Remnant-like Particle-Cholesterol Concentrations in Patients with Type 2 Diabetes Mellitus and End-Stage Renal Disease

Shaina Hirany, Dawn O’Byrne, Sridevi Devaraj, and Ishwarlal Jialal

Background: Lipid abnormalities contribute significantly to the increased risk of cardiovascular disease in diabetic and end-stage renal disease (ESRD) patients. Accumulating evidence supports a proatherogenic role for remnant lipoproteins. Thus, the aim of the present study was to compare remnant-like particle-cholesterol (RLP-C) in type 2 diabetic and ESRD patients with age- and gender-matched controls.

Methods: Using an immunoaffinity assay, we measured RLP-C concentrations in 48 type 2 diabetic patients with (n = 24) and without (n = 24) macrovascular complications, and 24 age- and gender-matched controls, as well as in 38 ESRD patients on hemodialysis (n = 19) and peritoneal dialysis (n = 19), and 19 age- and gender-matched controls.

Results: RLP-C correlated significantly with plasma triglycerides (TGs; r = 0.8). When compared with controls, RLP-C concentrations were significantly higher in type 2 diabetic patients with and without macrovascular complications (median, 0.22 and 0.17 mmol/L vs 0.14 mmol/L; P < 0.0002 and < 0.01, respectively); diabetic patients with macrovascular complications also had significantly higher RLP-C than diabetic patients without macrovascular complications (P <0.05). However, when RLP-C/TG ratios were computed, only diabetic patients with macrovascular complications showed significantly higher RLP-C/TG ratios compared with controls (P <0.05). Regarding ESRD, RLP-C concentrations were significantly increased in patients on both hemodialysis and peritoneal dialysis compared with controls (median, 0.23 and 0.21 mmol/L vs 0.13 mmol/L; P <0.0001). Whereas RLP-C was increased in ESRD patients on hemodialysis with TGs <2.26 mmol/L compared with controls, RLP-C/TG ratios were not significantly increased in these patients.

Conclusions: Type 2 diabetic patients with macrovascular disease demonstrated increased RLP-C and RLP-C/TG ratios, whereas ESRD patients showed only increased RLP-C concentrations.

Atherosclerotic cardiovascular disease is the leading cause of death in diabetes, particularly type 2 diabetes, and in patients with end-stage renal disease (ESRD). Dyslipidemia, characterized by hypertriglyceridemia, reduced HDL-cholesterol (HDL-C), and increased LDL-cholesterol (LDL-C), small dense LDL, and lipoprotein(a) may contribute to the accelerated atherosclerosis in these patients (1–4). Accumulating evidence also indicates that remnant lipoproteins promote atherosclerosis. Remnant lipoproteins are products of partially catabolized chylomicrons and VLDL from which some triglycerides (TGs) have been removed by the action of lipoprotein lipase and, to a lesser extent, by hepatic lipase. The resulting chylomicron and VLDL remnants, including intermediate-density lipoprotein (IDL), with reduced TGs but enriched cholesterol and apolipoprotein(a) E, are smaller in size and more dense, and are believed to be more atherogenic than the larger triglyceride-rich lipoproteins (TRLs) (5–8).

Several studies have implicated IDL concentrations with increased incidence or recurrence of coronary artery disease (CAD) (9–12). Increased IDL also has been found...
in diseases associated with accelerated or premature atherosclerosis, such as type III dyslipidemia, diabetes mellitus, chronic renal failure, and familial hypercholesterolemia (12–16). Ultracentrifugation and nondenaturing polyacrylamide gel electrophoresis methods have been used to separate small VLDL and IDL particles (13–17), but these methods are tedious and time-consuming and hence have limited applicability in clinical laboratories. Thus, development of simple and reliable methods for measurement of remnants for routine clinical laboratories is imperative.

The recent development by Nakajima et al. (18) of an immunoseparation method for measurement of remnant-like particles has allowed for a simpler routine method. In this assay, anti-human A1 and a unique apo B-100 mouse monoclonal antibody (J1-H) conjugated to Sepharose 4B removes HDL, chylomicrons, LDL, and most VLDL. The J1-H antibody does not recognize apo E-rich VLDL particles and TRLs containing apo B-48. Thus, chylomicron remnants and a fraction of VLDL enriched in apo E remain in the unbound fraction. These remnant-like particles (RLPs) are quantified by measuring cholesterol and TGs enzymatically in the unbound fraction.

With this assay, plasma RLP concentrations have been found to be significantly increased in patients with CAD compared with matched controls, and threefold increased concentrations have been demonstrated in type III dyslipidemia, a prototypic disorder of remnant metabolism (19–21). To date, there is little information with regard to RLP-cholesterol (RLP-C) concentrations in patients with diabetes and ESRD patients in American populations. In Asian populations, especially in those of Japanese ancestry, increased RLP-C, when compared with controls, has been reported in patients with impaired glucose tolerance and type 2 diabetes (22, 23) and in ESRD patients on hemodialysis (HD) (24, 25). In the present US-based population study, we evaluated RLP-C concentrations in type 2 diabetic patients with and without macrovascular complications and in patients with ESRD on HD and peritoneal dialysis (PD) compared with controls.

**Materials and Methods**

**LIPID AND LIPOPROTEIN ANALYSIS**

Lipid and lipoprotein concentrations were determined using Lipid Research Clinics methodology as described previously (26).

**RLP-C ASSAY**

For the RLP-C assay (18, 27), 300 μL of the immunoaffinity gel containing antibodies to apo A1 and B-100 were pipetted into separation cups containing steel balls and placed in a magnetic mixer manufactured by Otsuka Electronics. After the gel was allowed to settle for 5 min, 5 μL of blank (buffer), controls, or plasma was pipetted onto the surface of the gel and incubated with continuous mixing for 2 h at room temperature. After incubation, the gel was allowed to settle for 15 min, and 200 μL of the supernatant was placed into sample cups. Cholesterol concentrations were then measured using a peroxidase-based assay on the Cobas Mira S automatic analyzer (Roche Diagnostic Systems). The intra- and interassay precision (CVs <5%) has been reported previously (19).

**RLP-C MEASUREMENTS IN DIABETIC SUBJECTS**

The diabetic subjects were recruited from the diabetic clinic at Parkland Memorial Hospital and divided into two groups: (a) type 2 diabetic patients without macrovascular complications (n = 24), and (b) type 2 diabetic patients with macrovascular complications (n = 24). Age- and sex-matched healthy controls (n = 24) formed a third group. The criteria for macrovascular disease in diabetic patients included evidence of cardiovascular disease (clinical presentation and electrocardiographic evidence of myocardial infarction, positive stress tests, or coronary angiography), cerebrovascular disease (stroke, transient ischemic attacks, or magnetic resonance imaging evidence), or peripheral vascular disease (amputation, intermittent claudication, evidence of vascular disease with color-flow Doppler by B-mode ultrasound, amputation, or ankle-brachial index <0.8 and toe pressures <45 mmHg) (28). All subjects in the first group had no clinical evidence of atherosclerosis and normal baseline electrocardiograms. There were no significant differences in age, male/female ratio, and body mass index (BMI) among the three groups (Table 1). In this study, 40% were African-American, 44% were Caucasian, 1% were Asian, and 15% were Hispanic. Fasting blood samples were collected in EDTA tubes and stored at 4°C. RLP-C concentrations in the plasma were determined within 5 days of collection.

**Table 1. Subject characteristics, and lipid and lipoprotein profiles of controls and type 2 diabetic patients.**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 24)</th>
<th>DM patients (n = 24)</th>
<th>DM-MV patients (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>55.4 ± 7.2</td>
<td>57.3 ± 7.4</td>
<td>60.0 ± 6.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.5 ± 6.8</td>
<td>31.6 ± 6.0</td>
<td>31.7 ± 7.8</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>9/15</td>
<td>9/15</td>
<td>9/15</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.1 ± 0.8</td>
<td>5.0 ± 1.2</td>
<td>4.9 ± 0.9</td>
</tr>
<tr>
<td>Total TGs, mmol/L</td>
<td>1.3 ± 0.5</td>
<td>1.9 ± 1.2</td>
<td>1.6 ± 1.0</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.4 ± 0.8</td>
<td>3.2 ± 1.0</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.3 ± 0.4</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>RLP-C, mmol/L</td>
<td>0.15 ± 0.06</td>
<td>0.20 ± 0.10</td>
<td>0.23 ± 0.08</td>
</tr>
<tr>
<td>TGs &lt;2.26 mmol/L</td>
<td>0.17 ± 0.004</td>
<td>0.21 ± 0.06</td>
<td>0.23 ± 0.06</td>
</tr>
<tr>
<td>RLP-C/TG ratio</td>
<td>0.06 ± 0.03</td>
<td>0.06 ± 0.02</td>
<td>0.08 ± 0.03</td>
</tr>
</tbody>
</table>

- Results presented in SI units. To convert from SI units to mg/dL, multiply the values by 38.61 for cholesterol and 88.5 for TGs.
- Data are presented as mean ± SD.
- All controls had TGs <2.26 mmol/L.
- DM and DM-MV indicate type 2 diabetic patients without and with macrovascular complications, respectively.
- Compared with controls by Wilcoxon sign-rank test: *P <0.05; †P <0.005.
RLP-C MEASUREMENTS IN ESRD SUBJECTS
ESRD patients on HD (n = 19) and PD (n = 19) were recruited from the Renal Dialysis Unit at Parkland Memorial Hospital. Less than one-third were diagnosed with diabetes. Healthy controls (n = 19) formed the third group. The three groups were matched for age, gender, BMI, and racial background. In this study, 39% were African-American, 43% were Caucasian, and 18% were Hispanic. Causes of ESRD and exclusion criteria for the subjects have been described previously [Ref. (29) and Table 2]. Fasting blood samples were drawn before initiation of dialysis for HD patients. RLP-C concentrations were measured in fasting plasma as described above.

STATISTICS
The Kruskal–Wallis nonparametric test was used to assess overall differences between the groups. Differences within groups were compared by the Wilcoxon sign-rank test. Spearman rank correlation coefficients were used to assess relationships between RLP-C and lipid concentrations. Significance was set at <0.05. Analyses were performed using SAS (SAS Institute).

RESULTS
RLP-C CONCENTRATIONS IN CONTROL SUBJECTS
RLP-C concentrations in healthy control subjects for both the diabetic [mean, 0.15 ± 0.06 mmol/L (5.8 ± 2.2 mg/dL)] and the ESRD [mean, 0.14 ± 0.04 mmol/L (5.5 ± 1.7 mg/dL)] groups were consistent with previous reports by the present group and other investigators (19, 22, 30, 31).

RLP-C CONCENTRATIONS IN DIABETIC SUBJECTS
The characteristics and lipid and lipoprotein profiles of controls and diabetic patients are shown in Table 1. No significant differences were observed in age, gender, BMI, or lipid and lipoprotein concentrations between the control and diabetic groups for all subjects as well as the normotriglyceridemic groups (Table 1). The mean RLP-C concentrations in diabetic subjects with and without macrovascular complications and controls are shown in Table 1. RLP-C concentrations were significantly higher in the diabetic subjects with and without macrovascular complications compared with controls (P = 0.0001 and 0.01, respectively). In addition, diabetic patients with macrovascular complications had significantly higher RLP-C concentrations compared with diabetic patients without macrovascular complications (P <0.05). A positive correlation was observed between RLP-C and TG concentrations for controls (r = 0.37; P = 0.07) and diabetic patients without (r = 0.76; P <0.002) and with macrovascular complications (r = 0.6; P <0.002). In normotriglyceridemic patients [TGs <2.26 mmol/L (200 mg/dL)], RLP-C concentrations in diabetic patients with macrovascular complications were significantly higher compared with controls (P <0.0002), despite the TG concentrations being nonsignificant. There was a trend to significance in RLP-C (P = 0.07) in diabetic patients without complications compared with controls, despite TG concentrations being nonsignificant. To further account for the effect of TGs, RLP-C/TG ratios were computed. RLP-C/TG ratios were significantly increased only in type 2 diabetics with macrovascular complications compared with controls (P <0.02).

<table>
<thead>
<tr>
<th>Table 2. Subject characteristics, and lipid and lipoprotein profiles of Controls and ESRD patients. a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
</tr>
<tr>
<td><strong>HD patients</strong></td>
</tr>
<tr>
<td><strong>PD patients</strong></td>
</tr>
<tr>
<td>(n = 17)</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
</tr>
<tr>
<td>Male/female ratio</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
</tr>
<tr>
<td>TGs, mmol/L</td>
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<tr>
<td>LDL-C, mmol/L</td>
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<tr>
<td>HDL-C, mmol/L</td>
</tr>
<tr>
<td>RLP-C, mmol/L</td>
</tr>
<tr>
<td>RLP-C/TG ratio</td>
</tr>
</tbody>
</table>

a Data are presented as mean ± SD.

b All results are presented in SI units. To convert from SI units to mg/dL, multiply the values by 38.61 for cholesterol and 88.5 for TGs.

c,d Compared with controls by Wilcoxon sign-rank test: c P <0.05; d P <0.005.

RLP-C CONCENTRATIONS IN ESRD SUBJECTS
The subject characteristics and lipid and lipoprotein profiles of controls and ESRD patients on dialysis are shown in Table 2. Both dialysis groups showed significantly higher RLP-C concentrations compared with controls (P <0.0001); however, there were no significant differences in RLP-C concentrations between the two dialysis groups (P >0.05). A strong positive correlation was observed between RLP-C and TG concentrations for controls and dialysis patients (r = 0.7 and 0.9, respectively; P <0.001). Because both dialysis groups had significantly higher TG values compared with controls, RLP-C concentrations in normotriglyceridemic (TGs <2.26 mmol/L) patients were compared. In the normotriglyceridemic group, TG values were significantly higher only in PD patients (P <0.02) and not in HD patients compared with controls. Thus, RLP-C concentrations were compared between the HD and control groups. Again, RLP-C concentrations were significantly higher for HD (0.22 ± 0.11 vs 0.14 ± 0.04 mmol/L; P <0.05) compared with controls. However, RLP-C/TG ratios in ESRD patients on HD and PD failed to show any significant differences compared with controls (P >0.30).
Lipid abnormalities observed in diabetic and ESRD patients contribute significantly to the increased risk of cardiovascular disease, a major cause of mortality and morbidity. Increased LDL-C is the best known risk factor for CAD; however, accumulating evidence now exists to implicate TRL remnants as an atherogenic risk factor. In vitro evidence for potential proatherogenic effects of TRL remnants include impairment of endothelium-dependent vasorelaxation (32, 33), enhancement of platelet aggregation in whole blood (34, 35), and uptake by macrophages causing foam cell formation (36). Several case-controlled studies using ultracentrifugation have demonstrated TRL remnants as more closely associated to the progression and severity of CAD than LDL-C (12, 37). In type 2 diabetes, Kasama et al. (14) reported increased IDL-cholesterol in normolipidemic non-insulin-dependent diabetics, and Tkac et al. (15) found that the severity of angioigraphically evaluated CAD was positively related to the number of TRL particles. Accumulation of remnants in ESRD patients as reflected by increased IDL concentrations also have been reported (16).

Isolation of remnants by laborious ultracentrifugation techniques has hampered clinical studies. Thus, there is a need for more reliable methodologies. Recent development of an immunoaffinity method that uses a combination of anti-human A1 and a unique apo B-100 (J1-H) mouse monoclonal antibody has allowed routine isolation of chylomicron remnants and small VLDL enriched in apo E. Studies using this assay have confirmed the role of RLPs in the pathogenesis of atherosclerosis, demonstrating significantly increased RLP concentrations in normolipidemic men with CAD (19, 30), in patients with type III dyslipidemia (19–21), in sudden cardiac death (37), in vasospastic angina with nearly normal coronary arteries (38), and in patients with coronary artery restenosis after angioplasty (39). Kugiyama et al. (40) have shown that in 135 patients with CAD, patients with RLP-C concentrations in the highest tertile had a significantly higher probability of developing coronary events than did those with remnant concentrations in the lowest tertile. Furthermore, higher concentrations of remnants were a significant and independent predictor of developing coronary events in multivariate analyses that included extent of stenosis, age, sex, smoking, hypertension, diabetes, hypercholesterolemia, and hypertriglyceridemia as covariates. Further documentation of the atherogenicity of RLPs comes from the work of Takeichi et al. (37) in age-matched cases with sudden cardiac death, with and without advanced coronary atherosclerosis. These authors showed that the RLP-C concentration was the only variable that was statistically different between the two groups.

Whereas substantial data exist that relate RLPs and CAD, few studies have addressed RLP concentrations in chronic diseases such as diabetes and ESRD. Watanabe et al. (22) observed significantly higher RLP-C concentrations in Asian patients with impaired glucose tolerance and type 2 diabetes compared with controls with no marked differences in total cholesterol or LDL-C. However, although TG concentrations were higher in the diabetic groups and correlated well with RLP-C (r = 0.95), the RLP-C/TG ratio was not reported and the authors did not correct for the increased TGs. Shimizu et al. (23) reported increased RLP-C in type 2 diabetic patients with microalbuminuria and macroalbuminuria compared with those without albuminuria. However, no control population was studied, and TG concentrations were significantly higher in the diabetic patients with macroalbuminuria. The present study is the first to address RLP-C concentrations in diabetic patients with and without macrovascular complications compared with controls from a US-based population. Although both diabetic groups demonstrated increased RLP-C concentrations compared with controls, diabetic patients with macrovascular complications had significantly higher concentrations than diabetic patients without macrovascular complications. Furthermore, in normotriglyceridemia as well as when the RLP-C/TG ratio was used, which is a better measure of remnant status (20), only the diabetic macrovascular complications group showed significantly higher RLP-C concentrations. Diabetic vascular complications are a leading cause of mortality and morbidity; hence, increased RLP-C concentrations in this group substantiates its atherogenic role. More importantly, no significant differences existed in the well-known atherogenic risk factors of total cholesterol and LDL-C or TGs among the three groups. Thus, RLP-C concentrations or the RLP-C/TG ratio may be a better indicator of CAD risk than cholesterol and TGs in normolipidemic type 2 diabetic populations and maybe useful in monitoring the effect of drug therapy on this atherogenic lipoprotein.

Increased TGs are the primary lipid abnormality in ESRD, possibly because of increased production and decreased clearance of TRLs, leading to accumulation of TRL remnants. The present study is the first to address RLP concentrations in ESRD patients on both HD and PD. TG concentrations in both the HD and PD groups were significantly higher than in controls (twofold), and RLP concentrations were almost threefold higher than in controls, although no significant differences existed between the HD and PD groups. Similar increases in RLP concentrations previously have been reported in Asian ESRD patients on HD (24) compared with controls. In addition, Sekihara et al. (25) have reported that patients on HD with CAD had significantly higher RLP-C concentrations than HD patients without CAD. However, the concentrations were not compared with matched controls. There appear to be no reports on RLP-C concentrations in patients with ESRD on PD. A consistent feature of RLP-C is its strong positive correlation with total TGs reported here and in the studies reported above (r = 0.8) (24, 25). However, the study by Oda et al. (24) in HD subjects did not adjust for the significantly higher TG concentrations.
in the HD groups compared with controls. To examine the effect of RLPs independent of TG concentrations, we evaluated the RLP-C concentrations in normotriglyceridemic dialysis patients and computed RLP-C/TG ratios. RLP-C concentrations were again significantly higher in the HD group compared with controls in spite of TG concentrations being nonsignificant. However, the RLP-C/TG ratios failed to show any significant differences. This could possibly be attributable to the small sample size. Thus, it appears that only in normolipidemic HD patients, and not in PD patients, would RLP-C concentrations be a better indicator of CAD risk than TG alone.

In conclusion, this study demonstrates that the dyslipidemia of diabetic patients with and without macrovascular complications and of ESRD patients on HD and PD includes increased concentrations of potentially atherogenic RLPs. Moreover, the increases were more prominent in diabetic patients with macrovascular complications and in the normotriglyceridemic HD group. However, it is probable that the nature of the atherogenic RLPs may be different between the two groups and that the diabetic subjects may possess more chylomicron remnants, whereas the ESRD subjects have more VLDL remnants, and this warrants further study. Thus, the measurement of RLP-C by a reliable immunoaffinity assay may add to the assessment of CAD risk in normolipidemic patients, and could become part of the lipoprotein profile in patients with established risk factors for CAD, if future studies confirm its utility independent of plasma TGs.

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References


