CVD and which metabolic disorders should be monitored to follow therapeutic effects.

Dyslipidemia, characterized by elevated triglycerides levels and low HDL levels, is a hallmark of metabolic syndrome [6,7]. We have shown that remnant lipoproteins, triglyceride-rich lipoproteins, are a strong risk factor for CVD [8–13]. Recently, a simple and reliable technique for measurement of remnant-like lipoprotein particles cholesterol (RLP-C) using an immunoseparation method has been developed [8,14]. We

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and others have shown that high RLP-C levels are a strong risk factor for CVD [8–11]. We thus hypothesized that measurement of RLP-C levels might be helpful in the assessment of CVD risk in metabolic syndrome. A number of studies have shown that endothelial vasomotor dysfunction, a predictor of future coronary events, is often present in patients with metabolic syndrome or insulin resistance [15]. Furthermore, it is proposed that endothelial dysfunction itself may induce insulin resistance [16]. Thus, endothelial dysfunction is closely linked to pathogenesis of metabolic syndrome and it reflects multiple factors that contribute to CVD events in metabolic syndrome. In this study, we tested whether RLP-C levels are a risk factor for coronary artery disease (CAD) and endothelial vasomotor dysfunction in patients with metabolic syndrome. Furthermore, we examined the therapeutic effects of bezafibrate and atorvastatin on RLP-C levels and endothelial vasomotor dysfunction in a subgroup of the study patients.

2. Methods

2.1. Study patients

This study enrolled 132 consecutive patients with the metabolic syndrome and CAD who underwent cardiac catheterization for chest pain or ischemic changes detected by electrocardiogram. All patients had angiographic evidence of organic diameter stenosis of >70% of at least one major coronary artery (single-vessel disease, 48 patients; two-vessel disease, 45 patients; three-vessel disease, 29 patients; left main coronary artery disease, 10 patients). The metabolic syndrome was defined as the presence of three or more of the following abnormalities: waist circumference ≥85 cm in male and ≥90 cm in female (according to The Examination Committee of Criteria for Obesity Disease in Japan [17]), fasting glucose levels ≥110 mg/dL, triglyceride levels ≥150 mg/dL, HDL levels <40 mg/dL in male and <50 mg/dL in female, blood pressure ≥130/85 mmHg.

This study also enrolled 78 consecutive patients with metabolic syndrome but without CAD who underwent cardiac catheterization for atypical chest pain in the hospital during the same period as the patients with CAD. These non-CAD patients had angiographically normal coronary arteries (<10% stenosis) and normal left ventriculography and thereby formed a case control group to evaluate whether RLP-C levels as a risk factor differed between patients with and 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. Written informed consent was obtained from all patients before the study.

2.2. Lipid-lowering therapy

A subgroup of the consecutive study patients with metabolic syndrome and CAD ($n=40$) were randomly assigned to 4 weeks of oral atorvastatin (10 mg/day) or bezafibrate (400 mg/day). All of the patients were blinded to the content of the tablets. They were advised to adhere to their usual diet and lifestyle throughout the 4-week treatment period. Measurements of vasomotor function in the brachial artery and blood sampling were performed in the same manner after an overnight fast on the same morning before and at the end (4 weeks) of treatment. All medications were withdrawn 12 h before the measurements.

Table 1
Characteristics of study patients

	Without CAD ($n=78$)	With CAD ($n=132$)	p-value
Age (years)	64 ± 3.9	64 ± 3.8	NS
Gender male (%)	78	78	NS
waist (cm)	91 ± 4	92 ± 4	NS
Hypertension (%)	58	61	NS
DM (%)	64	70	NS
BMI (kg/m^2)	26.4 ± 2.9	26.6 ± 3.2	NS
Triglyceride (mg/dL)[a]	176 (149, 202)	184 (146, 241)	0.02
HDL-C (mg/dL)[a]	46 (38, 57)	43 (37, 51)	NS
LDL-C (mg/dL)	136 ± 22	136 ± 26	NS
RLP-C (mg/dL)[a]	5.9 (4.0, 7.4)	7.0 (5.1, 9.7)	<0.0001
HOMA-IR	2.3 ± 1.1	2.4 ± 1.1	NS
FFA (mg/dL)	561 ± 210	563 ± 287	NS
hsCRP (mg/dL)[a]	0.07(0.03, 0.12)	0.16 (0.08, 0.40)	<0.0001
IL-6 (pg/mL)	2.3 ± 1.3	3.5 ± 2.4	0.0002
TNF-α (pg/mL)	2.4 ± 1.3	2.8 ± 0.9	0.0002
Leptin (ng/mL)	11.3 ± 5.1	13.3 ± 5.9	0.01
FMD (%)	5.0 ± 1.6	3.8 ± 1.6	<0.0001

[a] Expressed as the median value (interquartile range). Other data are expressed as the mean value ±S.D. or frequency (%) of patients. Waist, waist circumference; DM, diabetes mellitus; BMI, Body mass index; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; FFA, free fatty acid; hsCRP, high sensitive C-reactive protein; IL-6, Interleukin 6; TNF-α, Tumor necrosis factor-α; RLP-C, remnant-like lipoprotein particles-cholesterol; FMD, flow-mediated dilatation of brachial artery.

2.3. Measurements of flow-mediated dilation (FMD) in brachial artery

Vasodilator responses in the brachial arteries were measured by use of B-mode ultrasound images with a 7.5-MHz linear array transducer (HP-5500, Phillips Corp., Tokyo, Japan) as validated previously by us as well as others [18,19]. Measurements were performed by two observers who were blinded to the study protocol and the subject grouping. The brachial artery was scanned in the antecubital fossa in a longitudinal fashion. Optimal brachial artery images were obtained between 1 and 5 cm above the antecubital crease. This location was marked, and all subsequent images were obtained at the same location. The exact distance of the measured point of the skin surface from the antecubital crease was recorded in each study subject to ensure that the same location of the brachial artery was measured at each time point. Gain setting

was optimized at the beginning of the study and was kept constant throughout the recording period. After baseline measurements of the diameter and flow velocity in the brachial artery, a blood pressure cuff was placed around the forearm and inflated with a pressure of 250–300 mmHg for 5 min, and then released. Diameter measurements during reactive hyperemia were taken 45–90 s after cuff deflation. Then, sublingual nitroglycerin (300 μg) was administered, and 3 min later the measurements were repeated. Images were recorded on a super-VHS videocassette recorder (model BR-S601M, Victor Corp., Tokyo, Japan), and brachial arterial diameters were measured from the tape with ultrasonic calipers, as described previously [18,19]. The response of the vessel diameter to reactive hyperemia (flow-mediated dilation; FMD) and nitroglycerin was expressed as a percentage increase in the diameter from the baseline value. The diameter responses were assessed at three points along a 10-mm length of the artery, and results were averaged. Blood flow was calculated by multiplying the velocity–time integral of the Doppler flow signal by heart rate and the vessel cross-sectional area. Increase in brachial blood flow was calculated as a maximum flow recorded in the first 15 s after cuff deflation and was expressed as a percentage increase in the flow from the baseline value.

2.4. Assays

At the beginning of the study, venous blood was obtained from all patients after a 12-h overnight fast. All patients ate standard Japanese meals (1900 kcal/d, 25% fat, 59% carbohydrate, and 16% protein) the day before blood sampling [8–11]. Serum was stored at 4 °C and was used for assays of lipoproteins and lipids within 3 days after sampling. The plasma and the remaining serum were stored at −80 °C until other assays were performed. RLP was isolated by application of the fasting serum to an immunoaffinity mixed gel that contained anti-apoA-1 and anti-apoB-100 monoclonal antibodies (Japan Immunoresearch Laboratories), according to the method, described in previous reports [8–14]. Levels of HDL-cholesterol, LDL-cholesterol, and triglyceride in fasting serum were measured as described previously [8–11].

Tumor necrosis factor-alpha (TNFα), interleukin (IL)-6, and leptin concentrations in fasting plasma were measured by enzyme linked immunosorbent assay (ELISA) with commercially available kits. High sensitive C-reactive protein (hsCRP) levels in the serum were assayed by rate nephelometry (Dade Behring). The insulin resistance index was assessed by homoeostasis model assessment for insulin resistance (HOMA-IR).

2.5. Statistical analyses

The levels of RLP-C, triglyceride, HDL-C, and hsCRP were not distributed normally, and therefore, these data were expressed as the median and range (25 and 75th percentiles) and were log-transformed when these data were statistically analyzed. The mean value and frequency (Table 1) were compared between two groups using the unpaired *t*-test and Chi-square analysis, respectively. For comparison of lipids and biochemical parameters before and after treatment with bezafibrate or atorbastatin (Table 4), two-way analysis of variance for repeated measures followed by post hoc testing with Sheffe's test was used. The assessment of independent association of risk factors (as independent variables) with CAD was performed by multivariate logistic regression analysis using the independent variables that had a significant difference between patients with and without CAD using an unpaired *t*-test and Chi-square analysis. The following factors were included as categorical variables: high levels of RLP-C (≥5.0 mg/dL according to our previous reports [8–11]); high levels of triglyceride (≥150 mg/dL); high levels of hsCRP (≥0.3 mg/dL; arbitrarily defined as the 75th percentile of the distribution of the levels in the study patients), and high levels of IL-6, TNFα, and leptin (≥2.6 pg/mL, 2.5 pg/mL, and 11 ng/mL, respectively; arbitrarily defined as the 50th percentile of the distribution of the respective levels in the study patients). The assessment of independent correlation of risk factors with FMD was performed by multivariate linear regression analysis using the independent variables that had a significant correlation with FMD in the univariate analysis. Statistical significance was defined as $p < 0.05$. Analyses were assessed in part using StatView 5.0 for Windows (Tokyo, Japan).

3. Results

3.1. Comparisons of risk factors between patients with and without CAD

Risk factor profiles in the study patients are shown in Table 1. The fasting levels of triglyceride, RLP-C, hsCRP, IL-6, TNFα, and leptin were significantly higher in CAD patients with metabolic syndrome compared with non-CAD patients with metabolic syndrome. Comparison of risk factors between the metabolic-syndrome patients with and without CAD using multivariate logistic regression analysis demonstrated that high levels of RLP-C, hsCRP, IL-6, TNFα, and leptin remained independent risk factors for the presence of CAD (Table 2). Moreover, high RLP-C levels had the strongest association with CAD among these covariates. In addition, high RLP-C levels were an independent risk factor for CAD in a subgroup of the patients with normotriglyceridemia (Table 2). It was clear that RLP-C levels in combination with high hsCRP levels had an incremental effect on the risk of CAD (Fig. 1, upper panel).

3.2. Correlation of risk factors with FMD in patients with metabolic syndrome and CAD

FMD was significantly impaired in the patients with CAD as compared with those without CAD, as shown in Table 1.

Table 2
Association of lipids and biochemical parameters with coronary artery disease

	All patients		Patients with normo-triglyceridemia	
	Relative risk (95% CI)	*p*-value	Relative risk (95% CI)	*p*-value
RLP-C (≥5.0 mg/dL)	7.0 (2.7–17)	<0.0001	6.8 (2.6–17)	<0.0001
Triglyceride (≥150 mg/dL)	0.4 (0.2–0.9)	NS	–	–
hsCRP (≥0.3 mg/dL)	6.5 (2.6–16)	<0.0001	4.2 (2.0–8.7)	0.0001
IL-6 (≥2.6 pg/mL)	2.5 (1.3–5.2)	<0.001	2.5 (1.2–5.2)	0.01
TNFα (≥2.5 pg/mL)	3.6 (1.7–7.7)	0.001	3.2 (1.5–6.8)	<0.01
Leptin (≥11 ng/mL)	2.1 (1.1–4.4)	<0.05	1.9 (0.9–4.0)	NS

CI, confidence interval. Other abbreviations as in Table 1.

FMD had a significant and inverse correlation with levels of RLP-C, triglyceride, hsCRP, IL-6, TNFα, and HOMA-IR in patients with metabolic syndrome and CAD using univariate linear regression analysis (Table 3). Further, FMD also had a positive correlation with HDL-C levels. FMD was comparable between patients with and without smoking status, diabetes, hypertension, or male sex as categorical risk factors (data not shown). Using multivariate linear regression analysis, FMD had an independent and inverse correlation with levels of RLP-C, triglyceride, and hsCRP after adjustment for levels of HDL-C, IL-6, and TNFα, and HOMA-IR (Table 3). Furthermore, RLP-C levels had a stronger association with FMD than triglyceride and hsCRP levels. It was clear that high RLP-C levels in combination with high hsCRP levels had an incremental effect on the risk of endothelial vasomotor dysfunction (Fig. 1, lower panel). Dilator response to nitrate was not correlated with either RLP-C levels or hsCRP levels ($r = 0.03$, p = NS, $r = 0.01$, and p = NS, respectively).

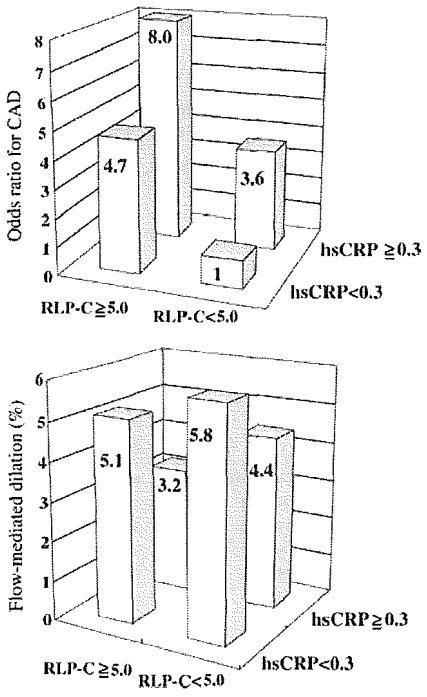

Fig. 1. Upper panel, incremental effect on odds ratios for CAD of the combination of higher levels of RLP-C and hsCRP. Lower panel, incremental effect on FMD of the combination of higher levels of RLP-C and hsCRP.

3.3. *Effects of treatment with bezafibrate or atorvastatin on FMD and other parameters in patients with metabolic syndrome and CAD*

Before treatment, there was not significant difference in FMD, lipid levels, and values of other parameters tested between patients treated with bezafibrate and atorvastatin (Table 4). Treatment for 4 weeks with bezafibrate or atorvastatin significantly decreased levels of RLP-C, triglyceride, LDL-C, hsCRP, IL-6, TNFα, and HOMA-IR, and increased HDL-C levels (Table 4). LDL-C levels had a greater decrease in the atorvastatin group than the bezafibrate group, while the other parameters had comparable changes from pretreatment values between the two treatment groups (Table 4). Also, bezafibrate and atorvastatin significantly improved FMD to a comparable degree after 4 weeks of therapy (Table 4). Using univariate linear regression analysis, the changes in FMD from baseline (post-treatment value minus pre-treatment

Table 3
Univariate and multivariate linear regression analyses; correlation of FMD with lipid and biochemical parameters and components of metabolic syndrome

	Univariate analysis		Multivariate analysis	
	r	*p*-value	Standardized regression coefficient	*p*-value
RLP-C	−0.64	<0.0001	−0.46	<0.0001
Triglyceride	−0.51	<0.0001	−0.3	0.002
hsCRP	−0.59	<0.0001	−0.26	0.002
HDL-C	0.39	0.02	0.07	NS
IL-6	−0.29	0.0002	−0.08	NS
TNFα	−0.25	0.001	−0.07	NS
HOMA-IR	−0.26	0.001	−0.12	NS
Leptin	−0.02	NS	–	–
Age	−0.13	NS	–	–
Waist circumference	−0.1	NS	–	–
BMI	0.02	NS	–	–
Systolic BP	0.01	NS	–	–

r, regression coefficient; FMD, flow-mediated dilation; BP, blood pressure. Other abbreviations as in Table 1.

Table 4
Changes of lipids and biochemical markers after treatment with bezafibrate or atorvastatin

	Bezafibrate ($n=20$)		Atorvastatin ($n=20$)	
	Before	After treatment	Before	After treatment
FMD (%)	4.02 ± 2.8	5.9 ± 3.4*	4.1 ± 2.9	6.0 ± 3.4*
RLP-C (mg/dL)[a]	9.2 (7.0, 12)	5.2 (4.6, 6.5)**	9.1 (7.2, 10.6)	5.3 (4.5, 6.7)**
Triglyceride (mg/d)[a]	200 (155, 253)	136 (80, 172)**	202 (171, 251)	141 (101, 163)**
HDL-C (mg/dL)[a]	45 (38, 53)	56 (43, 62)**	46 (37, 55)	51 (43, 61)**
LDL-C (mg/dL)	133 ± 24	119 ± 31**	139 ± 34	85 ± 23[b,**]
hsCRP (mg/Dl)[a]	0.23(0.12, 0.30)	0.09 (0.05, 0.1)**	0.3 (0.13, 0.40)	0.09 (0.03, 0.28)**
IL-6 (pg/mL)	2.6 ± 1.5	2.3 ± 1.1*	2.6 ± 1.7	2.2 ± 1.1*
TNFα(pg/mL)	2.31 ± 0.71	2.07 ± 0.44**	2.32 ± 0.70	2.02 ± 0.67**
HOMA-IR	2.2 ± 1.6	1.5 ± 0.7*	2.13 ± 0.9	1.68 ± 0.6*

FMD, flow mediated vasodilatation. Other abbreviations as in Table 1.
[a] Expressed as the median value (interquartile range). Other data are expressed as the mean value ± S.D.
[b] $p<0.01$ versus after treatment with bezafibrate.
* $p<0.05$.
** $p<0.01$ versus respective values before treatment.

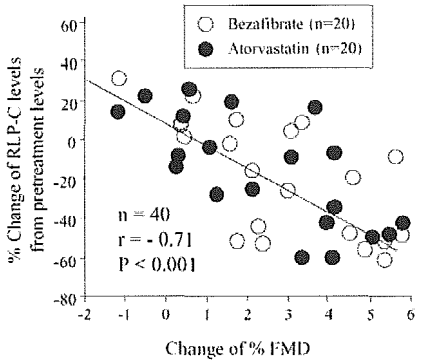

Fig. 2. Correlation of the change in FMD with the % change in RLP-C levels from pre-treatment values after treatment with bezafibrate (open circles) or atorvastatin (closed circles). The changes in FMD were calculated as the differences between pre-treatment and post-treatment values (post-treatment values minus pre-treatment values).

value) after the treatment with the lipid-lowering medications had a significant association with the % changes in levels of RLP-C, triglyceride, HOMA-IR, hsCRP, and IL-6 from their pre-treatment values after treatment (Fig. 2 and Table 5). Using multivariate linear regression analysis, the % changes in RLP-C levels had a significant and independent association with the changes in FMD after adjustment for the drugs assigned and the % changes in levels of triglyceride, HOMA-IR, hsCRP, and IL-6 (Table 5).

4. Discussion

This study showed that high RLP-C levels were the strongest risk factor for CAD and the impaired FMD in patients with metabolic syndrome independently of the covariates including the components of metabolic syndrome and the proinflammatory markers levels. Furthermore, this study showed that treatment with atorvastatin or bezafibrate induced rapid improvement of FMD in patients with metabolic syndrome. Also, treatment with atorvastatin or bezafibrate improved dyslipidemia including high RLP-C levels and decreased proinflammatory markers levels. The reduction of RLP-C levels after treatment with the lipid-lowering medications had a strong association with the improvement of FMD independently of drugs assigned and changes in levels of triglyceride, HOMA-IR, hsCRP, and IL-6. On the other hand, neither RLP-C levels nor other risk factors was correlated with dilator response to nitrates, an endothelium-independent dilation. Therefore, measurement of RLP-C is useful for the assessment of therapeutic effects on endothelial vasomotor dysfunction in metabolic syndrome.

We and others have shown that RLP-C levels are increased and predict future coronary events in patients with CAD and type II DM or insulin resistance [11]. Based on the mechanisms as described in previous reports, [6,7,11] increased flux

Table 5
Univariate and multivariate linear regression analyses; correlation between changes in FMD and lipids and biochemical parameters from baseline values after treatment

	Univariate analysis		Multivariate analysis	
	r	p-value	Standardized regression coefficient	p-value
RLP-C	−0.71	<0.0001	−0.66	<0.0001
Triglyceride	−0.46	<0.05	−0.31	NS
HOMA-IR	−0.41	<0.05	−0.2	NS
hsCRP	−0.55	<0.01	−0.58	<0.001
IL-6	−0.47	<0.01	−0.3	NS
TNFα	−0.3	NS	–	–
LDL-C	−0.13	NS	–	–
HDL-C	0.13	NS	–	–

r, regression coefficient. Other abbreviations as in Tables 1–3. Multivariate regression analysis included only the covariates that related to changes in FMD in the univariate analysis. Changes in FMD, post-treatment FMD minus pre-treatment FMD.

of free fatty acids from the periphery to the liver might cause hepatic production and secretion of triglyceride-rich VLDL, leading to increase in circulating levels of remnant lipoproteins in patients with metabolic syndrome. However, the causes of remnant lipoproteinemia in the metabolic syndrome are multifactorial and linked with each other and not simply a function of increased free fatty acid and flux to the liver. For example, a proinflammatory state intimately connects with dyslipidemia in metabolic syndrome [1,5]. Elevated levels of TNFα and IL-6, independent risk factor for CAD in the present patients with metabolic syndrome are known to increase triglyceride levels, [20,21] which could contribute to remnant lipoproteinemia. Furthermore, the present study demonstrated that high levels of hsCRP also were an independent risk factor for endothelial vasomotor dysfunction and CAD. When we categorized patients according to RLP-C levels and hsCRP levels at baseline, higher levels of RLP-C and hsCRP were additive in their effect on the risk of endothelial vasomotor dysfunction and CAD in patients with metabolic syndrome. Taken together, these results are compatible with previous data that chronic subclinical inflammation is an important factor in the pathogenesis of metabolic syndrome [22].

The present study showed that high RLP-C levels were an independent risk factor for CAD even in a subgroup of the patients with metabolic syndrome and normo-triglyceridemia. Furthermore, high RLP-C levels had a stronger association with endothelial vasomotor dysfunction than high triglyceride levels. Although RLP levels are closely and positively correlated with triglyceride levels, [8] the present data indicate that high RLP-C levels are a better marker of dyslipidemia than hyper-triglyceridemia in this syndrome.

As described in previous reports, [15,16] FMD is likely to be lower in the present non-CAD patients with metabolic syndrome as compared with control patients without metabolic syndrome and CAD in our database (5.0 ± 1.6 versus 7.7 ± 1.8, respectively) [18,19]. We showed recently that RLP increased the susceptibility of the coronary endothelium to oxidative stress, [8,13] leading to inhibition of nitric oxide-mediated dilation of human coronary arteries [8]. Furthermore, we showed that RLP upregulated the expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and tissue factor in cultured human endothelial cells [12]. In addition, there is evidence that RLP enhances platelets aggregation [23]. These proatherothrombogenic effects of RLP may explain the association of high RLP-C levels with the increased prevalence of CAD and endothelial dysfunction in metabolic syndrome. In this regard, high RLP-C levels could be a distinct patho-physiological feature of metabolic syndrome, and thus measurement of RLP-C is useful for identification of high-risk populations. 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 and therefore not applicable for clinical use. 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.

The present study showed that bezafibrate and atorvastatin, different types of lipid-lowering drugs, exerted beneficial effects on FMD, levels of RLP-C and triglyceride, HOMA-IR, and proinflammatory markers with comparable degree except for LDL-C levels. The beneficial effect on vasomotor function is consistent with a previous report, [24] but this previous study showed that the improvement of FMD was not correlated with changes in lipids profiles after treatment with fenofibrate or atorvastatin, though remnant lipoproteins levels were not assessed. Several reports have shown that both statin and fibrate are capable of improving insulin sensitivity through a reduction of triglyceride levels or their pleiotropic effects [25–27]. Thus, the reduction in HOMA-IR after treatment with atorvastatin and bezafibrate may be partly mediated through direct or indirect favorable effects of atorvastatin and bezafibrate on insulin sensitivity. It remains undetermined which of statins and fibrates, first-line lipid-lowering drugs, are more useful for preventing CVD in metabolic syndrome.

In conclusion, elevated levels of RLP-C are a risk factor for endothelial dysfunction and CAD in metabolic syndrome. Measurement of RLP-C is useful for assessment of CAD risk and therapeutic effects in metabolic syndrome.

Acknowledgement

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Increased Ambulatory Pulse Pressure is a Strong Risk Factor for Coronary Endothelial Vasomotor Dysfunction

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STRUCTURED ABSTRACT

OBJECTIVES: This study was aimed to determine the relationship between pulse pressure (PP) and coronary vasomotor dysfunction, a predictor of coronary events.
BACKGROUND: PP is a strong risk factor for coronary artery disease (CAD). However, the mechanisms by which an increase in PP affects the pathogenesis of CAD are unclear.
METHODS: Ambulatory blood pressure (BP) monitoring for 24 hr was performed in 103 consecutive patients with normal coronary angiograms (51 hypertensive and 52 normo-tensive; age, 42-70 years). The relationship between changes in coronary arterial diameter and blood flow during an intracoronary infusion of acetylcholine (ACh) (5, 10, 50 μg/min), and BP parameters and other traditional risk factors was evaluated using univariate and multivariate linear regression analyses.
RESULTS: With multivariate analyses, the 24 hr PP showed an inverse correlation with the epicardial coronary dilator response to ACh independently of other covariates including age, smoking, and 24 hr systolic BP in normo-tensive as well as hypertensive patients. Furthermore, multivariate analysis showed that the 24 hr PP was inversely and independently correlated with the increase in coronary blood flow in response to ACh. The dilator response of epicardial coronary arteries to nitrate was not significantly correlated with 24 hr PP.
CONCLUSIONS: Increased 24 hr PP is independently associated with endothelial vasomotor dysfunction in conduit and resistance coronary arteries irrespective of the presence of hypertension. Increased ambulatory PP may have an intimate relation to coronary endothelial vasomotor dysfunction.

KEY WORDS

Hypertension, Endothelium-dependent dilation, Risk factors, Pulse pressure, Ambulatory blood pressure monitoring.

CONDENSED ABSTRACT

The mechanisms by which increased pulse pressure (PP) is a risk for coronary artery disease are unclear. This study examined the relationship between changes in coronary arterial diameter and blood flow to acetylcholine, and blood pressure (BP) parameters in 103 patients. This study found that an increase in 24 hr PP measured by ambulatory BP monitoring was independently associated with endothelial vasomotor dysfunction, a predictor of future coronary events, in conduit and resistance coronary arteries irrespective of the presence of hypertension. Thus, increased PP may have an intimate association with coronary endothelial vasomotor dysfunction.

A List of Abbreviations and Their Definitions

PP = pulse pressure,
BP = blood pressure,
SBP = systolic blood pressure,
DBP = diastolic blood pressure,
ABPM = ambulatory blood pressure monitoring,
CAD = coronary artery disease,
ACh = acetylcholine,
HR = heart rate,
NO = nitric oxide

Introduction

Pulse pressure (PP), calculated as the difference between systolic and diastolic blood pressure (SBP and DBP, respectively), has been previously reported to be a stronger cardiovascular risk factor than SBP alone, especially in elderly hypertensive patients (1). Even in normo-tensive subjects, an increase in PP remains a powerful and independent predictor of cardiovascular risk, particularly for myocardial infarction (2). However, the underlying mechanisms by which an increase in PP plays a role in pathogenesis of coronary artery disease (CAD) remain unclear. A recent report (3) showed that an increase in PP was associated with endothelial vasomotor dysfunction, an independent predictor of future coronary events, in the resistance vessels downstream from the brachial artery in hypertensive patients. However, there is no information on the effects of PP on endothelial vasomotor functions in human coronary arteries in normo-tensive or hypertensive patients. Furthermore, the relationship between PP and endothelial vasomotor function in the coronary arteries may not be similar to the brachial artery because of the predominant role of DBP in the coronary circulation. Thus, the objective of this study was to determine whether an increase in PP might be associated with endothelial vasomotor dysfunction in the conduit and resistance vessels in the coronary circulation in both normo-tensive and hypertensive subjects.

Methods

Study Patients

Study subjects consisted of a consecutive series of 103 patients. Characteristics of the study subjects are shown in Table 1. They underwent diagnostic coronary angiography for atypical chest pain (95 subjects) or ST depression at rest or during exercise without chest pain (8 subjects) in Yamanashi University Hospital between January, 2002 and January, 2004. They fulfilled all of the following inclusion criteria: (1) angiographically normal coronary arteries (<5% narrowing after nitrate administration) and no coronary spasm (<50% decrease in epicardial coronary diameter from baseline and neither chest pain nor ischemic electrocardiographic change) after the intracoronary infusion of acetylcholine (ACh); (2) normal left ventriculography; (3) no left ventricular hypertrophy, verified by both electrocardiography and echocardiography; and (4) no history of myocardial infarction, congestive heart failure, valvular heart disease, secondary hypertension, stroke, renal dysfunction (serum creatinine concentration > 2.0 mg/dl) or other serious diseases. All medications that could have affected coronary vasomotor reactivity and BP were withdrawn > 3 days before the study. Hypertension was defined according to JNC-VI criteria (4): 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. Written informed consent was obtained from all study subjects before the study. The study was approved by the ethics committee at our institution.

Protocol for Coronary Angiography

After baseline angiography, incremental doses of ACh (5, 10, and 50 μg/min) were infused directly into the left coronary artery through the Judkins catheter for 2 min with a 5-min interval between each dose (5). Hemodynamic measurements and coronary angiography were repeated before and during each of the ACh infusions. After an additional 15 min, intracoronary injection of isosorbide dinitrate (1 mg) was performed. Two minutes after that, coronary angiography was performed in multiple projections in all study subjects.

Ambulatory BP Measurements

SBP, DBP, PP, and heart rate (HR) during daily activities were measured every 30 min for 24 hr, by the oscillometric method, using a noninvasive ambulatory BP monitoring system (TM-2425, A&D, Tokyo, Japan) (6). The daytime and nighttime mean values of SBP, DBP, PP, and HR during the 24-hour period were analyzed after reviewing the patients' diaries. We defined daytime as the period from the time they awoke to the time they went to sleep, and nighttime as the period during which they were sleeping (7). The daytime, nighttime, and 24 hr SBP, DBP, PP, and HR were the averages of all of the values obtained at 30-min intervals. Non-dipper hypertension was defined by the absence of the fall (>10%) in the nighttime mean SBP, and/or in DBP from the respective daytime values (7).

Quantitative Coronary Angiography and the Measurement of Coronary Blood Flow

A quantitative coronary angiographic study was performed in all of the study subjects with the Judkins technique in the morning when the patients were fasting, in the same manner as described previously (5). Measurement of luminal diameter of the left

anterior descending coronary artery at the mid segment was performed quantitatively by use of a computer-assisted coronary angiographic analysis system (Cardio 500, Kontron Instruments) by 2 observers blinded to the study protocol. Responses of the coronary artery diameter to infusions of ACh and nitrates were expressed as percent changes from baseline diameter measured on angiograms taken just before each infusion.
Blood flow velocity was measured in a subgroup of 56 consecutive subjects using a 0.014-in wire equipped with a Doppler crystal at its tip (Flow Wire, Cardiometrics), which was advanced through the Judkins catheter and carefully positioned in a straight proximal segment of the left anterior descending coronary artery to obtain a stable flow velocity signal (5). The stable peak flow velocity signals at baseline and during a 2-min infusion of ACh at doses of 5 and 10 μg/min were used for the analysis (Flow Map, Cardiometrics). Coronary blood flow (mL/min) was estimated from coronary blood flow velocity and arterial diameter by the following formula: 0.5 x averaged peak velocity (cm/min) x cross-sectional area (cm^2). The response of coronary blood flow to intracoronary infusions of ACh was expressed as a percentage change from the baseline blood flow just before ACh infusion.

Statistical Analysis

Results are expressed as the mean ± SD or percentage. The mean values of continuous variables were compared between the 2 groups using an unpaired t test and frequencies were compared by χ^2 analysis. Comparison of continuous variables among more than 3 groups was performed using one-way ANOVA. Linear regression analysis was used to determine the relationship between the coronary responses and all continuous variables. Multivariate linear regression analyses were also used to determine the relationship between coronary responses and 24 hr ambulatory PP; independent covariates included any continuous variable that was significantly correlated with the coronary responses in the univariate analysis. In addition, the multivariate analysis also included any categorical risk factor that led to a significant difference in coronary responses when patients with and without that the traditional risk factors were compared using an unpaired t test. The categorical variables were coded using the following dummy variables: 0 for the absence of the risk factor; or 1 for the presence of the risk factor. When correlation between coronary flow response and risk factors was analyzed in the multivariate analysis, only data from all patients were used because there were too few patients tested for coronary flow response in the various subgroups. A confidence level of $P < 0.05$ was considered statistically significant. Analyses were partially assessed using StatView 5.0 (SAS Institute, Cary, North Carolina).

Results

Comparisons of Clinical Characteristics and Parameters Among All Study Patients, Hypertensive and Normo-tensive Patients

None of the clinical characteristics or parameters except the BP parameters was significantly different among the 3 groups, all patients, hypertensive patients and normo-tensive patients (Table 1).

Correlations of Epicardial Coronary Diameter Responses With Clinical Characteristics and BP Parameters

Intracoronary infusion of ACh constricted the coronary arteries in a majority of patients and dilated the arteries in a small number of patients, resulting in an overall

constrictor response. Using univariate linear regression analysis, age and 24 hr ambulatory PP and SBP had a significant and inverse correlation with the dilator responses of epicardial coronary arteries to ACh at dose of 50 μg/min in all patients (Fig 1 and Table 2). Age and ambulatory PP and SBP also showed a significant correlation in the subgroup of normo-tensive patients, as shown in Table 2. As shown in Table 3, smokers had an impaired dilation or an enhanced constriction of epicardial coronary arteries to ACh as compared with nonsmokers in all 3 of the study groups (Table 3). Using multivariate linear regression analysis after adjustment for age, smoking status, and ambulatory SBP as covariates, 24 hr ambulatory PP remained significantly and inversely correlated with the coronary diameter response to ACh at dose of 50 μg/min in all patients, the hypertensive patients, and the normo-tensive patients (Table 4). Ambulatory PP also was independently correlated with the diameter responses to ACh at 5 and 10 μg/min in all patients as well as normo-tensive patients (standardized regression coefficient, 5 μg/min; – 0.47 and – 0.53, respectively; $P < 0.05$ in both. 10 μg/min; – 0.44 and – 0.47, respectively; $P < 0.05$ in both). The dilator response to nitrates was not significantly correlated with 24 hr ambulatory PP in either all patients or just the normo-tensive patients ($r = – 0.1$, P = NS; $r = – 0.12$, P = NS, respectively).

Correlation of Coronary Flow Responses With Clinical Characteristics and BP Parameters

Coronary blood flow was increased in response to ACh infusion in all patients studied. Using univariate linear regression analysis, age and ambulatory 24 hr PP and SBP had a significant and inverse correlation with percent increase of coronary flow in response to ACh at doses of 5 μg/min in all patients (Fig 2 and Table 2). Age and ambulatory PP also had a significant correlation in the subgroup of the normo-tensive patients (Table 2). Diabetic patients had an impaired increase of coronary flow to ACh as compared with non-diabetic patients in all of the 3 study groups (Table 3). In all of the patients, 24 hr ambulatory PP remained significantly and inversely correlated with the % increase of flow in response to ACh at doses of 5 μg/min using multivariate linear regression analysis after adjustment for age, ambulatory SBP, and diabetes (these covariates were significantly related to the flow response in the univariate linear regression analysis or the unpaired t test) (Table 4). Also, the independent and inverse correlation of 24 hr ambulatory PP with the % increase of flow in response to ACh at doses of 10 μg/min was observed in all of the patients (standardized regression coefficient = – 0.45, $P < 0.05$).

Discussion

The present study assessed the relation between PP and endothelial vasomotor function in human coronary arteries. Multivariate analyses indicated that increased ambulatory PP had a significant and independent correlation with abnormal vasomotor reactivity in both the conduit and resistance vessels in the coronary circulation, as demonstrated by impaired dilation or enhanced constriction of epicardial coronary arteries and by the impairment of the coronary blood flow increase in response to an intracoronary infusion of ACh. The epicardial coronary diameter responses to nitrates were not significantly correlated with ambulatory PP. Thus, the present results indicate that an increase in ambulatory PP has an independent association with endothelium-dependent vasomotor dysfunction in conduit and resistance coronary vessels in the coronary circulation.

The present study further showed that increased PP also had a significant and independent association with epicardial coronary vasomotor dysfunction in a subgroup of normo-tensive patients. This may be related to previous findings (2) that an increase in PP is a strong predictor of cardiovascular disease, especially myocardial infarction, independently of other BP parameters. However, prospective studies are required to determine the value of ambulatory PP for the prediction of future cardiac events in patients with preclinical hypertension in order to confirm the results of the present study. In contrast to the usefulness of ambulatory PP in the present study, office PP did not have a significant association with coronary endothelial vasomotor dysfunction. This is consistent with previous reports (1,8,9) that found 24 hr ambulatory PP monitoring superior to office PP measurement for predicting cardiovascular disease because ambulatory PP may more accurately reflect the dynamic interaction between the heart and the central stiff arteries during all of the patient's activities.

We and others have previously shown that coronary vasomotor regulation largely depends on endothelial nitric oxide (NO) activity (10,11). Thus, the decrease in coronary endothelial NO activity may be the underlying mechanism for the coronary endothelial vasomotor dysfunction in the present patients with increased ambulatory PP. The decrease in arterial NO might cause coronary artery spasm (12) and induction of various pro-atherothrombogenic molecules in the arterial walls (13), resulting in a high incidence of myocardial infarction.

PP is largely determined by 3 hemodynamic factors: arterial stiffness, stroke volume, and wave reflections (14). Among these factors, an increase in arterial stiffness most importantly contributes to the effects of an elevated PP on the risk of cardiovascular disease. An increase in extracellular matrix formation causes arterial stiffness, especially in central arteries, and it might largely contribute to the increase in PP in patients with age and hypertension (15). Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers attenuate extracellular matrix formation in addition to reducing SBP (15). Therefore, these drugs could reduce arterial stiffness, thereby effectively reducing PP as well as SBP.

In conclusion, increased 24 hr ambulatory PP has a strong and independent association with endothelial vasomotor dysfunction in the conduit and resistance vessels in the coronary circulation in normo-tensive as well as hypertensive patients. Increased ambulatory PP may have an intimate relation to coronary endothelial vasomotor dysfunction.

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Figure Legends

Fig 1.
Correlations of 24 hr ambulatory PP with % changes of epicardial coronary dilator responses to ACh (50 μg/min) in all the study patients, hypertensive patients, and normo-tensive patients.

Fig 2.
Correlations of 24 hr ambulatory PP with % changes of coronary blood flow responses to ACh (5 μg/min) in all the study patients, hypertensive patients, and normo-tensive patients.

Table 1. Study Patients' Characteristics

	All patients (n=103)	Hypertensive patients (n=51)	Normotensive patients (n=52)
Age (yrs)	62±11	63±11	61±12
Male (%)	45	45	44
Body mass index (kg/m^2)	24±4	25±5	24±3
Smoking (%)	45	49	41
Total cholesterol (mg/dL)	205±36	206±39	203±33
Diabetes mellitus (%)	26	30	22
Hypertension (%)	50	-	-
Non-dipper (%)	31	62*	-
Ambulatory daytime PP (mmHg)	50±11	54±13	47±8 †
Ambulatory daytime SBP (mmHg)	127±17	140±16*	119±12* †
Baseline coronary diameter (mm)	3.3±0.8	3.4±1.0	3.2±0.6
Baseline coronary flow (mL/min)	83±39	85±40	80±39

Data are expressed as mean±SD and percentage. *$p<0.05$ vs. All patients, † $p<0.05$ vs. Hypertensive patients.
PP; pulse pressure, SBP; systolic blood pressure.

Table 2. Relationships of coronary risk factors (continuous variables) with the % changes in diameter of epicardial coronary arteries and coronary blood flow response to ACh using univariate linear regression analysis

	% Change in Epicardial Diameter (ACh 50 μg/min)			% Change in Coronary Flow (ACh 5 μg/min)		
	All patients (n=103)	Hypertensive patients (n=51)	Normotensive patients (n=52)	All patients (n=56)	Hypertensive patients (n=29)	Normotensive patients (n=27)
Age	-0.50**	-0.44**	-0.53**	-0.31*	0.24	-0.41*
BMI	-0.08	-0.01	-0.10	0.04	-0.06	-0.13
Total cholesterol	0.06	0.09	0.04	-0.23	-0.33	-0.08
24hr PP	-0.46**	-0.47**	-0.44**	-0.40**	-0.38*	-0.44*
24hr SBP	-0.40**	-0.35*	-0.43**	-0.36*	-0.39*	-0.25
24hr DBP	-0.18	-0.03	-0.23	-0.18	-0.20	0.02
Office PP	-0.28	-0.27	-0.30	-0.29	-0.34	-0.17
Office SBP	-0.26	-0.20	-0.19	-0.29	-0.36	-0.19
Office DBP	0.04	0.22	0.23	-0.07	0.22	-0.06

Data are expressed as regression coefficient. *p<0.05, **p<0.01.
BMI, body mass index; PP, pulse pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 3. Comparisons of % changes in diameter of epicardial coronary arteries and coronary blood flow response to ACh using categorical variables to classify patients

	% Change in Epicardial Diameter (ACh 50 μg/min)					
	All patients (n=103)		Hypertensive patients (n=51)		Normotensive patients (n=52)	
	Presence	Absence	Presence	Absence	Presence	Absence
Male	-13±7	-10±6	-15±5	-12±6	-12±8	-9±7
Smoking	-14±6	-9±6*	-15±5	-11±6*	-13±7	-8±7*
Diabetes	-14±5	-11±7	-15±5	-12±6	-11±5	-10±8
Hypertension	-13±6	-10±8				
Non-dipper Hypertension			-12±6	-14±5		
	% Change in Coronary Flow (ACh 5 μg/min)					
	All patients (n=56)		Hypertensive patients (n=29)		Normotensive patients (n=27)	
	Presence	Absence	Presence	Absence	Presence	Absence
Male	67±58	59±47	45±45	58±47	89±64	59±49
Smoking	63±57	62±48	45±45	59±46	83±65	65±51
Diabetes	21±35	77±49*	24±39	73±39*	11±10	80±56*
Hypertension	53±46	72±57				
Non-dipper Hypertension			51±50	54±39		

Data are expressed as mean±SD. *p<0.05 vs. presence of respective factors.

Table 4. Multiple linear regression analysis for the association of risk factors with relative changes in epicardial coronary diameters and coronary flow response to ACh

	% Change in Epicardial Diameter (ACh 50 μg/min)			% Change in Coronary Flow (ACh 5 μg/min)
	All patients (n=103)	Hypertensive patients (n=51)	Normotensive patients (n=52)	All patients (n=56)
Age	-0.45**	-0.34*	-0.51**	-0.14
24hr PP	-0.36*	-0.50**	-0.36*	-0.50**
Smoking	-0.42**	-0.39*	-0.54**	
Diabetes				-0.45**
24hr SBP	-0.19	0.09	-0.05	-0.22

Data are expressed as standardized regression coefficient. *p<0.05, **p<0.01.
PP, pulse pressure; SBP, systolic blood pressure.