Association of Remnant Lipoprotein Levels With Impairment of Endothelium-Dependent Vasomotor Function in Human Coronary Arteries

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Background—It remains undetermined whether triglyceride-rich lipoproteins are an independent risk factor for atherosclerosis.

Methods and Results—The correlation of responses of coronary arterial diameter (quantitative coronary angiography) and coronary blood flow (intracoronary flow wire technique) to intracoronary infusion of acetylcholine (10 and 50 μg/min) with coronary risk factors including remnant lipoprotein levels was statistically analyzed in 106 consecutive subjects with normal coronary angiograms. Remnant lipoproteins were isolated from fasting blood with an immunoaffinity mixed gel containing anti–apolipoprotein (apo) A-1 and anti–apoB-100 monoclonal antibodies. In multivariate stepwise regression analysis, remnant lipoprotein levels had the most significant correlation with abnormal epicardial coronary vasomotor responses to acetylcholine infusion, reflected by impaired dilation or constriction of the epicardial coronary arteries, and the levels also had an inverse and independent correlation with the coronary blood flow increase in response to acetylcholine. In a subgroup of 53 consecutive subjects, constrictor responses of epicardial coronary diameters to intracoronary infusion of N^G-monomethyl-L-arginine (50 μmol/min for 4 minutes) at baseline, reflecting the presence of coronary nitric oxide bioactivity, had an inverse and independent correlation with remnant lipoprotein levels by use of multivariate analysis.

Conclusions—Remnant lipoprotein levels were independently associated with abnormal endothelium-dependent vasomotor function in large and resistance coronary arteries in humans, indicating that remnant lipoproteins may impair endothelial vasomotor function in human coronary arteries. The decrease in coronary nitric oxide bioactivity may be responsible in part for the inhibitory effects of remnant lipoproteins. (Circulation. 1998;97:2519-2526.)

Key Words: acetylcholine ■ lipoproteins ■ endothelium-derived factors ■ endothelium ■ hyperlipoproteinemia

Endothelial dysfunction is known to be an early event in atherosclerotic development and an important contributor to the pathogenesis of coronary artery disease. The arterial response to ACh is determined by the balance between the dilator action of endothelium-derived substances, including NO, and the direct constrictor action of ACh on smooth muscle. Thus, endothelial dysfunction leads to impaired dilation or constriction of coronary arteries, and it also leads to impairment of the increase in coronary blood flow in response to intracoronary infusion of ACh. We and others have demonstrated that intracoronary infusion of L-NMMA, an inhibitor of NO synthase, constricted coronary arteries at baseline and decreased the dilator response or augmented the constrictor response of arteries to intracoronary infusion of ACh, reflecting the presence of coronary NO bioactivity. Furthermore, risk factors for coronary artery disease primarily inhibit coronary NO bioactivity in humans.

It remains controversial whether triglycerides and triglyceride-rich lipoproteins are independent risk factors for atherosclerosis. However, there is increasing clinical evidence showing that high levels of remnant lipoproteins, derived from VLDL and chylomicrons, are associated with the progression of coronary atherosclerosis, and isolated remnant lipoproteins are reported to be taken up by macrophages and to cause foam cell formation in vitro experiments. Thus, remnant lipoproteins might cause endothelial vasomotor dysfunction and increase the risk of coronary atherosclerosis in patients with hypertriglyceridemia. It has been difficult to assay levels of remnant lipoproteins, because they have heterogeneous properties. However, we have recently developed a simple and reliable method to isolate...
remnant lipoproteins by an immunoaffinity mixed gel of anti-\(\text{apoA-I}\) and anti-\(\text{apoB-100}\) monoclonal antibodies.\(^{15-18}\)

Thus, this study was performed to determine, by use of multivariate analysis of risk factors for atherosclerosis, whether levels of remnant lipoproteins isolated by the gel may have a significant relation to human coronary endothelial dysfunction in a large number of subjects.

### Methods

#### Study Subjects Enrolled for Analyses of Coronary Vasomotor Function

Study subjects, who were examined for association of coronary risk factors with coronary vasomotor function, consisted of a consecutive series of 106 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 on rest or exercise ECG without chest pain (11 subjects) in Kumamoto University Hospital between January 1995 and March 1997. They fulfilled all of the following inclusion criteria: (1) angiographically normal coronary arteries (<5% narrowing after nitrate administration) and no coronary spasm after intracoronary infusion of \(\text{ACh}\); (2) normal left ventriculography; (3) no left ventricular hypertrophy, verified by ventriculography; and (4) no history of myocardial infarction, congestive heart failure, valvular heart disease, or other serious diseases. All lipid-lowering drugs and other medications that could have affected coronary vasomotor reactivity were withdrawn ≥7 days before the study. Written informed consent was obtained from all study subjects before the study. The study was approved by the ethics committee at our institution.

#### Assessment of Coronary Risk Factors

The following clinical information and fasting levels of lipids, lipoproteins, and apo were collected from all study subjects: age, sex, body mass index, history of smoking (defined as smoking ≥10 cigarettes/d within 1 month before the study), history of hypertension (blood pressure ≥140/90 mm Hg or requiring antihypertensive medication), history of diabetes mellitus (according to World Health Organization criteria), family history of coronary heart disease, remnant lipoprotein cholesterol, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, \(\text{Lp(a)}\), and apo (\(\text{A-I}\), \(\text{A-II}\), \(\text{B}\), \(\text{C-II}\), \(\text{C-III}\), and \(\text{E}\)). We statistically analyzed all of these risk factors for association with coronary vasomotor function using both univariate and multivariate statistical analyses. In subgroups of study subjects, fasting and postprandial plasma insulin levels (42 subjects), postprandial remnant levels (21 subjects), and waist-to-hip ratios (20 subjects) were obtained and used for statistical analysis of the association with epicardial coronary diameter: response to \(\text{ACh}\) by use of univariate statistical analysis, because the number of subjects in each study subgroup was too small to be used for multivariate statistical analysis. After admission to our hospital, all of the study patients ate a standard meal for ≥5 days before cardiac catheterization, when blood sampling for the assays was performed. The energy content of the standard meal was 1900 kcal/d (55 g fat [25% of total energy], 270 g carbohydrate [59%), and 70 g protein [16%]; the ratio of polyunsaturated fatty acids to saturated fatty acids was 1:1.3). Blood was obtained for assays after an overnight fast and before heparinization at the time of the cardiac catheterization. Serum levels of total cholesterol and triglycerides were measured by enzymatic methods.\(^{16,18}\) HDL cholesterol levels in serum were measured with the use of polyethylene glycol–modified enzymes and sulfated N\(-\)G\(-\)monomethyl L\(-\)arginine (L-NMMA) high-performance liquid chromatography (HPLC) assay. Serum levels of apo (\(\text{A-I}\), \(\text{A-II}\), \(\text{B}\), \(\text{C-II}\), \(\text{C-III}\), and \(\text{E}\)) and \(\text{Lp(a)}\) were measured by an immunoturbidimetric technique using commercially available kits (apo: Daiichi; \(\text{Lp(a)}\): Chugai Pharmaceutical). LDL cholesterol was calculated according to the Friedewald formula.\(^{21}\) The calculated values of LDL cholesterol in 3 subjects with triglyceride levels >400 mg/dL were excluded from the data of LDL cholesterol in the study subjects. After subjects had fasted overnight, plasma insulin levels were determined before and 60 and 120 minutes after a 7.5-g oral glucose load in a subgroup of 42 study subjects. Plasma insulin levels were measured by a radioimmunoassay kit (Eiken).

### Isolation and Characterization of Remnant Lipoproteins

Remnant lipoproteins in the fasting state were isolated by application of fasting serum to the immunoaffinity mixed gel that contained anti-\(\text{apoA-I}\) and anti-\(\text{apoB-100}\) monoclonal antibodies (Japan Immunoresearch Laboratories), and the unbound fraction containing remnant lipoproteins was eluted with PBS.\(^{15-18}\) Contents of cholesterol and triglycerides in the isolated fractions were measured by enzymatic methods.\(^{16,18}\) VLDL and LDL were isolated by ultracentrifugation from EDTA plasma when subjects were in the fasting state. The bound VLDL fraction was obtained by elution with 1.0 mol/L acetic acid–0.5 mol/L NaCl after elution of the unbound VLDL fraction from the immunoaffinity column to which VLDL was applied. In a subgroup of 21 study subjects, postprandial remnant lipoprotein levels were also measured in serum obtained 5 hours after subjects had eaten a test meal (400 kcal/m\(^2\) body surface area: 87% fat, 8% carbohydrate, 5% protein, and 0.5 ratio of polyunsaturated fatty acids to saturated fatty acids: Johmoh Shokuhin). Lab gel electrophoresis, electrophoretic mobility on agarose gel, and HPLC of the prepared lipoproteins were examined in the same manner as reported previously.\(^{15,16,18}\)

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### TABLE 1. Characteristics of the Study Subjects With Normal Coronary Angiograms

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remnant cholesterol, mg/dL</td>
<td>4.5 ± 0.4 (0.5–21.2)</td>
</tr>
<tr>
<td>Smoking, No. of subjects, % of total</td>
<td>37, 35%</td>
</tr>
<tr>
<td>Age, y</td>
<td>58 ± 1.2 (24–78)</td>
</tr>
<tr>
<td>Diabetes, No. of subjects, % of total</td>
<td>15, 14%</td>
</tr>
<tr>
<td>Hypertension, No. of subjects, % of total</td>
<td>28, 26%</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>56/50</td>
</tr>
<tr>
<td>Body mass index</td>
<td>22 ± 2.0 (18.2–29.1)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>196 ± 3.3 (112–284)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>129 ± 6.8 (39–459)</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>122 ± 3.0 (32–214)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>51 ± 1.3 (26–97)</td>
</tr>
<tr>
<td>ApoA-I, mg/dL</td>
<td>126 ± 2.1 (69–184)</td>
</tr>
<tr>
<td>ApoA-II, mg/dL</td>
<td>31 ± 0.5 (18–53)</td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>99 ± 2.2 (52–148)</td>
</tr>
<tr>
<td>ApoC-II, mg/dL</td>
<td>3.8 ± 0.2 (1–12.6)</td>
</tr>
<tr>
<td>ApoC-III, mg/dL</td>
<td>9.7 ± 0.5 (2.2–26.9)</td>
</tr>
<tr>
<td>ApoE, mg/dL</td>
<td>5.6 ± 0.2 (2.8–19.1)</td>
</tr>
<tr>
<td>(\text{Lp(a)}), mg/dL</td>
<td>18.6 ± 1.5 (3–91)</td>
</tr>
</tbody>
</table>

No. of subjects, % of total

n=106. Values in parentheses are range of data.
Quantitative Coronary Angiography and 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 in our previous reports. 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 coronary artery diameter to infusions of ACh, L-NMMA, and vitamin C were expressed as percent changes from baseline diameter measured on angiograms taken just before each infusion.

Coronary flow velocity was measured in a subgroup of 45 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. The stable peak flow velocity signals at baseline and during a 2-minute infusion of ACh at a dose of 10 µg/min were used for 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 × averaged peak velocity (cm/min) × cross-sectional area (cm²). The response of coronary blood flow to intracoronary infusion of ACh (10 µg/min) was expressed as a percentage change from the value of the baseline blood flow just before ACh infusion.

Study Protocols

After baseline angiography, incremental doses of ACh (10 and 50 µg/min) were infused directly into the left coronary artery through the Judkins catheter for 2 minutes with a 5-minute interval between the 2 doses. Hemodynamic measurements and coronary angiography were repeated at each of the ACh infusions. Fifteen minutes after completion of intracoronary injection of ACh, L-NMMA (50 µmol/min for 4 minutes) was infused into the left coronary artery through the Judkins catheter in a subgroup of 53 consecutive subjects, as described in our previous report. L-NMMA infusion was performed at a rate of 2 mL/min, and measurement of systemic hemodynamics and coronary angiography were performed before and at the last 30 seconds of the infusion. In a separate subgroup of the 6 consecutive subjects who had remnant levels above the 75th percentile (>5.1 mg/dL) and who did not have prior infusion of L-NMMA, vitamin C was infused directly (10 mg/min for 13 minutes) into the left coronary artery through the Judkins catheter 15 minutes after completion of intracoronary injection of ACh. At the end of vitamin C infusion, ACh (50 µg) was simultaneously infused into the left coronary artery in the same manner as the first ACh infusion. Measurement of systemic hemodynamics and coronary angiography were performed before and at the end of combined infusion of vitamin C and ACh. After an additional 15 minutes, intracoronary injection of isosorbide dinitrate (1 mg) or intravenous injection of nitroglycerin (250 µg) was performed. Two minutes after that, coronary angiography was performed in multiple projections in all study subjects.

Patients With Myocardial Infarction

In another group of 75 consecutive patients with myocardial infarction who were admitted at Kumamoto University Hospital from January 1995 to March 1997, the possible association of remnant lipoprotein levels with coronary artery disease was statistically analyzed in comparison with the 106 study subjects with normal coronary angiograms. Criteria for myocardial infarction included characteristic ECG changes and elevation of creatinine kinase enzyme to more than twice the upper limit of normal. Coronary angiography and left ventriculography were performed after myocardial infarction in all 75 patients, and the findings supported the diagnosis of myocardial infarction (single-vessel disease, 24 patients; 2-vessel disease, 26 patients; 3-vessel disease, 25 patients). After admission to our hospital, all of the patients with myocardial infarction took the same standard meal as the subjects with normal coronary angiograms for ≥5 days before blood sampling. Blood was obtained after an overnight fast ≥4 weeks after the onset of myocardial infarction. Lipid-lowering drugs were withdrawn ≥7 days before blood sampling.

Drugs

L-NMMA was obtained from Wako Chemicals and vitamin C was from Takeda Pharmaceutical. All drugs were dissolved in physiological saline and then steriley filtered before use. All drug solutions were kept at 37°C during the procedure.

Statistical Analysis

Data are expressed as mean ± SE unless otherwise indicated. Multivariate linear regression analyses were performed on all subjects with normal coronary angiograms to assess the independent correlation of coronary arterial responses to infusions of ACh and L-NMMA with the following coronary risk factors: age; sex; body mass index; histories of smoking, hypertension, and diabetes mellitus; family history of coronary heart disease; and fasting serum levels of total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, Lp(a), apo5, and remnant lipoprotein cholesterol. Multiple logistic regression analysis was performed on patients with myocardial infarction and subjects with normal coronary angiograms to identify the coronary risk factors that differed independently between them. This analysis included the following variables: age; sex; body mass index; histories of smoking, hypertension, and diabetes mellitus; family history of coronary heart disease; and fasting serum levels of total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, Lp(a), and remnant lipoprotein cholesterol. Sex (coded as 1 for male, 0 for female), history of smoking, history of hypertension, history of diabetes mellitus, and family history of coronary heart disease were included as categorical variables. The other risk factors were included as continuous variables. Values of the levels of remnant lipoproteins, triglycerides, apoC-II, apoC-III, apoE, and Lp(a) were log-transformed for statistical analysis because of a skewed distribution. Paired or unpaired Student’s t test was used for comparison of the 2 means. A value of P<0.05 was considered statistically significant. Analyses were performed in part with the use of SPSS Professional Statistics 6.1 for the Macintosh (SPSS Japan Inc).

Results

Measurements of Remnants in Subjects With Normal Coronary Arteriograms

Distribution of remnant lipoprotein cholesterol levels in the fasting state in subjects with normal coronary angiograms was skewed and shifted to lower levels, with a mean level of 4.5±0.4 mg/dL (range, 0.5 to 21.2 mg/dL). From SDS-PAGE analysis, the unbound lipoprotein fraction isolated from the fasting serum by the immunoaffinity mixed gel was found to be enriched in apoE and contained little apoB-48, a marker for chylomicron-derived lipoproteins; apoA-I and apoA-II were not detectable in the isolated unbound lipoprotein fraction, as shown in Figure 1. HPLC profiles showed that the unbound fraction from hypertriglyceridemic subjects consisted mainly of lipoproteins with particle sizes in the range of VLDL, as shown in Figure 2. Furthermore, agarose gel electrophoretograms showed that the unbound lipoprotein fraction isolated by the immunoaffinity mixed gel had β or slow pre-β mobility, whereas VLDL had fast pre-β mobility, as shown in Figure 3. The lipid compositions were not significantly different between the unbound lipoprotein fraction and the bound VLDL fraction (data not shown).
Fasting remnant lipoprotein levels also correlated strongly with postprandial remnant levels (6.6 ± 1.8 mg/dL) 5 hours after subjects ate the test meal (n = 21, r = 0.621, P = 0.0001 by univariate linear regression analysis). Multivariate statistical analysis showed that levels of triglycerides, apoC-III, apoE, total cholesterol, and apoB were independently correlated with levels of remnant lipoprotein cholesterol and that triglyceride levels had the most significant correlation with remnant levels (partial regression coefficient [partial r] = 0.767, P < 0.0001).

**Responses of Epicardial Coronary Diameter**

Univariate statistical analysis showed that the coronary diameter response to ACh infusion (50 μg) was significantly correlated with age and fasting levels of remnant cholesterol, triglycerides, apoC-II, apoC-III, apoE, total cholesterol, LDL cholesterol, apoB, HDL cholesterol, and plasma insulin. The constrictor response of the epicardial coronary arteries to ACh (50 μg) was significantly correlated with postprandial remnant levels (n = 21, r = 0.401, P = 0.01 by linear regression analysis). Linear regression analysis (Figure 4) showed a significant correlation between fasting remnant levels and the epicardial coronary diameter response to ACh (50 μg). Infusion of ACh (50 μg) dilated the epicardial coronary arteries in most subjects with remnant levels below the 25th percentile (<2.43 mg/dL), whereas it constricted the arteries in most of the subjects with remnant levels above the 75th percentile (>5.1 mg/dL), as shown in Figure 5. The dilator response to nitrates (endothelium-independent vasodilators) was not significantly different between subjects with remnant levels below the 25th percentile and above the 75th percentile (also shown in Figure 5). In multivariate regression analysis (Table 2), fasting levels of remnant cholesterol and LDL cholesterol, age, and smoking history were independently correlated with the response of coronary diameter to the infusion of ACh (50 μg), and remnant levels had the most significant correlation with diameter response to ACh. Response of coronary diameter to ACh at the dose of 10 μg also had a significant and independent correlation with remnant lipoprotein levels (partial r = −0.595, P < 0.001 by multivariate statistical analysis). There was no significant association of dilator response of epicardial coronary diameters to nitrates with any risk factors by multivariate statistical analysis.

Vitamin C infusion improved the coronary vasomotor response to ACh (50 μg) in the 6 subjects with remnant levels above the 75th percentile (>5.1 mg/dL) (percent change of diameter from baseline in response to ACh, 12 ± 2.4% constriction before vitamin C versus 7.1 ± 1.4% constriction after vitamin C; n = 6, P = 0.01). Intracoronary infusion of ACh (50 μg) and vitamin C infusion had no significant effects on heart rate and mean blood pressure.

**Responses of Coronary Blood Flow**

Coronary blood flow was increased in response to ACh infusion in all subjects studied. Univariate analysis showed that the percent increase in the flow response to ACh (10 μg) had a significant inverse correlation with fasting levels of remnant cholesterol, triglycerides, apoC-III, apoE, total cholesterol, and LDL cholesterol. Linear regression analysis (Figure 6) showed that the coronary flow increase in response to ACh was progressively suppressed with an increase in remnant levels. Multivariate analysis (Table 2) showed that remnant levels and LDL cholesterol levels were independently correlated with the flow response to ACh infusion (10 μg) and that remnant levels had a stronger correlation with the flow response. The increase in flow response to ACh at this dose (10 μg) is unlikely to be affected by the change of epicardial coronary diameter because the degree of the change was modest (from 12% constriction to 26% dilation, with a mean of 6.7% dilation from baseline values).

**Response of Epicardial Coronary Arterial Diameter to L-NMMA**

Intracoronary infusion of L-NMMA decreased arterial diameter in all subjects studied. Univariate linear regression analysis showed that the levels of remnant cholesterol, total
cholesterol, LDL cholesterol, apoB, triglycerides, and apoE were significantly and inversely correlated with the decrease in epicardial coronary diameter in response to L-NMMA infusion. A history of smoking, diabetes mellitus, or hypertension was associated with a significantly inhibited constrictor response of the epicardial coronary diameter to L-NMMA.

Linear regression analysis (Figure 7) showed that the epicardial coronary constrictor response to L-NMMA infusion was progressively suppressed with an increase in remnant levels. Multivariate linear regression analysis (Table 2) showed that level of remnant lipoproteins and histories of smoking and diabetes mellitus were independently correlated with the constrictor response of the epicardial coronary diameter to L-NMMA infusion and that remnant levels had the most significant correlation with response to L-NMMA. Intracoronary infusion of L-NMMA showed no appreciable effects on heart rate and mean blood pressure, as described in previous reports.5,6

Association of Remnant Lipoprotein Levels With Myocardial Infarction

Remnant lipoprotein levels in the fasting state were significantly higher in patients with myocardial infarction than in subjects with normal coronary angiograms (7.6 ± 0.8 versus 4.5 ± 0.4 mg/dL, respectively; P = 0.0001 by use of unpaired t test). Among the coronary risk factors examined, remnant lipoprotein levels (P = 0.01), diabetes mellitus (P = 0.0003), hypertension (P = 0.03), sex (P = 0.001), smoking (P = 0.01), LDL cholesterol levels (P = 0.01), and Lp(a) levels (P = 0.008) differed significantly and independently between patients with myocardial infarction and subjects with normal coronary angiograms, using multiple stepwise logistic regression analysis with forward stepwise selection.

Discussion

The present study was the first to assess the relation of remnant lipoprotein levels to responses of coronary endothelial vasomotor function in a large series of subjects. Multi-

![Figure 3](image-url)  
**Figure 3.** Agarose gel (1%) electrophoretograms. Lane 1, VLDL; 2, LDL; 3, remnant lipoproteins (unbound fraction) in the fasting state; 4, remnants (unbound fraction) in the postprandial state (5 hours after a meal); 5, fasting plasma; and 6, postprandial plasma. VLDL, LDL, and plasma were from a normolipidemic subject; remnants were from a subject with diabetes mellitus.  

![Figure 4](image-url)  
**Figure 4.** Correlation between remnant cholesterol levels and percent changes from baseline values in epicardial coronary diameters in response to intracoronary infusion of ACh (50 μg). Values of remnant lipoprotein cholesterol were log-transformed because of a skewed distribution. In [mg/dL] indicates loge [mg/dL]. Statistical analysis was performed by linear regression analysis.
variate analyses indicated that levels of remnant lipoproteins had a significant and independent correlation with abnormal vasomotor reactivity in large and resistance coronary arteries, as reflected by impaired dilation or constriction of epicardial coronary arteries and by impairment of coronary blood flow increase in response to intracoronary infusion of ACh. The epicardial coronary diameter response to nitrates was not significantly correlated with remnant lipoprotein levels. Thus, the present results indicate that remnant lipoproteins have an important and causative role in abnormalities of endothelium-dependent vasomotor function in large and resistance coronary arteries in humans. We and others5,6 have previously reported that intracoronary infusion of L-NMMA, an inhibitor of NO synthase, constricts the coronary arteries under resting and ACh-stimulated conditions in vivo in humans and that endothelium-derived NO is an important determinant of epicardial coronary arterial response to ACh. Using multivariate analysis, we showed in the present study that the constrictor response of epicardial coronary arterial diameter to intracoronary infusion of L-NMMA at basal conditions, reflecting basal coronary NO bioactivity, was independently decreased with an increase in remnant lipoprotein levels. Thus, these results suggest that the increase in remnant levels may cause a decrease in coronary NO bioactivity, leading to impairment of endothelium-dependent dilation in human coronary arteries. Remnant lipoproteins may be oxidatively modified in the arterial intima and cause an increase in the susceptibility of coronary endothelium to oxidative stress, which may play a role in the genesis of coronary endothelial dysfunction in subjects with high remnant lipoprotein levels. This is supported by the present observation that vitamin C, an antioxidant, improved coronary vasomotor function in subjects with high remnant lipoprotein levels.

The current method using immunoaffinity mixed gel containing anti–apoA-1 and anti–apoB-100 monoclonal antibodies has been reported to be capable of isolating apoE-rich VLDL particles containing apoB-100 together with chylomicron remnants containing apoB-48, neither of which binds to the immunoaffinity gel.15–18 Our unique anti–apoB-100 monoclonal antibody has been shown to recognize apoB-100 in LDL and most VLDL but not in apoE-enriched VLDL.15,16,18 Because these lipoproteins also lack apoA-1, remnant lipoproteins can be isolated in the unbound fraction by use of this immunoaffinity mixed gel. According to analyses with SDS-PAGE, HPLC profiles, agarose gel electrophetograms, and composition of lipids, the present unbound lipoprotein fraction in blood obtained after a fast, isolated by the immunoaffinity gel, had slow pre-mobility, particle sizes mainly in the range of VLDL, enrichment in apoE relative to VLDL, and little apoB-48, all of which are properties expected in VLDL remnants.11,18,22 In addition, these properties closely mimic those of β-VLDL from patients with type III hyperlipoproteinemia, as observed in previous reports.15–18,22 Thus, it is concluded that the unbound

**Figure 5.** Percent changes in epicardial coronary diameters from baseline values in response to intracoronary infusion of ACh (50 μg, left) and to isosorbide dinitrate and nitroglycerin (Nitrates, right) in subjects with remnant cholesterol levels <25th percentile (open bars) and >75th percentile (solid bars).

**TABLE 2. Multivariate Statistical Analyses for Associations of Coronary Risk Factors With Coronary Arterial Responses to ACh and L-NMMA**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Partial r</th>
<th>P</th>
<th>Partial r</th>
<th>P</th>
<th>Partial r</th>
<th>P</th>
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<tr>
<td>Remnants</td>
<td>−0.661</td>
<td>&lt;0.0001</td>
<td>−0.634</td>
<td>&lt;0.0001</td>
<td>−0.533</td>
<td>&lt;0.0001</td>
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<tr>
<td>Smoking</td>
<td>−0.366</td>
<td>0.001</td>
<td>−0.162</td>
<td>NS</td>
<td>−0.423</td>
<td>0.01</td>
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<td>Age</td>
<td>−0.313</td>
<td>0.002</td>
<td>−0.187</td>
<td>NS</td>
<td>−0.220</td>
<td>NS</td>
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<tr>
<td>LDL cholesterol</td>
<td>−0.362</td>
<td>0.001</td>
<td>−0.408</td>
<td>0.001</td>
<td>−0.290</td>
<td>NS</td>
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<tr>
<td>Diabetes</td>
<td>−0.224</td>
<td>NS</td>
<td>−0.229</td>
<td>NS</td>
<td>−0.398</td>
<td>0.01</td>
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<tr>
<td>Hypertension</td>
<td>−0.172</td>
<td>NS</td>
<td>−0.101</td>
<td>NS</td>
<td>−0.320</td>
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<td>Sex</td>
<td>−0.128</td>
<td>NS</td>
<td>−0.221</td>
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<td>Total cholesterol</td>
<td>−0.235</td>
<td>NS</td>
<td>−0.268</td>
<td>NS</td>
<td>−0.234</td>
<td>NS</td>
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<tr>
<td>Triglycerides</td>
<td>−0.142</td>
<td>NS</td>
<td>−0.322</td>
<td>NS</td>
<td>−0.127</td>
<td>NS</td>
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<tr>
<td>HDL cholesterol</td>
<td>+0.262</td>
<td>NS</td>
<td>+0.225</td>
<td>NS</td>
<td>+0.264</td>
<td>NS</td>
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</table>

Partial r indicates partial regression coefficient.
lipoprotein fraction isolated from fasting blood by the immunofinity mixed gel is almost identical to VLDL remnants.

Most remnant lipoproteins, however, are reported to be cleared rapidly from the circulation by hepatic uptake in animal experiments and in studies with normolipidemic subjects. High levels of remnant lipoproteins in overnight fasting blood in some of the present subjects may be caused by enhanced hepatic secretion of VLDL or delayed clearance of the remnants, which may possibly occur in hypertriglyceridemic patients. Previous reports showed that levels of triglyceride-rich lipoproteins in the postprandial state were a better predictor of the presence of coronary artery disease than those in the fasting state. In the present study, remnant lipoprotein levels, however, were measured in overnight fasting blood to avoid postprandial variability of lipid and lipoprotein levels including remnant lipoprotein levels, and fasting remnant levels were found to be statistically well correlated with coronary vasomotor dysfunction. Considering the present results that fasting remnant levels were significantly correlated with postprandial levels, the fasting levels may reflect the postprandial increase in remnant levels. The present method using the immunoinfinity gel seems to have a good theoretical basis and could be useful to quantify levels of the “injurious” and “atherogenic” triglyceride-rich lipoproteins.

Coronary endothelial dysfunction is shown to have an important role in the pathogenesis of coronary artery disease. In the present study showed that remnant lipoprotein levels are independently associated with the risk of myocardial infarction. Therefore, the present results imply that remnant lipoproteins could cause atherothrombotic development as well as abnormal coronary vasomotor reactivity in hypertriglyceridemic subjects. Thus, measurement of remnant lipoprotein levels, in addition to cholesterol-rich lipoproteins, and therapy to reduce remnant lipoprotein levels, such as exercise, diet, reduction in obesity, and triglyceride-reducing drugs, may be necessary in patients with ischemic heart disease.

In conclusion, remnant lipoprotein levels have a significant and independent correlation with impaired endothelial function in large and resistance coronary arteries in humans. The decrease in coronary NO activity associated with the increase in remnant levels may contribute in part to this correlation.

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