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.

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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Study patients. This study at Kumamoto University Hospi-
tal involved consecutive enrollment of 120 patients with

type II DM and CAD who underwent cardiac catheteriza-
tion for chest pain or ischemic changes detected by electro-
cardiography. 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

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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
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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 insti-
tution.

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 lip-
oprotein (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 revascu-
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when progression of angiographic coronary stenosis was
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changes lasting >10 min, despite full medication. The need for

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<tr>
<th>Abbreviations and Acronyms</th>
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<tbody>
<tr>
<td>apo = apolipoprotein</td>
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<tr>
<td>CAGB = coronary artery bypss graft surgery</td>
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<tr>
<td>CAD = coronary artery disease</td>
</tr>
<tr>
<td>DM = diabetes mellitus</td>
</tr>
<tr>
<td>HbA1c = glycosylated hemoglobin</td>
</tr>
<tr>
<td>HDL = high-density lipoprotein</td>
</tr>
<tr>
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</tr>
<tr>
<td>PCI = percutaneous coronary intervention</td>
</tr>
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<td>RLP = remnant-like lipoprotein particles</td>
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<td>VLDL = very-low-density lipoprotein</td>
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 METHODS

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tal involved consecutive enrollment of 120 patients with
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changes lasting >10 min, despite full medication. The need for

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>With CAD (n = 120)</th>
<th>Without CAD (n = 120)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>65.6 ± 8.4</td>
<td>65.6 ± 8.0</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>75/45</td>
<td>76/44</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.2 ± 2.8</td>
<td>23.9 ± 3.6</td>
<td>NS</td>
</tr>
<tr>
<td>Cigarette smoker (%)</td>
<td>69 (58%)</td>
<td>55 (46%)</td>
<td>NS</td>
</tr>
<tr>
<td>Systemic hypertension (%)</td>
<td>64 (53%)</td>
<td>59 (49%)</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>202 ± 39</td>
<td>183 ± 34</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>44 ± 14</td>
<td>50 ± 15</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>128 ± 36</td>
<td>112 ± 30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>151 ± 71</td>
<td>123 ± 48</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hemoglobin A₁c (%)</td>
<td>7.2 ± 1.5</td>
<td>6.5 ± 1.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RLP cholesterol (mg/dl)*</td>
<td>5.8 (3.1–6.2)</td>
<td>3.7 (2.5–4.7)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*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.
High RLP cholesterol levels (>4.7 mg/dl) 2.2 1.2–6.4 <0.05
High hemoglobin A1c 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.

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 (HbA1c) 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 HbA1c 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 HbA1c 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 PCI; 6 CAGBs; 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

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

Data are presented as the number (%) of patients.

ACE = angiotensin-converting enzyme; HMG-CoA = 3-hydroxy-3-methyl-glutaryl-coenzyme A; NS = not significant.
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 HbA1c levels and 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 HbA1c 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 HbA1c 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. 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.

**References**