Plasma phospholipid transfer protein, cholesteryl ester transfer protein and lecithin:cholesterol acyltransferase in end-stage renal disease (ESRD)

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Abstract
Background. Chronic kidney disease (CKD) results in accelerated atherosclerosis that is primarily caused by inflammation, oxidative stress and impaired triglyceride and HDL metabolisms. Several plasma proteins including phospholipid transfer protein (PTLP), cholesteryl ester transfer protein (CETP) and lecithin:cholesterol acyltransferase (LCAT) affect HDL metabolism. PLTP transfers phospholipids and free cholesterol from triglyceride-rich lipoproteins to HDL, phospholipids between HDL particles and facilitates cholesterol efflux from cells. CETP catalyzes the transfer of cholesteryl esters from HDL to LDL in exchange for triglycerides, and LCAT catalyzes esterification of free cholesterol on the surface of HDL. Given the role of these proteins in the regulation of HDL metabolism, we examined the effect of ESRD on plasma PLTP, CETP and LCAT.

Methods. A group of 21 stable ESRD patients maintained on haemodialysis and a group of 21 age-matched normal control individuals were included in the study. Plasma apolipoprotein A-1, PLTP, CETP and LCAT levels were measured.

Results. Plasma triglyceride concentration was elevated and plasma HDL cholesterol, apolipoprotein A-1 and LCAT concentrations were significantly reduced, whereas plasma PLTP and CETP concentrations and activities were unchanged in the ESRD patients.

Conclusions. These findings point to acquired LCAT and Apo A-1 deficiencies and tend to exclude dysregulation of PLTP or CETP in the pathogenesis of HDL abnormalities in haemodialysis patients.

Keywords: CETP; HDL metabolism; haemodialysis; LCAT; PL

Introduction
Chronic kidney disease (CKD) is associated with accelerated atherosclerosis and a high risk of death from cardiovascular complications [1]. The atherogenic diathesis in CKD is accompanied by and, in part, due to inflammation, oxidative stress and dyslipidaemia [2–4]. CKD-induced dyslipidaemia is marked by elevated plasma triglyceride (TG) and very low-density lipoprotein (VLDL) concentrations, impaired VLDL and chylomicron clearance, accumulation of intermediate-density lipoprotein (IDL) and chylomicron remnants, diminished HDL cholesterol and Apo A-1 concentrations, impaired HDL maturation, increased HDL triglyceride and elevated plasma pre-beta HDL [2,5].

HDL metabolism is, in part, regulated by a number of plasma and cell-associated proteins. These include, but are not limited to lecithin:cholesterol acyltransferase (LCAT) that catalyzes esterification of free cholesterol in the plasma, Apo A-1 that is the principal apolipoprotein constituent of HDL, cholesteryl ester transfer protein (CETP) that mediates transfer of cholesteryl ester from HDL to LDL in exchange for triglyceride, and phospholipid transfer protein (PLTP) that is involved in transfer of phospholipids and conversion of HDL particles [2,6,7].

A number of earlier studies have attempted to explore the underlying mechanisms of the reduction of plasma HDL cholesterol (HDL-C) concentration, impaired HDL maturation, altered HDL composition and increased plasma concentration of lipid-poor pre-beta HDL particles in CKD. These studies have demonstrated diminished plasma LCAT activity and Apo A-1 concentrations in patients with advanced renal failure [8] and down-regulation of hepatic LCAT and Apo A-1 gene expressions in experimental animals [9–11]. However, the available data on the potential role of PLTP and CETP in the pathogenesis of dyslipidaemia of CKD are limited.

PLTP is a member of the lipid transfer protein gene family that includes CETP, lipopolysaccharide-binding protein (LBP) and bactericidal permeability-increasing protein (BPI) [12]. PLTP facilitates the transfer of phospholipids and free cholesterol from the surface of triglyceride-rich lipoproteins (undergoing lipolysis) to HDL [13,14]. In addition, PLTP can transfer phospholipids between different HDL particles and thereby convert HDL-3 to both larger and smaller lipid-poor (pre-beta) HDL particles [15–17].
CETP plays a central role in HDL metabolism. It shuttles cholesterol ester from HDL to apolipoprotein B-containing particles in exchange for triglycerides. This exchange results in the reduction of HDL cholesterol and elevation of VLDL and LDL cholesterol [18]. Increased CETP activity may also modify the anti-inflammatory and anti-oxidant properties of HDL and thus contribute to oxidative stress and chronic inflammation, major players in the pathogenesis of atherosclerosis [19–23].

In view of the role of PLTP and CETP in the regulation of HDL metabolism and their potential link to inflammation and atherosclerosis, we sought to examine plasma PLTP and CETP abundance and activity in stable haemodialysis-dependent patients with end-stage renal disease (ESRD), a condition marked by impaired HDL metabolism, inflammation and accelerated atherosclerosis.

### Subjects and methods

The study protocol was approved by Human Subjects Institutional Review Board of the University of California, Irvine, and completed with the assistance of the University of California General Clinical Research Center.

**Subjects**

A total of 21 stable patients with ESRD maintained on haemodialysis for a minimum of 3 months were recruited for the study. Blood access during dialysis consisted of arterio-venous fistula or PTFE grafts in all patients. None of the patients had indwelling catheters and none had received intravenous iron preparations or antibiotics during the 2 weeks preceding the study. Haemodialysis therapy was performed three times weekly using cellulose acetate dialyzers. Individuals with evidence of acute or chronic infection or acute intercurrent illnesses were excluded. Medical history, systolic and diastolic blood pressures, body weight, inter-dialytic weight change, routine monthly laboratory data and dialysis prescription including dialyzer type and medications were recorded. A group of 21 normal age-matched subjects were used as controls. Random non-fasting blood samples were obtained from haemodialysis access in the ESRD group (immediately before dialysis) and by venipuncture in the control group.

**Measurements of plasma lipids**

Plasma total cholesterol, triglyceride, HDL and LDL cholesterol were measured by the Clinical Laboratory facilities at the University of California Irvine Medical Center as follows: plasma total cholesterol and triglycerides were measured by the Beckman Coulter DXC 800 instrument (Beckman Coulter Inc., Fullerton, CA, USA), plasma HDL and LDL cholesterol were measured by electrophoretic fractionation using the SPIFE 3000 system (Helena Laboratories Corp., Beaumont, TX, USA).

**Measurements of PLTP**

The plasma PLTP protein level was measured by western blot analysis using a rabbit polyclonal anti-PLTP antibody (Novus Biologicals Inc., Littleton, CO, USA). PLTP activity was measured using the BioVision PLTP activity Assay Kit (Mountain View, CA, USA) and the SpectraMax M5 plate reader (Molecular Devices, Sunnyvale, CA, USA) and expressed as millimole of phospholipid transferred per litre plasma per hour.

**Measurements of CETP**

Plasma CETP protein concentration was measured by western blot analysis using a polyclonal rabbit anti-CETP antibody. CETP activity was measured using a fluorescent CETP activity Assay Kit (BioVision Inc.) and the SpectraMax M5 plate reader PLTP and expressed as millimole of neutral lipid transferred per litre plasma per hour.

### Results

#### General data

Data are summarized in Table 1. There were 10 men in the control group and 13 in the ESRD group. Body mass index (BMI) was comparable in the two groups. Among the ESRD group, 11 (52%) patients had documented atherosclerotic cardiovascular disease and 7 (33%) patients were treated with statin preparations. Nine of the ESRD patients had coronary artery disease of whom five had a history of congestive heart failure, two had a history of myocardial infarctions, two had undergone coronary artery bypass surgery and two individuals had peripheral vascular disease. One patient had isolated peripheral vascular disease and another had a history of stroke. None of the individuals in the normal control group had evidence of atherosclerotic cardiovascular disease, and none was on statin therapy. The underlying causes of ESRD were diabetic nephropathy in 12, hypertension in 3 and chronic glomerulonephritis in 5 patients and polycystic kidney disease in 1. The types of vascular access included A-V fistulas in 14 and A-V grafts in 7 patients. As expected, serum creatinine, blood urea nitrogen and phosphorus concentrations were significantly higher in ESRD patients compared to the control group. Blood haemoglobin concentration was significantly lower, whereas serum

### Table 1. Biochemical parameters in normal control and ESRD groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 21)</th>
<th>ESRD (n = 21)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mg/dL)</td>
<td>13.3 ± 1.0</td>
<td>69.2 ± 4.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.85 ± 0.18</td>
<td>10.45 ± 0.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.14 ± 0.24</td>
<td>9.10 ± 0.20</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>3.20 ± 0.16</td>
<td>5.43 ± 0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>iPTH (pg/mL)</td>
<td>40.0 ± 3.8</td>
<td>293.9 ± 57.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>14.03 ± 0.38</td>
<td>11.72 ± 0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>32.6 ± 6.2</td>
<td>209.9 ± 23.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>18.6 ± 2.1</td>
<td>26.8 ± 2.1</td>
<td>0.012</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.9 ± 0.14</td>
<td>3.8 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Kt/V</td>
<td>–</td>
<td>1.53 ± 0.16</td>
<td>–</td>
</tr>
</tbody>
</table>

**Measurements of apolipoprotein A-I concentration**

Apo A-I protein abundance was determined using an ELISA kit purchased from AlerChek Inc. (Portland, Maine, UK) and a SpectraMax M5 plate reader as specified in the manufacturer’s protocol.

**Measurements of LCAT concentration**

Plasma LCAT protein concentration was determined using an EIA kit from Alpco Diagnostics (Salem, NH, USA) and a SpectraMax M5 plate reader following the manufacturer’s instructions.

**Measurements of plasma cytokine concentrations**

Plasma concentrations of IL-6, IL-8 and TNF alpha were determined using the Millipore 13-plex inflammatory cytokine panel (Billerica, MA, USA) run on the Luminex 100 IS system. Data were analysed using the MiraiBio MasterPlex QT Version: 0.1.171 software (San Francisco, CA, USA).

**Data analysis**

Data are expressed as mean ± SE (unless indicated otherwise). Student’s t-test, Wilcoxon rank-sum test and regression analysis were used in the statistical analysis of the data as appropriate. Pearson’s correlation coefficients were calculated using the CORR Procedure from SAS 9.1.3 Software (Cary, NC, USA). P values <0.05 were considered significant.
ferritin level and transferrin saturation were higher in ESRD patients than the corresponding values found in the control group. However, serum albumin and calcium concentrations in the ESRD patients were not significantly different than those observed in the control group. The mean Kt/V value in the ESRD patients was >1.5, reflecting an adequacy of dialysis regimen in the study participants.

**Lipid profile**

Data are summarized in Table 2 and Figure 1. Total cholesterol, LDL cholesterol and HDL cholesterol concentrations were lower in ESRD patients compared to the normal control group. The reduction in HDL-cholesterol level in ESRD patients was accompanied by a marked reduction in serum Apo A-1 concentration (75 ± 2.6 versus 126 ± 9.6 mg/dL, *P* = 0.03). A significant correlation was found between Apo A-1 and HDL-C concentration among the study population (*r* = 0.54, *P* = 0.05). As expected plasma triglyceride concentration and VLDL cholesterol concentration were elevated in the ESRD group. No significant difference was found in lipid values among statin-treated (LDL = 75 ± 30 mg/dL, HDL = 42 ± 13 mg/dL) and un-treated (LDL = 66 ± 21, HDL = 36 ± 15 mg/dL) ESRD patients.

**PLTP and CETP data**

Data are illustrated in Figures 2 and 3. Plasma PLTP concentration and PLTP activity were unchanged in haemodialysis patients when compared to those found in the control subjects. Likewise, CETP abundance and activity in haemodialysis patients were similar to those found in the normal control group. Weak correlations were found between plasma PLTP (*r* = 0.41, *P* = 0.06) and CETP (*r* = 0.38, *P* = 0.10) activities with their corresponding concentrations. No significant difference was found in the PLTP or CETP activity to concentration ratios between the ESRD and control groups. Similarly, no significant correlation was found between PLTP or CETP values and either age, BMI, HDL-C or VLDL-C in either group. However, CETP activity was positively correlated with triglyceride levels within the ESRD group (*r* = 0.49, *P* = 0.05).

**LCAT data**

Data are illustrated in Figure 4. Plasma LCAT concentration in ESRD patients (4.82 ± 0.45 µg/mL) was significantly lower than that found in the normal control group (8.04 ± 0.58 µg/mL, *P* < 0.001). A significant correlation was found between LCAT concentration and HDL-C among the study population (*r* = 0.60, *P* = 0.02).

**Cytokine data**

Data are shown in Figure 5. Compared with the control group, the ESRD group exhibited a marked elevation of plasma IL-6, IL-8 and TNF alpha concentrations. No significant correlation was found between plasma PLTP activity and IL-6 (*R* = 0.01, *P* = 0.92), IL-8 (*R* = 0.09, *P* = 0.63) or TNF alpha (*R* = 0.3166, *P* = 0.10). Likewise, no significant correlation was found between plasma CETP activity and IL-6 (*R* = −0.12, *P* = 0.51), IL-8 (*R* = −0.28, *P* = 0.13) or TNF alpha (*R* = −0.31, *P* = 0.09).

**Discussion**

ESRD results in profound lipid disorders that stem largely from the dysregulation of HDL and triglyceride-rich
lipoproteins. ESRD is consistently associated with reduced HDL cholesterol, increased HDL triglyceride, decreased plasma apoA-1 and impaired maturation of cholesterol-poor HDL-3 [2]. Impaired maturation of HDL in ESRD is largely due to down-regulation of LCAT which plays an important role in the HDL-mediated uptake of cholesterol from extra-hepatic tissues [2]. The triglyceride enrichment of HDL is primarily due to the deficiency of hepatic lipase that catalyzes the hydrolysis and removal of triglycerides from HDL [2].

Increased CETP can lower HDL cholesterol and raise HDL triglyceride and increased PLTP can reduce the HDL level and elevate the VLDL level. Thus, increased CETP and PLTP can produce a lipid profile similar to that caused by CKD. For this reason, it is tempting to hypothesize that CKD-induced dyslipidaemia could be, in part, due to up-regulation of these transfer proteins. However, we found no difference in either activity or abundance of PLTP or CETP between the haemodialysis-dependent ESRD patients and normal control individuals included in the present study. These findings tend to exclude dysregulation of PLTP and CETP as the major mediators of altered metabolism of HDL and other lipoproteins in the ESRD population.

In an earlier study, Schlitt et al. [24] found increased PLTP activity in haemodialysis patients when compared with their control group. The reason for the apparent disparity between the results of the latter study with that of the present study is unclear. It is of note that differences in the method used for the measurement of PLTP activity can significantly affect the results of the study [25]. However, this is an unlikely explanation for the difference in the PLTP activity data between the two studies. This is because in both studies PLTP activity was measured by commercially available kits that utilized similar methods. Instead the disparity appears to be due to differences in the populations studied. While the haemodialysis patients included in the two studies had similar characteristics and lipid profiles, the control groups used were different. For instance, 34% of the control subjects included in the study reported by Schlitt et al. had hypertension; some had diabetes mellitus or were treated with statin preparations. In contrast, individuals with acute or chronic illnesses and those receiving medications were
excluded from the present study. Additionally, the control group used in the former study had elevated mean LDL cholesterol (160 mg/dL) which was approximately twice that was found in our controls (84 mg/dL). Thus, differences in the control groups may account for the observed differences.

Few studies have reported on CETP concentration in haemodialysis patients. In concert with the findings of the present study, Kimura et al. found no significant difference in plasma CETP concentration between haemodialysis patients and normal subjects [26,27]. The present study revealed no significant difference in either plasma CETP activity or concentration between stable haemodialysis-dependent ESRD patients and the normal control individuals.

Studies aimed at exploring the role of CETP in cholesterol metabolism and cardiovascular outcomes in patients with ESRD have been inconclusive. Seiler et al. [28] measured CETP activity in 69 haemodialysis subjects and prospectively assessed cardiovascular events and mortality. The authors found no difference in baseline CETP activity between patients with and without cardiovascular disease. Likewise, no correlation was found between CETP activity and cardiovascular events or death at 4 years. In contrast, Kimura et al. reported that Japanese haemodialysis patients with vascular disease had lower CETP concentrations when compared to those without cardiovascular disease. Additionally, in those patients with high HDL and CETP levels, there was a significantly lower prevalence of cardiovascular disease [26,29]. Thus, the role of CETP in cardiovascular complications in haemodialysis-dependent ESRD population remains unclear.

As expected, plasma HDL cholesterol was markedly reduced in our ESRD patients. This was associated with and largely due to diminished plasma concentration of Apo A-1, which is the principal apolipoprotein constituent of HDL, as well as a marked reduction in LCAT concentration. LCAT is a key constituent of HDL which serves a dual function as phospholipase-2 and acyl-CoA cholesterol acyltransferase [9,10,30]. LCAT plays a crucial role in reverse cholesterol transport and HDL maturation. Thus, the observed LCAT deficiency contributes to diminished HDL cholesterol and impaired HDL maturation in patients with advanced CKD. Earlier studies have shown diminished plasma LCAT enzymatic activity in ESRD patients [8]. The present study demonstrates that the reduction in LCAT activity shown in the latter studies is due to the reduction in LCAT concentration as opposed to inhibition of its activity by the uraemic milieu. An earlier study from this laboratory demonstrated that LCAT deficiency in animals with experimental CKD is associated with and, at least in part, due to down-regulation of hepatic gene expression of this enzyme [9].

There is evidence that inflammation results in significant changes in plasma PLTP and CETP levels and that alteration in PLTP and CETP expressions or activities can modify the response to pro-inflammatory stimuli [31–37]. The ESRD patients employed in the present study exhibited a significant increase in plasma concentration of inflammatory mediators, IL-6, IL-8 and TNF alpha. However, as noted above, despite the prevailing systemic inflammation, plasma PLTP and CETP levels were unchanged in the study population and did not significantly correlate with the measured pro-inflammatory cytokines.

In conclusion, the present study revealed marked reductions in plasma Apo A-1 and LCAT but no change in either PLTP or CETP concentration or activity in stable haemodialysis-dependent ESRD patients. These findings confirm the role of Apo A-1 and LCAT deficiencies and exclude the significant participation of PLTP and CETP in the pathogenesis of dyslipidaemia in this population.

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Conflict of interest statement. None declared.

References

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**Implementing a home haemodialysis programme without adversely affecting a peritoneal dialysis programme**

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**Abstract**

**Background.** As the population with stage 5 CKD grows, the associated costs of providing dialysis care increase. Due to the high costs of these therapies, home haemodialysis is enjoying a renaissance in many jurisdictions. However, concerns persist as to whether home haemodialysis programmes grow at the expense of other home therapies such as peritoneal dialysis. This study attempts to look at the impact of a new home haemodialysis programme on an existing peritoneal dialysis programme in the province of British Columbia.

**Methods.** Using the provincial renal database in British Columbia (PROMIS), all patients receiving dialysis were tracked over the years preceding the implementation of a home haemodialysis programme and following its implementation. Rate of growth by specific dialysis modality


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