

LEVELS OF CHOLESTERYL ESTERS AND OTHER LIPIDS IN THE PLASMA OF PATIENTS WITH END-STAGE RENAL FAILURE

Michael P.T. Gillett, PhD; Enyioma N. Obineche, MD, FRCP; Mohammed S. Lakhani, MSc; Abdishakur M. Abdulle, BSc; Iradj Amirlak, MD, FAAP; Mona Al Rukhaimi, MD, MRCP; Mustafa N. Suleiman, MD, FRCP

Background: The importance of plasma lipid abnormalities in chronic renal failure (CRF) is well recognized, but surprisingly little attention has been given to the study of some plasma lipid fractions, including cholesteryl esters (CE) and phospholipids, which might be expected to be important factors in the pathogenesis of the disease.

Materials and Methods: Fasting blood samples were taken from 25 control subjects and 53 CRF patients (29 predialysis and 24 on hemodialysis). Samples were analyzed for urea nitrogen, creatinine, triacylglycerols, total and individual phospholipids, total and free cholesterol, as well as cholesterol bound to very low-, low- and high-density lipoproteins (VLDL, LDL and HDL). Plasma CE was calculated and expressed as a percentage of total cholesterol.

Results: Over half of the patients had CE levels more than two standard deviations below the control value. In this subgroup of low CE patients, total, LDL- and HDL-cholesterol levels were also significantly lower than for controls, while levels of phosphatidylcholine and lysophosphatidylcholine were decreased and increased, respectively. In patients with high CE, no significant lipid abnormalities were observed.

Conclusion: In this study, CE was an excellent marker for lipid disturbances—if CE was high, then the other lipid fractions were normal, but if CE was low, most other lipid fractions were abnormal. The changes noted appear to be consequences of or related to deficiency of the plasma enzyme lecithin-cholesterol acyltransferase.

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Key Words: Plasma lipids, cholesteryl esters, chronic renal failure, lecithin-cholesterol acyltransferase.

The importance of plasma lipid and lipoprotein abnormalities in chronic renal failure (CRF) is recognized for several different reasons. First, ischemic heart disease is associated with hyperlipoproteinemias and is the major cause of death in patients on maintenance dialysis.¹⁻⁴ The incidence of cardiovascular complications is also abnormally elevated in predialysis patients with CRF.⁵ These types of findings have led in recent years to an increase in various aggressive hypolipidemic therapeutic measures being used in dialysis patients.⁶⁻¹¹ Second, it has long been thought that abnormalities in lipid and lipoprotein metabolism may be involved not just in the initial injury to the kidney, but also in ongoing processes that eventually lead to end-stage renal failure.¹²⁻¹⁵ Finally, there is growing evidence that dyslipoproteinemia in the pre-transplant patient may be a contributing factor in allograft failure.¹⁶⁻¹⁸

Most studies in renal disease have measured the main plasma lipids such as total cholesterol and triacylglycerols, together with high-density lipoprotein (HDL) cholesterol or other measures of lipoproteins. Comparatively little information is available concerning other lipid fractions, such as cholesteryl esters (CE) or phospholipids that may play a role in renal disease pathogenesis. Specifically, these lipid fractions may be affected by characteristic alterations in lipid metabolism that occur in CRF, such as decreased activities of key enzymes. One such enzyme is lecithin-cholesterol acyltransferase (LCAT), deficiency of which has been shown to occur in renal disease.¹⁹⁻²⁴ In the present study, we reassessed plasma lipids and lipoproteins, including both free cholesterol and CE as well as phospholipids, in patients with end-stage renal failure drawn from the multiracial population of the United Arab Emirates. The results suggest that a decreased concentration of CE relative to total cholesterol occurs commonly in renal failure and is associated with other lipid changes, while patients with high levels of cholesteryl esters show essentially normal levels of other lipids and lipoproteins.

Materials and Methods

A total of 53 male patients (mean age 52±13 years) with end-stage renal failure were included in this study, 24 of

From the Departments of Biochemistry, Internal Medicine and Paediatrics, Faculty of Medicine and Health Sciences, UAE University, Al Ain, Abu Dhabi, and the Nephrology Unit, Dubai Hospital, Dubai, United Arab Emirates.

Address reprint requests and correspondence to Dr. Gillett: Department of Biochemistry, FMHS, UAE University, P.O. Box 17666, Al Ain, Abu Dhabi, United Arab Emirates.

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TABLE 1. Levels of cholesteryl esters (CE), urea and creatinine in plasma samples from apparently healthy controls and patients with chronic renal failure (CRF).

Variable	Controls (n=25)	CRF high CE (n=22)*	CRF low CE (n=31)
CE (%)	71.3±0.9	73.8±3.1 ^a	57.7±13.9 ^{ab}
Urea nitrogen (mmol/L)	3.39±1.23	19.5±7.4 ^a	22.5±9.6 ^a
Creatinine (μmol/L)	81.0±30.7	490.9±268.3 ^a	504.2±236.7 ^a

The patients have been divided into two groups: those with CE more than two standard deviations below the mean control value and those with levels above this cut-off point. Significance of differences between patient groups and controls: ^a*P*<0.001; significance of differences between patient groups: ^b*P*<0.001.

TABLE 2. Levels of different lipid fractions in plasma samples from apparently healthy controls and patients with end-stage renal failure.

Lipid levels (mmol/L)	Controls (n=25)	CRF high CE (n=22)	CRF low CE (n=31)
TC	5.02±1.07	4.88±1.48	3.93±1.38 ^{bd}
FC	1.52±0.30	1.27±0.39	1.61±0.68
HDL-TC	0.96±0.27	0.98±0.32	0.68±0.32 ^{cd}
LDL-TC	3.61±0.95	3.53±1.40	2.58±0.95 ^{ce}
VLDL-TC	0.45±0.26	0.37±0.31	0.51±0.48
TG	1.49±0.58	1.33±0.73	1.92±1.69
TPL	2.75±0.58	2.32±0.59 ^a	2.29±0.73 ^a

TC=total cholesterol; FC=free cholesterol; HDL=high-density lipoprotein; LDL=low-density lipoprotein; VLDL=very low density lipoprotein; TG=triacylglycerols; TPL=total phospholipids; significance of differences between patient groups and controls: ^a*P*<0.05, ^b*P*<0.001, ^c*P*<0.001; significance of differences between patient groups: ^d*P*<0.05, ^e*P*<0.01.

TABLE 3. Relative concentrations of individual plasma phospholipids for apparently healthy controls and patients with end-stage renal disease.

Phospholipids (% TPL)	Controls (n=25)	CRF high CE (n=22)	CRF low CE (n=31)
LPC	5.6±1.8	6.1±2.1	3.8±1.7 ^{bd}
SM	20.6±2.6	20.3±2.7	20.0±5.0
PC	70.0±3.0	68.7±4.7	71.6±5.7 ^c
PE	3.8±0.9	5.0±2.4 ^a	5.2±2.3 ^a

LPC=lysophosphatidylcholine (lysolecithin); SM=sphingomyelin; PC=phosphatidylcholine (lecithin); PE=phosphatidylethanolamine; significance of differences between patient groups and controls: ^a*P*<0.05, ^b*P*<0.01; significance of difference between patient groups: ^c*P*<0.05, ^d*P*<0.001.

whom were undergoing maintenance hemodialysis (from 6 months to two years), while the rest were predialysis patients. The reference group consisted of 25 apparently healthy men (mean age 49±15 years) with no history of renal or any other serious disease. Before their inclusion, all the patients and normal individuals were informed about the research before they gave their consent for both blood samples and personal details to be collected. The experimental protocol of this study was approved by the Faculty of Medicine and Health Sciences Ethical Committee of the United Arab Emirates University.

Blood samples were taken in the early morning for all subjects after overnight fasting and for hemodialysis subjects both before and immediately after a dialysis session. The samples were anticoagulated with disodium

EDTA and kept on ice until delivered to the laboratory, usually within four hours. The blood samples were centrifuged to prepare plasma, several aliquots of which were batched and stored at -70°C for subsequent analysis. Commercially available kits (Boehringer Mannheim, now Roche Diagnostics, Germany) were used to measure creatinine, blood urea nitrogen, HDL cholesterol in fresh plasma and triacylglycerols, and free and total cholesterol in thawed samples of previously frozen plasma. Total and individual phospholipids in plasma were measured, as described by Gillett and Besterman.²⁵ Very low-density lipoprotein (VLDL) was separated by preparative ultracentrifugation using a Type 25 angle-head rotor and L7-65 instrument (both Beckman Instruments Inc., Fullerton, CA, USA) and the cholesterol content measured using the kit described above. Low-density lipoprotein (LDL) cholesterol was calculated as the total plasma cholesterol - HDL and VLDL cholesterol. The concentration of CE was taken as the difference between total and free cholesterol, and was expressed as a percentage of total cholesterol. In healthy individuals, this percentage is very constant,²⁶ and patients were subdivided into two groups according to whether they had high or low CE, defined as below two standard deviations (SD) of the normal value. All results were presented as mean values ±SD and the significance of differences between parameters for the patients and the reference population was determined using Student's *t*-test.

Results

In the control group, the mean CE was 71.3%±0.9% and values below 69.5% (mean -2 SD) in CRF patients were considered as being low CE, and patients with values of 69.5% or above as being high CE. In total, 31 out of 53 of patients (60%) had low CE according to this criterion, but they did not differ significantly in terms of creatinine and urea levels from the patients with high CE, as shown in Table 1. A much smaller proportion of patients on maintenance hemodialysis (42%) showed decreased CE than did predialysis patients (76%). No correlation was apparent between CE levels and levels of either creatinine or urea, either for the whole patient group or for the predialysis group. When compared both with controls and with patients with high levels of CE, patients with low CE showed significantly decreased levels of total cholesterol and phospholipids, LDL cholesterol and HDL cholesterol, while triacylglycerols and VLDL cholesterol were also increased, but not significantly (Table 2). Levels of free cholesterol were unchanged in patients with low CE, but in patients with high CE, free cholesterol and total phospholipids were both significantly lower than for controls. For low CE patients, the relative concentrations of lysophosphatidylcholine (lysolecithin, LPC) and phosphatidylcholine (lecithin, PC) were decreased and increased, respectively (Table 3). Also both groups of

TABLE 4. Levels of different lipid fractions in plasma samples from hemodialysis patients with high or low plasma CE measured before and immediately after a single routine dialysis session.

Lipid levels	High CE (n=12)		Low CE (n=10)	
	Before	After	Before	After
CE (%)	74.6±2.7	73.0±5.1	56.6±16.0	63.4±14.2
TC (mmol/L)	4.53±1.73	4.76±1.42	3.84±1.09	4.45±1.49
FC (mmol/L)	1.14±0.43	1.28±0.44	1.60±0.48	1.57±0.59
HDL-TC (mmol/L)	0.99±0.36	1.16±0.42	0.73±0.41	0.89±0.41
LDL-TC (mmol/L)	3.19±1.65	3.29±1.36	2.50±0.77	3.11±1.16
VLDL (mmol/L)	0.34±0.36	0.31±0.40	0.61±0.76	0.45±0.43
TG (mmol/L)	1.40±0.89	1.38±1.01	2.70±2.75	1.91±1.34
TPL (mmol/L)	2.16±0.66	2.63±0.61	2.30±0.78	2.63±0.78
LPC (%)	6.1±1.5	6.9±1.8	3.6±1.6	4.3±2.0
SM (%)	19.8±2.9	21.2±2.0	19.5±8.0	20.5±4.3
PC (%)	70.3±2.9	67.4±3.6	74.5±5.7	71.0±1.5
PE (%)	4.0±2.1	4.5±2.5	4.1±1.5	3.6±1.5

patients showed increased levels of phosphatidyl-ethanolamine, but no significant changes in sphingomyelin. Additionally, for 22 of the 24 hemodialysis patients, lipid status was evaluated before and after a routine dialysis session (Table 4). Of this group, 12 had high CE and 10 had low CE. No significant changes in lipid levels occurred after hemodialysis in either of these subgroups, although in the low CE patients there was a tendency for the relative concentrations of CE to increase, as did LPC, while PC decreased.

Discussion

Even though alterations in lipid metabolism can be detected in the early stages of renal disease, it is known that there are big differences in the pattern and severity of lipid disorders in patients at an advanced stage of the disease.²⁷ Clearly, the present study shows that UAE patients with end-stage renal disease are also variable in their lipid status. Many appear to be normolipidemic, but more than half seem to have some form of dyslipoproteinemia. However, in the present population, there is no evidence for hypercholesterolemia in CRF and instead, total cholesterol and LDL-cholesterol levels have been found to be either normal or significantly decreased.

In this paper, the relative concentration of plasma CE was used as the basis for subdividing patients. This parameter successfully split the patients into those with low CE who showed hypocholesterolemia, including both decreased LDL and HDL cholesterol and those with high CE, no hypocholesterolemia and normal LDL and HDL cholesterol. The low CE group also showed an increased tendency towards hypertriglyceridemia. However, both triacylglycerol and VLDL-cholesterol levels were quite variable in this relatively small group of patients, and the differences in their concentrations from normal were not significant. From these results in the UAE patients, therefore, the factors which may be most important in

increasing the risk of atherosclerotic complications are low HDL and high VLDL levels, but not high LDL levels. However, no information is as yet available on qualitative changes in LDL in these patients. Clearly, risk may increase even if there is no overall increase in total LDL concentration, but instead a shift towards increases in the small dense LDL which are thought to be particularly atherogenic.²⁷⁻²⁹

Since nearly all of the circulating CE are generated within the circulation by the action of LCAT,³⁰ it could be that this enzyme and the acquired decrease in its activity in CRF are important in the development and progression of kidney disease. Although there have been many reports of acquired LCAT deficiency in renal disease,¹⁹⁻²⁴ there appears to have been very little interest in this phenomenon in recent years. Indeed, at least one recent study has suggested that LCAT is normal in dialysis patients,³¹ but other recent studies also report low activity,^{32,33} and differences may actually reflect the employment of different methodologies used to measure the activity of this enzyme.

In some studies, the effects of hemodialysis on LCAT activity have been studied directly in blood samples taken before and immediately after a single dialysis session. Again, the results are also not always consistent between different studies. Thus, Chan et al.²¹ showed that LCAT activity in hemodialysis patients, already low, was further decreased by dialysis, and this effect was attributed to inhibition by fatty acids, the concentration of which increases because of heparinization.³⁴ Other studies indicate that the direct effect of hemodialysis has been to increase LCAT activity slightly when measured with the endogenous lipoproteins acting as substrates, but not with exogenous lipoprotein substrates.²³ In this latter study and in those of Bories et al.²² and Walker et al.,³¹ LCAT activity measured with endogenous lipoprotein substrates was not significantly different from controls. Although LCAT has not been measured in the present study, the presence of low levels of CE in patient plasma is indicative of decreased LCAT activity.²⁶ This is also supported by the observation that low CE was twice as frequent in predialysis CRF patients than in hemodialysis patients. Furthermore, when low CE patients were dialysed, there was an increase in CE, although in this small study the increase was not significant, nor was it known how long this increase in CE lasted after dialysis. Clearly, hemodialysis has an effect on CE metabolism and LCAT, but further studies with more patients will be needed to clarify this effect.

However, because LCAT has an important and central role in plasma lipoprotein metabolism, the effects of a significant drop in LCAT enzyme activity in CRF may be seen as a wide range of lipid and lipoprotein changes. These include not just decreases in the LCAT product lipids, CE and LPC, and increases in the reactant lipids, cholesterol and PC, but also increased triacylglycerols. In turn, these lipid changes reflect changes in composition

and/or concentration of different lipoprotein fractions and can also lead to alterations in lipid composition of membranes, such as in red blood cells.²⁴

The results of this preliminary study of CE and phospholipids in patients with renal disease appear to be sufficiently interesting to warrant a more extensive and detailed review of blood lipid status in CRF patients and apparently healthy control subjects drawn from the UAE population. Such a study should concentrate on some of the little-studied lipid parameters in CRF, including membrane lipids in erythrocytes and lymphocytes and actual measurement of LCAT activity using a method that does not rely on either endogenous or exogenous lipoproteins.

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