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Acquired lecithin:cholesterol acyltransferase deficiency as a major factor in lowering plasma HDL levels in chronic kidney disease

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Abstract. Calabresi L, Simonelli S, Conca P, Busnach P, Cabibbe M, Gesualdo L, Gigante M, Penco S, Veglia F, Franceschini G (Università degli Studi di Milano, Milan; Niguarda Ca' Granda Hospital, Milan; University of Bari "Aldo Moro", Bari; University of Foggia, Foggia; Niguarda Ca' Granda Hospital, Milan; Monzino Cardiologic Institute, IRCCS, Milan; Italy). Acquired lecithin:cholesterol acyltransferase deficiency as a major factor in lowering plasma HDL levels in chronic kidney disease. *J Intern Med* 2014; doi: 10.1111/joim.12290.

Objectives. It has been suggested that a low plasma high-density lipoprotein cholesterol (HDL-C) level contributes to the high cardiovascular disease risk of patients with chronic kidney disease (CKD), especially those undergoing haemodialysis (HD). The present study was conducted to gain further understanding of the mechanism(s) responsible for the low HDL-C levels in patients with CKD and to separate the impact of HD from that of the underlying CKD.

Methods. Plasma lipids and lipoproteins, HDL subclasses and various cholesterol esterification parameters were measured in a total of 248 patients with CKD, 198 of whom were undergoing HD treatment and 40 healthy subjects.

Results. Chronic kidney disease was found to be associated with highly significant reductions in plasma HDL-C, unesterified cholesterol, apolipoprotein (apo)A-I, apoA-II and LpA-I:A-II levels in both CKD cohorts (with and without HD treatment). The cholesterol esterification process was markedly impaired, as indicated by reductions in plasma lecithin:cholesterol acyltransferase (LCAT) concentration and activity and cholesterol esterification rate, and by an increase in the plasma preβ-HDL content. HD treatment was associated with a further lowering of HDL levels and impaired plasma cholesterol esterification. The plasma HDL-C level was highly significantly correlated with LCAT concentration ($R = 0.438$, $P < 0.001$), LCAT activity ($R = 0.243$, $P < 0.001$) and cholesterol esterification rate ($R = 0.149$, $P = 0.031$). Highly significant correlations were also found between plasma LCAT concentration and levels of apoA-I ($R = 0.432$, $P < 0.001$), apoA-II ($R = 0.275$, $P < 0.001$), LpA-I ($R = 0.326$, $P < 0.001$) and LpA-I:A-II ($R = 0.346$, $P < 0.001$).

Conclusion. Acquired LCAT deficiency is a major cause of low plasma HDL levels in patients with CKD, thus LCAT is an attractive target for therapeutic intervention to reverse dyslipidaemia, and possibly lower the cardiovascular disease risk in these patients.

Keywords: cholesterol esterification, chronic kidney disease, HDL, LCAT.

Introduction

Chronic kidney disease (CKD) dramatically increases cardiovascular disease (CVD) risk, which increases markedly with declining kidney function, and is highest in patients undergoing haemodialysis (HD) [1, 2]. Although patients with CKD suffer from multiple comorbid conditions known to increase CVD risk, such as hypertension and diabetes [3], their enhanced CVD mortality cannot be fully explained by these risk factors [4]. Dyslipidaemia, a major CVD risk factor in the

general population, is frequently observed amongst patients with CKD. Although dyslipidaemia in CVD patients is mainly characterized by increased plasma levels of the atherogenic low-density lipoprotein cholesterol (LDL-C), plasma LDL-C levels are commonly within or below the recommended limits in patients with CKD, and the dyslipidaemia is mainly characterized by moderate reductions in the anti-atherogenic high-density lipoprotein cholesterol (HDL-C) [5].

HDL metabolism is regulated by a number of plasma and cell-associated proteins. It is initiated in the liver and intestine, with the secretion of lipid-poor apolipoprotein (apo)A-I, which then acquires phospholipids and cholesterol through interaction with the ATP-binding cassette A1 transporter to form nascent preβ-HDL [6]. Once in the circulation, preβ-HDL particles interact with LCAT. LCAT esterifies cholesterol, synthesizes nonpolar cholesteryl esters and generates mature spherical α-HDL. preβ-HDL particles have a short plasma half-life, being cleared rapidly through the kidney [7], whereas the turnover of mature α-HDL is much slower. Cholesterol esterification by LCAT thus plays a central role in intravascular HDL metabolism and in the determination of plasma HDL structure and concentration.

A limited number of studies, mainly in small series of patients, have investigated HDL and plasma cholesterol esterification in CKD; however, all were conducted in patients with CKD on HD (CKD-HD), thus hindering the separation of the impact of HD from that of the underlying CKD. These studies demonstrated reductions in plasma apoA-I concentrations in patients with CKD-HD, compared with subjects with normal renal function [8]. A reduced capacity of plasma from patients with CKD-HD to esterify cholesterol within an exogenous substrate (i.e. reduced LCAT activity [9]), probably reflecting a reduction in plasma LCAT concentration [8], was reported in patients with CKD-HD 30 years ago [10]. The hypothesis that this results in impaired plasma cholesterol esterification, which is determined by the interaction of LCAT with endogenous lipoproteins [9], is supported by the accumulation in plasma of small preβ-HDL [11], but remains to be firmly established.

The aim of the present study was to comprehensively evaluate and compare HDL and cholesterol esterification in a large cohort of patients with CKD-HD, advanced CKD patients not requiring HD and healthy subjects to (i) gain further understanding of the mechanism(s) responsible for the low HDL levels in patients with CKD-HD and (ii) separate the impact of HD from that of underlying CKD.

Materials and methods

Subjects

A total of 198 consecutive stable patients with CKD-HD receiving maintenance HD for a minimum of 6 months who attended the Dialysis Unit of the Niguarda Ca' Granda Hospital in Milan, Northern Italy (n = 142) or the Renal, Dialysis and Transplantation Unit, University Hospital in Foggia, Southern Italy (n = 56) were enrolled in the study. The causes of renal disease were as follows: glomerulonephritis (n = 48, 24.2%), cystic renal disease (n = 22, 11.1%), diabetic nephropathy (n = 32, 16.2%), vascular and hypertensive nephropathy (n = 32, 16.2%), infectious/obstructive interstitial nephropathy (n = 16, 8.1%), renal neoplasia leading to an anephric state (n = 3, 1.5%) and other (n = 25, 12.6%) or unknown causes (n = 20, 10.1%).

In addition, 50 consecutive patients with CKD not requiring HD (at CKD stages G4 and G5, as defined by an estimated glomerular filtration rate (GFR) of 15–29 mL min^{-1} and <15 mL min^{-1}, respectively [12, 13]), were recruited amongst those attending the outpatient predialysis clinic of the Niguarda Ca' Granda Hospital. The estimated GFR in these individuals ranged from 6.2 to 28.6 mL min^{-1}. The causes of renal disease were as follows: glomerulonephritis (n = 16, 32%), cystic renal disease (n = 5, 10%), diabetic nephropathy (n = 7, 14%), vascular and hypertensive nephropathy (n = 10, 20%), infectious/obstructive interstitial nephropathy (n = 3, 6%) and other (n = 6, 12%) or unknown causes (n = 3, 6%).

Finally, 40 subjects free of kidney damage (and without abnormalities of blood or urine composition or of imaging test results), recruited amongst blood donors attending the Servizio Immunoematologico Trasfusionale of the Niguarda Ca' Granda Hospital, matched for age and gender, served as control subjects (controls). The estimated GFR values in these individuals ranged from 62.3 to 113.7 mL min^{-1}.

Exclusion criteria for both patients and controls were liver dysfunction, thyroid disorders or acute

infectious diseases. A detailed medical history, including age, gender, weight, height, body mass index (BMI), diabetes mellitus, hypertension, past or current smoking and current medications, was obtained from all participants. Hypertension was defined as brachial blood pressure ≥140/80 mmHg and/or current anti-hypertensive treatment. Alcohol consumption did not exceed 15 g per day for any individual, and none followed a regular exercise training programme. Patients with diabetic received insulin once or twice daily (doses from 10 to 35 IU) or oral antidiabetic agents. None of the subjects received corticosteroids or immunosuppressive agents. Patients with CKD-HD were appropriately administered phosphate binders and vitamins; most of these patients also received erythropoiesis-stimulating agents. Patients were dialysed with conventional high-flux HD, for at least 3.5 h, three times per week using bicarbonate-containing dialysis fluid. Blood flow, dialysate flow rate, dialyser model and treatment time were tailored to individual patients to achieve the target equilibrated urea Kt/V ≥1. The study was approved by the local ethics committee.

Biochemical analyses

Fasting blood samples were collected from the HD access in patients with CKD-HD (immediately before dialysis) and by venipuncture in patients with CKD and controls. Blood was collected into tubes containing Na_2-EDTA (final concentration 1 mg mL^{-1}), and plasma was prepared by low-speed centrifugation at 4 °C. Plasma aliquots and blood cells were immediately frozen at −80 °C. Plasma total and unesterified cholesterol, HDL-C and triglyceride levels were determined with standard enzymatic techniques. LDL-C was calculated using the Friedewald formula. Plasma apoA-I, apoA-II and apoB levels were determined by immunoturbidimetry. ApoE phenotyping was performed by isoelectric focusing [14].

The plasma levels of lipoprotein particles containing only apoA-I (LpA-I) and of particles also containing apoA-II (LpA-I:A-II) were determined by electroimmunodiffusion (Sebia Italia, Florence, Italy) [15]. HDL particle size was evaluated by nondenaturing polyacrylamide gradient gel electrophoresis (GGE), using precast 4–30% acrylamide gels (CBS Scientific, San Diego, CA, USA) [15]. The plasma preβ-HDL content was assessed by two-dimensional electrophoresis, where agarose gel electrophoresis was followed by nondenaturing GGE and subsequent immunoblotting for apoA-I [16].

Cholesterol esterification in plasma was assessed by calculating the unesterified/total cholesterol ratio [9]. The esterification of cholesterol within endogenous lipoproteins [cholesterol esterification rate (CER)], or incorporated into an exogenous standardized substrate (LCAT activity), was determined as previously described [9]. Plasma LCAT and cholesterylester transfer protein (CETP) concentrations were measured by immunoenzymatic assay [17]. The *LCAT* gene was amplified and sequenced using an automatic sequencer CEQ2000 DNA Analysis System (Beckman Coulter, Fullerton, CA, USA), as previously described [9].

Statistical analyses

Continuous variables are reported as mean ± SD, and categorical variables as number and percentage. Non-normally distributed variables were log transformed before analysis. Data were compared amongst groups by one-way ANOVA (numerical variables) or chi-squared test (categorical variables). Associations between variables were assessed by Pearson's correlation.

To separate the impact of HD from that of the underlying CKD on the measured variables, we included a specific term for each of the two conditions (CKD and CKD-HD) in a multivariable ANCOVA model; data were further adjusted for age, sex, BMI, smoking status, hypertension, diabetes and use of lipid-modifying agents. The final results of this analysis were expressed as adjusted beta coefficients and 95% confidence intervals. *P*-values below 0.05 were considered statistically significant. All tests were two-sided and were performed using SAS v. 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Subjects

The characteristics of the study participants (patients and controls) are shown in Table 1. There was no difference in the sex distribution amongst the three groups of subjects. The patients with CKD-HD and CKD were older and had lower BMI values than controls. The prevalence of smokers was similar amongst the three groups; hypertension and diabetes were more common amongst patients than controls. Overall, 81 (41%) patients with CKD-HD, 19 (38%) patients with CKD and

Table 1 *Demographic, clinical and lipid/lipoprotein data in the study participants*

	CKD-HD	CKD	Controls	*P**
n	198	50	40	
Sex, M/F	124/74	33/17	24/16	0.906
Age, years	67.0 ± 13.3	68.5 ± 13.7	60.6 ± 6.1	0.006
BMI, kg m^{-2}	22.8 ± 4.2	23.9 ± 3.4	24.7 ± 2.8	0.006
Smoking status, %				
Never	59	65	50	0.100
Former	19	31	38	
Current	22	4	12	
Hypertension, %	71	92	23	<0.0001
Diabetes, %	15	14	0	0.005
Total cholesterol, mg dL^{-1}	156.8 ± 43.9	180.5 ± 39.6	211.5 ± 38.3	<0.0001
Unesterified cholesterol, mg dL^{-1}	52.7 ± 14.0	52.6 ± 11.1	56.8 ± 9.1	0.118
LDL cholesterol, mg dL^{-1}	85.5 ± 37.4	99.7 ± 34.0	131.5 ± 33.2	<0.0001
HDL cholesterol, mg dL^{-1}	41.3 ± 13.2	50.2 ± 12.3	59.2 ± 11.8	<0.0001
ApoA-I, mg dL^{-1}	92.3 ± 20.5	119.2 ± 18.0	134.7 ± 17.5	<0.0001
ApoA-II, mg dL^{-1}	20.3 ± 5.4	22.8 ± 4.2	26.4 ± 3.7	<0.0001
ApoB, mg dL^{-1}	79.6 ± 28.9	100.7 ± 27.4	119.4 ± 32.7	<0.0001
Triglycerides, mg dL^{-1}	150.7 ± 83.1	153.4 ± 71.0	103.5 ± 44.4	0.001

CKD, chronic kidney disease; CKD-HD, patients with chronic kidney disease undergoing haemodialysis; controls, healthy subjects; BMI, body mass index; Apo, apolipoprotein; M, male; F, female.
Data are expressed as mean ± SD, unless otherwise stated. **P* values, as assessed by one-way ANOVA or chi-squared test, as appropriate; those for lipid/lipoprotein variables were adjusted for sex, age, BMI, smoking status, hypertension, diabetes and use of lipid-modifying agents.

none of the controls were taking statin medication (either pravastatin or simvastatin). Because several factors, such as the presence of diabetes, being overweight and taking lipid-lowering drugs, may affect some HDL and cholesterol esterification measurements, all biochemical data were adjusted for confounding variables, as specified in the Methods. One patient with CKD-HD carried a mutated *LCAT* allele; no *LCAT* gene mutations were found amongst patients with CKD and controls. *apoE* allele frequencies were similar in the three groups of subjects.

All subsequent analyses were performed after adjustment for age, sex, BMI, smoking status, hypertension, diabetes and use of lipid-modifying agents.

Plasma lipids and lipoproteins

Plasma lipid, lipoprotein and apolipoprotein levels in the three groups are shown in Table 1. Average total cholesterol, LDL-C and apoB levels were lower and triglyceride levels were higher in patients than controls. Average HDL-C, apoA-I and apoA-II levels were also lower in patients than controls (Fig. 1). No differences were found in the average plasma unesterified cholesterol levels.

The independent effects of HD and of the underlying CKD on plasma lipids and lipoproteins were tested by multivariable ANCOVA models, with further adjustment for age, sex, BMI, smoking status, hypertension, diabetes and use of lipid-modifying agents; the adjusted beta coefficients and 95% confidence intervals are shown in Table 2. CKD was associated with highly significant reductions in the average plasma total, LDL and HDL cholesterol, apoA-I, apoA-II and apoB levels, and with a smaller but still significant reduction in plasma unesterified cholesterol and a significant increase in plasma triglyceride levels. HD treatment was associated with a further significant reduction in total and HDL cholesterol, apoA-I, apoA-II and apoB levels, with no effect on unesterified cholesterol, LDL-C and triglyceride levels.

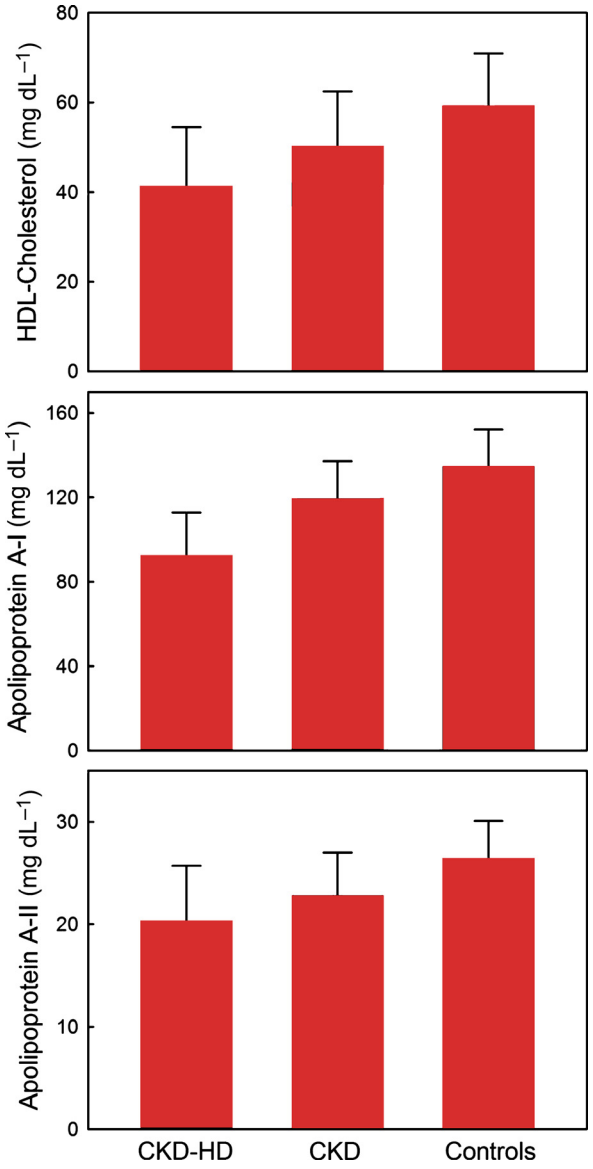

Fig. 1 *Plasma HDL cholesterol, apolipoprotein (apo)A-I and apoA-II levels in patients with chronic kidney disease (CKD) undergoing haemodialysis (CKD-HD, n = 198), patients with CKD (n = 50) and healthy subjects (controls, n = 40). Data are mean ± SD.*

HDL particles and cholesterol esterification

The plasma levels and size of HDL particles in the three groups are shown in Table 3. The distribution of HDL particles in those containing only apoA-I (LpA-I) and those containing both LpA-I and apoA-II (LpA-I:A-II) was significantly different amongst the three study groups. Patients with CKD showed a selective reduction in LpA-I:A-II particles compared with controls, whereas a reduction in both LpA-I and LpA-I:A-II particles (of greater magnitude in the latter) was observed in patients on HD. Patients had a significantly greater plasma preβ-HDL content compared with controls (Fig. 2); there was no significant difference in plasma HDL2 and HDL3 size.

Plasma cholesterol esterification was impaired in patients compared with controls, as indicated by the significantly greater plasma unesterified to total cholesterol ratio, and lower CER and LCAT activity and concentration (Fig. 2). LCAT specific activity, calculated as the ratio between LCAT activity and concentration, was similar amongst the three groups (controls: 7.32 ± 1.59 nmol h^{-1} μg^{-1}; CKD: 6.56 ± 2.12 nmol h^{-1} μg^{-1}; CKD-HD: 6.79 ± 2.89 nmol h^{-1} μg^{-1}; $P = 0.405$). Plasma CETP concentration was significantly different amongst the three groups of subjects.

The independent effects of HD and of the underlying CKD on HDL parameters are shown in Table 4. CKD had no effect on plasma LpA-I levels, but was associated with a highly significant reduction in plasma LpA-I:A-II levels and with a significant increase in the plasma preβ-HDL content. HD had no additional effect on plasma LpA-I and LpA-I:A-II levels, but slightly further increased plasma preβ-HDL content. Neither CKD nor HD treatment significantly affected HDL particle size except for a modest reduction in average HDL3 size associated with HD treatment.

Chronic kidney disease was associated with significant reductions in plasma CER and LCAT activity and concentration; the expected increase in the unesterified to total cholesterol ratio was of borderline statistical significance. CKD had no effect on plasma CETP levels. HD treatment was associated with a further, highly significant increase in the plasma unesterified to total cholesterol ratio, largely attributable to a reduction in the plasma LCAT activity and concentration. HD had no effect on plasma CER and was associated with a significant reduction in plasma CETP levels.

In the entire cohort, a lower plasma HDL-C level was highly significantly correlated with lower plasma LCAT concentration ($R = 0.438$, $P < 0.001$), LCAT activity ($R = 0.243$, $P < 0.001$) and CER ($R = 0.149$, $P = 0.031$). Highly significant correlations were also found between plasma LCAT concentration and levels of apoA-I ($R = 0.432$,

Table 2 *Independent effects of chronic kidney disease and haemodialysis on plasma lipid/lipoprotein levels in the study participants*

	CKD		HD	
	β	*P*	β	*P*
Total cholesterol, mg dL^{-1}	−40.20 (−61.40, −18.90)	0.0003	−20.70 (−35.50, −5.90)	0.0070
Unesterified cholesterol, mg dL^{-1}	−7.51 (−13.74, −1.29)	0.0190	0.30 (−4.04, 4.64)	0.8900
LDL cholesterol, mg dL^{-1}	−37.60 (−56.50, −18.80)	0.0001	−12.50 (−25.60, 0.60)	0.0600
HDL cholesterol, mg dL^{-1}	−10.70 (−16.90, −4.50)	0.0009	−8.10 (−12.40, −3.70)	0.0003
ApoA-I, mg dL^{-1}	−15.80 (−25.50, −6.10)	0.0020	−25.70 (−32.40, −18.90)	<0.0001
ApoA-II, mg dL^{-1}	−4.00 (−6.36, −1.65)	0.0010	−2.87 (−4.52, −1.23)	0.0007
ApoB, mg dL^{-1}	−26.60 (−41.80, −11.50)	0.0007	−15.20 (−25.80, −4.60)	0.0050
Triglycerides, log mg dL^{-1}	0.15 (0.05, 0.24)	0.0030	−0.02 (−0.09, 0.04)	0.5200

CKD, chronic kidney disease; HD, haemodialysis; Apo, apolipoprotein.
Data are reported as beta coefficients and 95% confidence intervals, as assessed by ANCOVA. Beta coefficients and *P* values for HD and CKD are mutually adjusted, and also adjusted for sex, age, body mass index, smoking status, hypertension, diabetes and use of lipid-modifying agents.

Table 3 *HDL and cholesterol esterification in the study participants*

	CKD-HD	CKD	Controls	*P**
LpA-I, mg dL^{-1}	43.1 ± 12.8	55.2 ± 15.9	50.1 ± 13.2	<0.0001
LpA-I:A-II, mg dL^{-1}	49.2 ± 13.6	64.0 ± 14.4	84.6 ± 12.7	<0.0001
Pre-β HDL, %	17.1 ± 4.7	15.8 ± 4.7	13.1 ± 3.2	<0.0001
HDL_2 size, nm	11.2 ± 0.4	11.1 ± 0.2	11.2 ± 0.3	0.267
HDL_3 size, nm	8.8 ± 0.4	8.9 ± 0.2	8.8 ± 0.3	0.372
Unesterified/total cholesterol	0.34 ± 0.05	0.29 ± 0.04	0.27 ± 0.02	<0.0001
CER, nmol mL^{-1} h^{-1}	30.2 ± 11.2	28.9 ± 10.7	38.5 ± 8.5	0.0001
LCAT activity, nmol mL^{-1} h^{-1}	26.1 ± 9.9	30.0 ± 8.8	36.0 ± 6.4	<0.0001
LCAT concentration, μg mL^{-1}	4.01 ± 0.92	4.64 ± 0.75	5.05 ± 0.73	<0.0001
CETP concentration, μg mL^{-1}	1.36 ± 0.35	1.61 ± 0.36	1.57 ± 0.34	<0.0001

CKD, chronic kidney disease; HD, haemodialysis; Apo, apolipoprotein; controls, healthy subjects; CER, cholesterol esterification rate; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesterylester transfer protein.
Data are expressed as mean ± SD. **P* values assessed by ANCOVA, adjusted for sex, age, body mass index, smoking status, hypertension, diabetes and use of lipid-modifying agents.

$P < 0.001$), apoA-II ($R = 0.275$, $P < 0.001$), LpA-I ($R = 0.326$, $P < 0.001$) and LpA-I:A-II ($R = 0.346$, $P < 0.001$). The plasma LCAT concentration was strongly positively correlated with plasma LCAT activity ($R = 0.261$, $P < 0.001$), but was not a limiting factor in CER determination as no significant correlation was found between the two variables ($R = -0.014$, $P = 0.838$).

Discussion

Dyslipidaemia has been linked to the increased CVD risk observed in patients with CKD, especially those undergoing HD treatment. The present results confirm previous findings [5] that levels of plasma total and LDL cholesterol are lower in patients with CKD compared with those in healthy individuals. Plasma apoB levels are also markedly reduced, indicating a lower number of circulating LDL particles. Also consistent with previous findings [5], the dyslipidaemia in patients with CKD in the present study was mostly characterized by both a moderate reduction in HDL-C and a moderate increase in plasma triglyceride levels. Whereas plasma triglycerides did not differ between the two patient with CKD groups, HD treatment was

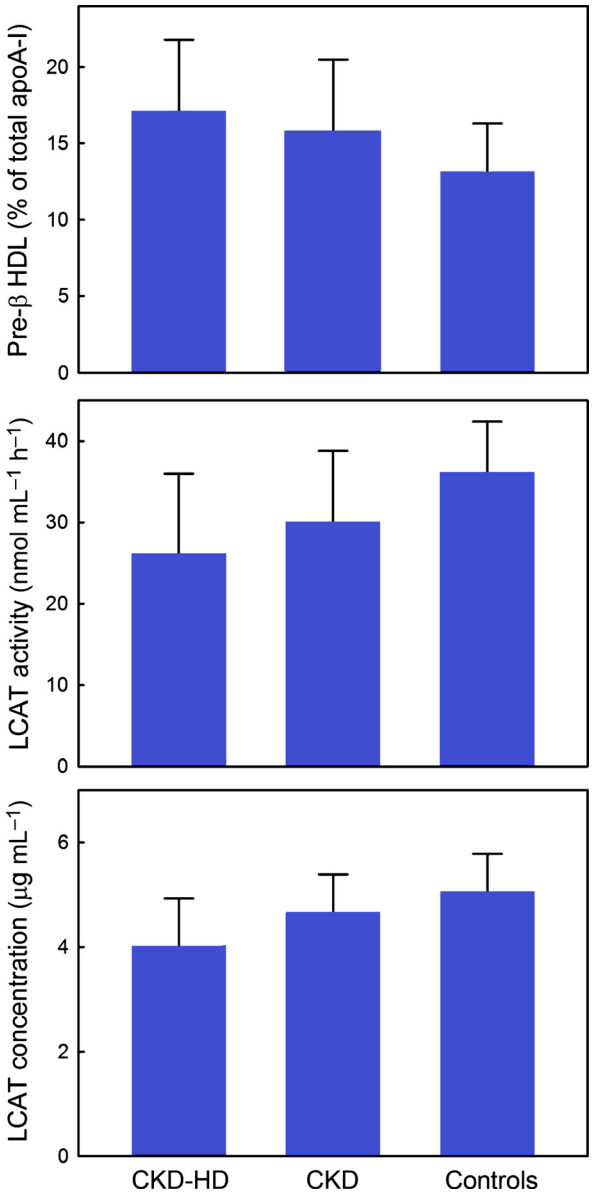

Fig. 2 *Plasma lecithin:cholesterol acyltransferase (LCAT) activity, LCAT concentration and preβ-HDL content in patients with chronic kidney disease (CKD) undergoing haemodialysis (CKD-HD, n = 198), patients with CKD (n = 50), and healthy subjects (controls, n = 40). Data are mean ± SD.*

associated with a further reduction in plasma HDL-C compared patients with CKD not undergoing HD.

Several abnormalities in HDL particles were found in the present study in patients with predialysis CKD, which persisted, and generally worsened with HD treatment. These included low plasma HDL apolipoprotein levels, a low content of LpA-I: A-II particles and a high content of preβ-HDL compared to healthy subjects. This abnormal HDL profile mirrors that found in individuals with genetic LCAT deficiency [9] and probably reflects the accumulation in plasma of cholesteryl ester-poor, apoA-I-containing discoidal HDL particles [18], which cannot mature into spherical HDL because of defective cholesterol esterification [19]. Indeed, plasma LCAT concentration and activity were reduced in patients with CKD in the present study, leading to defective plasma cholesterol esterification, as indicated by the elevated plasma unesterified/total cholesterol ratio. These findings are all consistent with a common metabolic defect as a major cause of the low plasma HDL level in patients with genetic LCAT deficiency and in those with CKD, both those who are and are not undergoing HD treatment. The reduced LCAT concentration/activity leads to defective cholesterol esterification and impaired preβ-HDL maturation, ultimately resulting in accelerated catabolism of LpA-I:A-II particles [20]. Reduced hepatic apoA-I production, as found in uraemic rats [21], is likely to minimally contribute to the low plasma HDL levels of patients with CKD. The rate of ApoA-I synthesis was found to be normal in both patients with CKD-HD [22] and in individuals with LCAT deficiency [20]. Moreover, defective apoA-I production would lead to impaired formation of nascent preβ-HDL particles [19], which instead would accumulate in plasma of patients with CKD.

In genetic LCAT deficiency, impaired cholesterol esterification is due to either normal hepatic secretion of an LCAT protein with dysfunctional activity, or to defective hepatic LCAT production [23]; however, the cause(s) of the defect in cholesterol esterification in patients with CKD appear to be more complex. The combination of low plasma LCAT concentration with normal LCAT specific activity indicates an acquired defect in hepatic production, and/or enhanced catabolism, of a normal enzyme as a leading cause of the impaired plasma cholesterol esterification. Indeed, it has been demonstrated that downregulation of hepatic *LCAT* gene expression can explain the reduced plasma LCAT concentration and activity in experimental CKD [24]. A similar mechanism may also operate in humans. No data are presently available on LCAT expression in patients with CKD, but a high molecular weight inhibitor of

Table 4 *Independent effects of chronic kidney disease and haemodialysis on HDL and cholesterol esterification in the study participants*

	CKD		HD	
	β	*P*	β	*P*
LpA-I, mg dL^{-1}	5.63 (−0.95, 12.22)	0.1000	−10.92 (−15.51, −6.33)	<0.0001
LpA-I:A-II, mg dL^{-1}	−21.40 (−28.40, −14.40)	<0.0001	−14.70 (−19.60, −9.90)	<0.0001
Pre-β HDL, %	2.70 (0.46, 4.94)	0.0190	1.58 (0.01, 3.14)	0.0500
HDL_2 size, nm	−0.17 (−0.35, 0.01)	0.0730	0.10 (−0.03, 0.23)	0.1300
HDL_3 size, nm	0.13 (−0.04, 0.31)	0.1300	−0.17 (−0.29, −0.04)	0.0090
Unesterified/total cholesterol	2.20 (−0.05, 4.45)	0.0570	4.40 (2.84, 5.97)	<0.0001
CER, nmol mL^{-1} h^{-1}	−13.3 (−19.31, −7.20)	<0.0001	4.14 (−1.29, 9.58)	0.1300
LCAT activity, nmol mL^{-1} h^{-1}	−4.94 (−9.71, −0.18)	0.0394	−5.72 (−9.78, −1.66)	0.0050
LCAT concentration, μg mL^{-1}	−0.53 (−0.97, −0.09)	0.0190	−0.59 (−0.90, −0.29)	0.0002
CETP concentration, μg mL^{-1}	0.08 (−0.11, 0.27)	0.4000	−0.24 (−0.38, −0.11)	0.0007

CKD, chronic kidney disease; HD, haemodialysis; CER, cholesterol esterification rate; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesterylester transfer protein.
Data are reported as beta coefficients and 95% confidence intervals, as assessed by ANCOVA. Beta coefficients and *P* values for HD and CKD are mutually adjusted, and also adjusted for sex, age, body mass index, smoking status, hypertension, diabetes and use of lipid-modifying agents.

apoA-I expression has been detected in plasma samples from patients with this condition [25]. Nevertheless, the impaired plasma cholesterol esterification in patients with CKD is also caused by a defective interaction between the enzyme and its lipid/lipoprotein substrates, as indicated by the reduced CER (a measure of the substrate efficiency of the LCAT reaction), independent of the reduced plasma LCAT concentration. The reason(s) for this defective interaction are not clear and may be linked to oxidative modification of HDL [26], leading to impaired LCAT reactivity with the lipoprotein substrate [27].

An aim of the present study was also to separate the impact of HD from that of underlying CKD on HDL abnormalities and defective cholesterol esterification, by comparing, for the first time, patients with CKD undergoing HD with those who do not require this treatment. The results indicate that alterations already present in patients with CKD before HD are generally aggravated in those undergoing treatment, with a further reduction in plasma LCAT concentration and activity, and a consequent additional effect on plasma HDL levels and particle distribution. This indicates that the HD treatment *per se* has little effect in initiating the series of metabolic events that ultimately lead to a reduction in plasma HDL levels.

The cross-sectional design of this study limits our ability to assess the directionality of observed associations. Furthermore, the recruitment of predialysis patients with CKD stages G4 and G5 limits the significance of present findings for patients with advanced disease, precluding any consideration of the impact of early kidney damage. Ongoing large prospective studies will address some of to these issues.

Whether or not the acquired LCAT deficiency with consequent HDL abnormalities found in patients with CKD in the present study may contribute to the enhanced CVD risk remains to be determined. In the general population, the association between a low plasma HDL-C level and an increased CVD risk is well established, and is present even in individuals with recommended plasma LDL-C levels [28] (i.e. similar to those found in the present CKD cohorts). However, the metabolic cause of the low HDL level is acquiring greater importance in determining HDL-mediated CVD risk, as Mendelian randomization and intervention studies failed to demonstrate an association between exposure to high plasma HDL-C, due to a polymorphism in the endothelial lipase gene [29], or treatment with a CETP inhibitor [30], and decreased CVD events. The present findings clearly identify a secondary LCAT defect as the metabolic cause of the low HDL

level in CKD. Whether this contributes to the high CVD risk in patients with CKD remains to be established [31].

In summary, we have demonstrated in the present study that a defect in plasma cholesterol esterification, secondary to acquired LCAT deficiency, is a major factor in reducing plasma HDL levels and altering HDL composition in patients with CKD. Given the role of a low HDL level in the progression of CKD [32], therapeutic interventions to alleviate the acquired LCAT defect, with either small-molecule LCAT activators [33] or recombinant human LCAT [34, 35], may help to reverse the dyslipidaemia, slow the progression of the disease, and thus possibly reduce the high CVD risk in patients with CKD.

Conflict of interest statement

Laura Calabresi and Loreto Gesualdo are consultants to MedImmune. All other authors have no conflict of interest to declare.

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