see commentary on page 779

Hemodialysis reduces plasma apolipoprotein C-I concentration making VLDL a better substrate for lipoprotein lipase

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Apolipoprotein Cs (apoC-1, apoC-II, and apoC-III) are lipoprotein components that have regulatory effects on enzymes involved in lipoprotein metabolism. Owing to their low molecular weights, apoCs can adsorb onto and/or pass through dialysis membranes. Our study determines the consequence of hemodialysis (HD) on plasma concentrations of apoCs and on the activities of enzymes modulated by apoCs. Plasma samples were collected from 28 patients with chronic renal failure before and after HD. Plasma apoC-II levels were unchanged, whereas apoC-III levels were slightly decreased in post-dialysis plasmas. The apoC-I content was markedly reduced during HD. This was due to a significant decrease in the apoC-I content of very low-density lipoprotein (VLDL), whereas the apoC-I content of high-density lipoprotein (HDL) was unchanged. Although HDL bound apoC-I is thought to inhibit cholesterol ester transfer protein, no change in the ability of pre- and post-dialysis VLDL to interact with the transfer protein were observed. Complementary experiments confirmed that VLDL-bound apoC-I has no transfer protein inhibitory potential. In contrast, an increase in the ability of post-dialysis apoC-I-poor VLDL to act as substrate for lipoprotein lipase (LPL) was found compared to pre-dialysis VLDL. Our study shows that apoC-I losses during HD might be beneficial by improving the ability of VLDL to be a substrate for LPL thus improving plasma triglyceride metabolism.

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Coronary artery disease is a major cause of mortality and morbidity in hemodialysis (HD) patients.[1,2] Among other risks factors, such as hypertension or diabetes, an altered lipoprotein metabolism is thought to contribute significantly to the accelerated development of atherosclerotic lesions in these patients.[3] The typical features of this dyslipidemia are elevated triglyceride (TG) concentrations and low high-density lipoprotein (HDL) cholesterol levels. In contrast, total and low-density lipoprotein (LDL) cholesterol levels usually remain in the normal range.[4–7] Although the etiology of the HD-associated dyslipidemia has not been fully established, alterations of several enzymes involved in lipoprotein metabolism are commonly observed. In particular, lipoprotein lipase (LPL) and hepatic lipase activities are significantly decreased.[8] As observed earlier in patients with hypertriglyceridemia[4] increased plasma TG level *per se* might constitute a major contributor to elevated cholesteryl ester transfer protein (CETP) activity in HD patients, and both HD *per se* and chronic renal failure would contribute in a complementary manner to these abnormalities.[4] During HD, heparin that is used as an anticoagulant to prevent clotting in the extra corporeal devices releases LPL from its binding sites at the vascular endothelium. It has been suggested that repeated heparinization induces a subsequent leakage of LPL that exceeds the rate of enzyme synthesis and thereby causes a depletion of LPL pool.[9–11] Besides lipase activities, increased CETP-mediated transfers of TGs from very low-density lipoprotein (VLDL) to LDL and HDL were also observed in HD patients. In the two latter cases, abnormalities were attributed mainly to the HD procedure, which led to both LPL loss and CETP inhibitor deficiency.[4,12] Elevated concentrations of apoC-III in VLDL is also known to constitute a prominent feature of dyslipidemia through LPL inhibition in patients with chronic renal feature.[13,14] In this case, lipid abnormalities should be considered as a reflect of chronic renal failure and not a consequence of the HD treatment.

ApoCs, including apoC-I, apoC-II, and apoC-III, are low molecular weight apolipoproteins, and they are components of chylomicrons, VLDL, and HDL where they exert specific

biological functions. In contrast to apoC-III, the metabolism of apoC-I and apoC-II in HD has not been investigated in an extensive manner. Nevertheless, alterations in apoC-I and apoC-II levels in HD are important to consider because of their potent inhibitory or stimulatory effects on a variety of receptors and enzymes involved in lipoprotein metabolism. ApoC-I inhibits the uptake of TG-rich lipoproteins via hepatic receptors, particularly the LDL receptor-related protein.[15,16] In addition, ApoC-I is a weak activator of lecithin cholesterol acyl transferase,[17,18] and it has been reported to inhibit plasma phospholipase A2,[19] CETP,[20] LPL, and hepatic lipase.[21,22] ApoC-II is a physiological activator of LPL and as such it is required for efficient lipolysis of TG-rich lipoproteins in the circulation.[23] Although mostly associated with circulating lipoprotein particles, apo Cs are able to transfer freely between lipoprotein particles through aqueous diffusion,[24] and due to their low molecular weight, they can diffuse through and/or absorb on dialysis membranes. ApoC leakage during the dialysis procedure might then contribute to alterations in lipoprotein metabolism in HD patients.

The aim of this study was to assess, in a comprehensive way, the influence of HD *per se* on apoCs and to evaluate the consequences on enzymes activities and plasma lipids. It is reported that the plasma apoC-I concentration is mostly modified in HD patients with chronic renal failure, resulting from a significant loss of VLDL-associated apoC-I during HD. Whereas pre- and post-dialysis VLDL did not differ in their ability to interact with CETP, a significant increase in the ability of post-dialysis, apoC-I-deprived VLDL to act as substrate for LPL was observed, suggesting an additional, yet unrecognized benefit of HD.

RESULTS

Effects of HD on biochemical parameters

Biochemical parameters for the 28 patients are given in Table 1. Before HD, urea and creatinine levels in plasma were 250 and 800% above the normal physiological range, respectively. As expected, the 4-h dialysis procedure induced an approx. 70% decrease in urea and creatinine concentrations. Total protein concentration in plasma was increased by 10% after HD ($P<0.001$ Wilcoxon), reflecting the hemoconcentration that is induced by fluid losses.

As compared with normal values, total cholesterol, HDL cholesterol, and apo A-I concentrations were in the normal physiological range in patients before HD, whereas the mean TG concentration was increased (Table 1). Interestingly, all the plasma lipid parameters were increased in similar proportions (approx. 10%) after HD ($P<0.001$ Wilcoxon), and levels of one given parameters before and after HD were remarkably similar when values were corrected for variation in total protein concentration (Table 1). Overall, these data indicate that the dialysis procedure *per se* has no selective impact on standard lipid parameters, and concomitant 10% increases in total cholesterol, TGs, HDL cholesterol, and total proteins after HD were actually due to hemoconcentration.

Apolipoprotein C concentrations before and after HD

Plasma concentrations of apolipoproteins C-I, C-II and C-III were determined before and after HD by a specific enzyme-linked immunosorbent assay (ELISA) (apoC-I) or by immunoturbidimetry (apoC-II and apoC-III). Values were expressed either as apolipoprotein concentration or as apolipoprotein to total protein ratio in order to take into account the hemoconcentration phenomenon. Whereas apoC-II concentrations were significantly increased after HD (Table 2, Figure 1), no significant change was observed when values were corrected for hemoconcentration. In contrast, although no significant changes in the apoC-III concentration were observed before and after HD, a slight, significant decrease appeared when values were corrected by hemoconcentration, indicating a significant loss of apoC-III during dialysis. Apo C-I concentrations decreased readily after dialysis and magnitude of changes was even greater when corrected for hemoconcentration with approximately a 20% decrease as compared with values before HD ($P<0.0001$). Concentrations of apo Cs (apo C-I, apo C-II, apo C-III) did not differ between two groups of patients with ($n=9$) or without ($n=19$) hypolipidemic medication

Table 1 | Biochemical and plasma lipid parameters in pre- and post-dialysis plasmas

	Before hemodialysis	After hemodialysis	Normal range
Urea (mmol/l)	25±5.8	7.92±2.44[a]	3.2–7.5
Creatinine (μmol/l)	926±185.4	355±95.9[a]	71–133
Protein (g/l)	69±7.1	79±10.8[a]	63–82
Total cholesterol (mmol/l)	4.28±1.05	4.69±1.06[a]	3.1–6.2
Total cholesterol/protein (μmol/g)	61.1±13.0	60.5±13.9	
Triglycerides (mmol/l)	2.04±1.13	2.26±1.14[a]	0.30–1.60
Triglycerides/protein (μmol/g)	29.6±15.8	28.8±14.3	
HDL cholesterol (mmol/l)	1.06±0.28	1.22±0.34[a]	0.90–1.60
HDL cholesterol/protein (μmol/g)	15.4±4.2	15.6±4.4	
Apolipoprotein A-I (g/l)	1.03±0.20	1.19±0.33[a]	1.10–1.80
Apo A-I/protein (mg/g)	14.84±2.80	15.15±3.75	

HDL, high-density lipoprotein.
Blood samples were collected immediately before and after hemodialysis. Urea, creatinine, and total protein were measured on Vitros 950 analyzer. Total cholesterol, triglycerides, apolipoprotein A-I, and HDL cholesterol were measured on a Dimension X-pand analyzer as described in 'Materials and methods'. Statistics by Wilcoxon test.
[a]$P<0.0001$ vs before hemodialysis.

Table 2 | Apolipoprotein C concentrations in pre- and post-dialysis plasmas

	Before hemodialysis	After hemodialysis
Plasma apo C-I (mg/l)	137.7±58.4	124.4±46.5[a]
Plasma apo C-I (mg/g prot)	2.03±0.97	1.60±0.64[b]
Plasma apo C-II (mg/l)	36.1±10.7	39.5±12.3[a]
Plasma apo C-II (mg/g prot)	0.53±0.17	0.50±0.14
Plasma apo C-III (mg/l)	176±49	180±51
Plasma apo C-III (mg/g prot)	2.59±0.79	2.29±0.61[a]
HDL apo C-I (mg/l)	19.2±5.4	22.0±6.2[a]
HDL apo C-I (mg/g apo A-I)	18.3±4.9	18.3±4.5
HDL apo C-III (mg/l)	107±38	103±39
HDL apo C-III (mg/g apo A-I)	103±36	85±33
VLDL apo C-I (mg/l)	87.7±58.7	58.0±52.6[a]
VLDL apo C-I (mg/g apo B)	187±113	104±101[b]
VLDL apo C-III (mg/l)	91±52	133±87
VLDL apo C-III (mg/g apo B)	217±80	205±134

HDL, high-density lipoprotein; VLDL, very low-density lipoprotein.
Blood samples were collected immediately before and after hemodialysis. HDL and VLDL were isolated by sequential ultracentrifugation. Apolipoprotein C-I concentrations were determined by a specific ELISA. Apo A-I, Apo B, ApoC-II, and apoC-III concentrations were determined by immunoturbidimetric assays on a Dimension X-Pand.
[a]$P<0.01$ vs before hemodialysis.
[b]$P<0.001$ vs before hemodialysis.

(predialysis: 1.72±0.86 vs 2.21±1.00 mg apo C-I/g protein, respectively; NS. Post-dialysis: 1.42±0.62 vs 1.67±0.65 mg apo C-I/g protein, respectively; NS). When patients with an hypolipidemic medication were excluded, a significant decrease in apoC-I after HD was still observed (2.21±1.00 mg apo C-I/g protein before dialysis vs 1.67±0.65 mg apo C-I/g protein after dialysis; $P<0.01$), indicating that differences in the whole population studied, before and after dialysis, were independent of treatments.

HD reduced VLDL-bound but not HDL bound apoC-I

In the plasma compartment, ApoC-I is mainly bound to HDL, and to a lesser extent to VLDL. In order to determine whether HD has a differential impact on ApoC-I contents of HDL and VLDL, both lipoprotein subclasses were isolated and apoC-I was determined in each fraction by ELISA. As shown, in Figure 2, the apoC-I content of HDL (expressed as apoC-I to apo A-I ratio) was the same before and after HD, whereas the apoC-I content of VLDL (expressed as apoC-I to apo B ratio) was markedly decreased (by 30%), as compared with values before HD (Figure 3). This was confirmed by polyacrylamide gradient gel electrophoresis and Western blot analyses, showing a marked reduction in VLDL apoC-I after HD (Figures 4a and b). ApoC-III in VLDL and HDL was also determined in pre- and post-dialysis plasmas. As shown in Table 2, slight decreases of the apoC-III concentrations were observed in both VLDL and HDL but differences did not reach the statistical significance. Time course analysis of the apo C-I content of VLDL was performed in patients undergoing HD at times 0, 2, and 24 h after initiation of the dialysis session. Again, a significant, 20% decrease in the apo C-I content of VLDL was found 2 h after initiation of HD, with normalization of the apo C-I levels after 24 h (Figure 5).

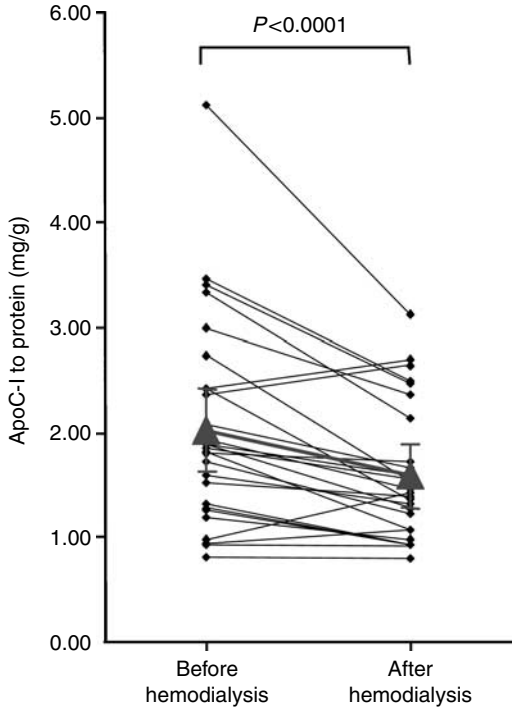

Figure 1 | ApoC-I content in total blood plasma before and after HD. Blood samples were collected immediately before and after HD. Apolipoprotein C-I concentrations were determined by a specific ELISA as described in 'Materials and Methods'. Results are expressed as apoC-I to total protein ratio. Values are mean±s.d. (bold type). *$P<0.01$ Wilcoxon test.

CETP mass concentration and activity before and after HD

As apoC-I was recently identified as a major physiological inhibitor of CETP, we determined whether CETP activity was modified by HD. As shown in Table 3, whereas CETP mass concentration was significantly increased after HD, no changes in CETP activity or CETP mass concentration were observed in total plasma before and after dialysis when corrected for hemoconcentration.

Effect of apoC-I on the ability of VLDL to act as substrates for CETP

As HD decreased VLDL-apoC-I but not HDL-apoC-I, we directly measured the ability of pre- and post-dialysis VLDL to act as substrate for CETP. As shown in Figure 6, the rate of exchange of cholesteryl esters was similar with pre- and post-dialysis VLDL. These data suggest therefore that unlike HDL apoC-I,[20] VLDL apoC-I is not a major determinant of their ability to interact with CETP. In order to confirm further that the CETP inhibitory potential was lost when apoC-I is associated with VLDL, CETP inhibition was measured at different apoC-I to human VLDL ratio. In the presence of constant amounts of apoC-I, but increasing amounts of VLDL, apoC-I inhibited CETP only at very high, extra physiological ratio (apoC-I to TG molar ratio 0.06 to 0.03), whereas at lower, physiological apoC-I to VLDL ratios (apoC-I to TG molar ratio below 0.015) no CETP blockade occurred (Figure 7).

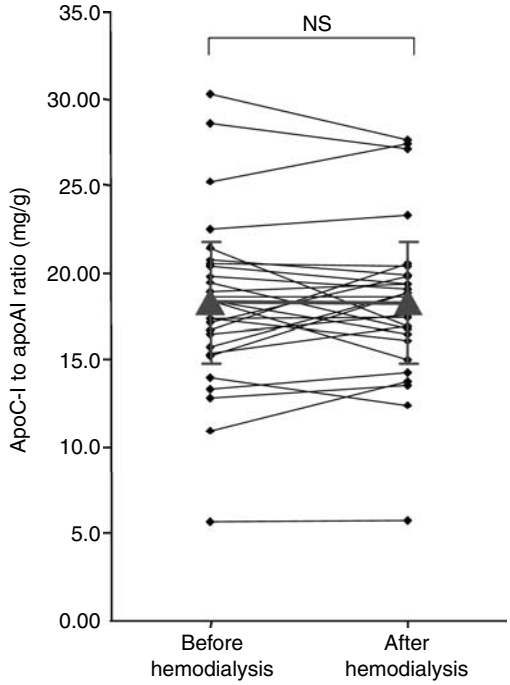

Figure 2 | ApoC-I content of HDL before and after HD. Blood samples were collected immediately before and after HD, and HDL were isolated by sequential ultracentrifugation as described in 'Materials and Methods'. Apolipoprotein C-I concentrations were determined by a specific ELISA as described in 'Materials and Methods'. Results are expressed as apoC-I to apoA-I ratios. Values are mean ± S.D. (bold type). NS: no significant differences by Wilcoxon test.

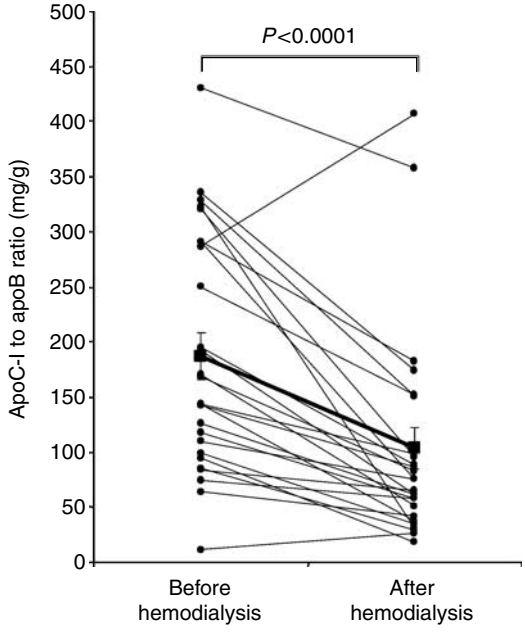

Figure 3 | ApoC-I content of VLDL before and after HD. Blood samples were collected immediately before and after HD, and VLDL were isolated by sequential ultracentrifugation as described in 'Materials and Methods'. Apolipoprotein C-I concentrations were determined by a specific ELISA as described in 'Materials and Methods'. Results are expressed as apoC-I to apoB ratio. Values are mean ± s.d. (bold type). *$P < 0.01$ Wilcoxon test.

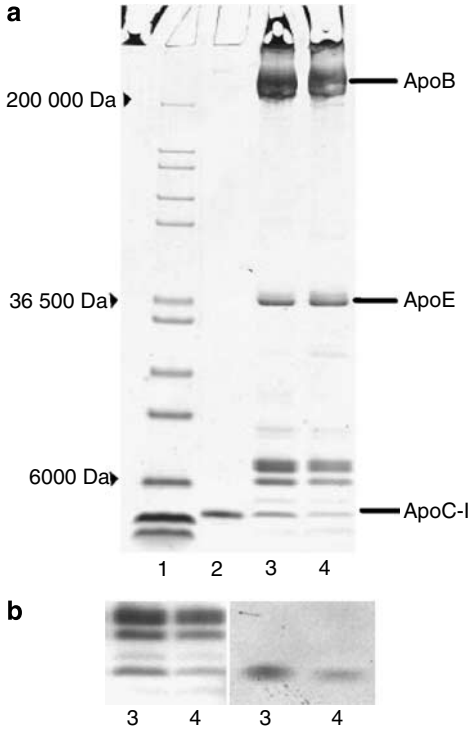

Figure 4 | Apolipoprotein composition of pre- and post-dialysis VLDL. VLDL were isolated by sequential ultracentrifugation from pre- or post-dialysis plasmas, and their apolipoprotein contents were analyzed on an sodium dodecyl sulfate-polyacrylamide gradient gel (**a**) by coomassie staining or (**b**) by Western blot analysis using anti apolipoprotein C-I antibodies. 1: Molecular weight markers, 2: purified apoC-I, 3: pre-dialysis VLDL, 4: post-dialysis VLDL.

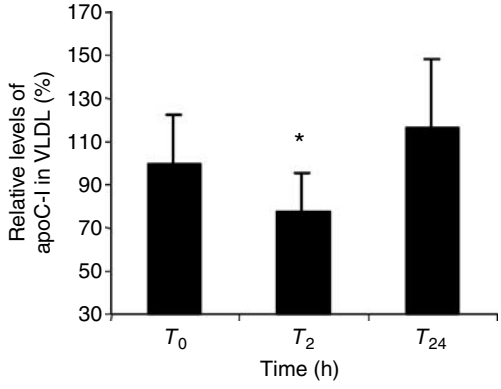

Figure 5 | Time course analysis of the apo C-I content of VLDL. Blood samples were collected before ($t = 0$) and after the dialysis session (2 and 24 h). VLDL were isolated by sequential ultracentrifugation, and apolipoprotein C-I concentrations were determined by a specific ELISA as described in 'Materials and Methods'. ApoC-I level in VLDL was compared to 100%, $t = 0$ controls. Values are mean ± s.d. *$P < 0.01$ *vs* T0; Wilcoxon test.

Post-dialysis VLDL is better substrate for LPL than pre-dialysis VLDL

Besides CETP, apoC-I is able to modulate the activity of several enzymes involved in lipoprotein metabolism. In particular, recent studies have demonstrated that the

Table 3 | CETP activity and mass concentration in pre- and post-dialysis plasmas

	CETP activity fluorescence a.u.	Specific CETP activity (fluorescence/mg CETP)	CETP mass (mg/l)	CETP to total protein ratio (μg/g protein)
Before hemodialysis	2444±486	1228±189	2.04±0.43	29.8±5.9
After hemodialysis	2539±496	1143±214	2.28±0.51[a]	28.6±7.5

Blood samples were collected immediately before and after hemodialysis. CETP activity and mass were measured as described in Materials and Methods. Statistics by Wilcoxon test.
[a]$P<0.0001$ vs before hemodialysis.

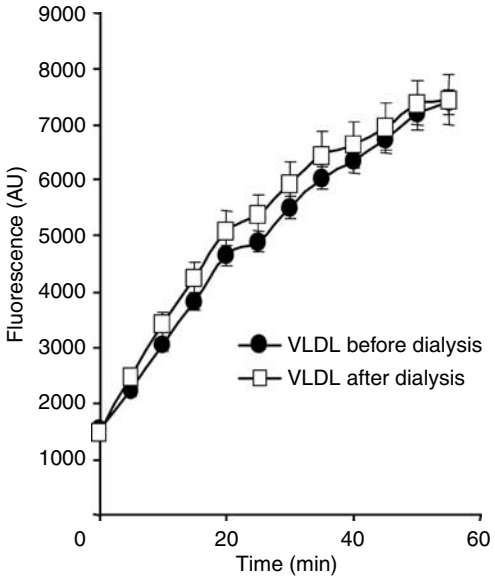

Figure 6 | Comparison of the ability of pre- or post-dialysis VLDL to interact with CETP. VLDL were isolated by sequential ultracentrifugation from pre- or post-dialysis plasmas. Cholesteryl ester transfer activity was determined as the rate of transfer of fluorescent NBD-cholesteryl esters from labeled liposome donors to HDL acceptors in the presence of a purified CETP fraction (final protein concentration, 8 μg) along a 3-h incubation period at 37°C (see 'Materials and Methods'). Blank values were obtained with homologous, incubated mixtures to which no purified CETP was added. Values are mean±s.d. of three determinations. No significant differences were observed by Wilcoxon test.

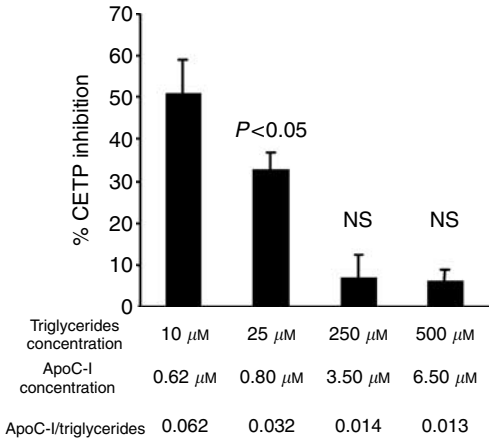

Figure 7 | Effect of VLDL concentrations on the CETP inhibitory potential of apoC-I. Cholesteryl ester transfer activity was determined as the rate of transfer of fluorescent NBD-cholesteryl esters from labeled liposome donors (phospholipids, 5 μmol/l) to VLDL acceptors (range, 10–500 μmol/l) in the presence of purified CETP (8 μg) in a final volume of 200 μl. Incubations were conducted for 3 h at 37°C in the absence or in the presence of purified apoC-I (concentration, 0.5 μmol/l). ApoC-I to TGs ratio was calculated by taking into account both the quantity of purified apo C-1 added to the incubation media (0.5 μM) and the VLDL-associated apoC-I (0.012 mol apo C1/mole TG). Percentage of CETP inhibition (vertical bars) was calculated by comparing the initial transfer rate in the presence of apoC-I to the initial transfer rate with no apoC-I added. Transfer rates were determined from the linear, initial portion of the time course curves. Values are mean±s.d. of three determinations. *Significantly different from mixtures containing 0.5 μmol/l of apoC-I, $P<0.05$; Mann–Whitney test.

potential hyperlipidemic effect of apoC-I overexpression was linked to its ability to inhibit LPL.[22] Therefore, we measured the ability of pre- and post-dialysis VLDL to act as substrate for LPL by an *in vitro* assay. LPL activity was expressed as the amount of free fatty acids (FFA) released from VLDL incubated with purified LPL. As shown in Figure 8, a significant, twofold increase in FFA release was observed with post-dialysis VLDL as compared with pre-dialysis VLDL ($P<0.01$). These data indicate therefore that, unlike for CETP activity, the association of apoC-I with VLDL in proportions found *in vivo* (apoC-I to TG molar ratio of approximately 0.01) has the ability to modify the interaction of VLDL with LPL.

DISCUSSION

Most of dialysis membranes are permeable to small size proteins and significant losses of small proteins such as β2 microglobulin (MW = 11 800 Da) are known to occur during HD.[25,26] As compared with β2 microglobulin, apolipoprotein Cs are even smaller proteins (molecular weight ranging from 6500 to 8500 Da). In addition, and despite their amphipatic nature and their association with large size lipoproteins, apo Cs are able to exchange freely between lipoprotein particles by diffusion through the aqueous phase.[24] Overall, it appears that apoCs are most likely to pass easily through and/or to adsorb on dialysis membranes and then to be lost partly during HD. The latter point was specifically addressed in this study by measuring plasma apoC content before and after HD of patients with chronic renal failure. Among apolipoprotein Cs, plasma apoC-I concentration underwent the most pronounced decrease during the HD procedure, with only a slight decrease in apoC-III and no change in apoC-II. In addition, levels of apolipoproteins with higher molecular weight, such as apo A-I remained unchanged after HD. Interestingly, it has been demonstrated that the adsorption/filtration of proteins during HD is dependent not only on

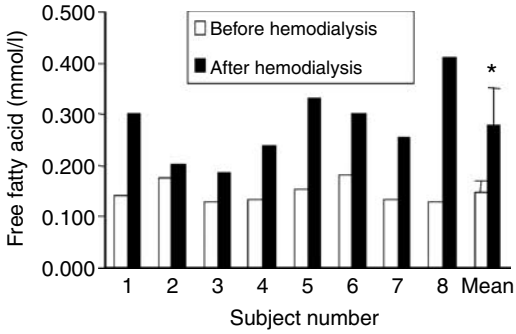

Figure 8 | Comparison of the ability of pre- or post-dialysis VLDL to act as substrates for LPL. VLDL were isolated by sequential ultracentrifugation from pre- or post-dialysis plasmas. VLDL (60 nmol TGs/assay) were incubated in the presence of purified LPL (purified from bovine milk, Sigma) in 10 μl Tris-buffer (0.1 M Tris, pH 8.5) at 37°C for 6 min. The reaction was stopped by the addition of Triton X-100, vortexing and cooling on ice. A blank sample was obtained in parallel by adding Triton before the addition of LPL and maintenance on ice. Aliquots of the samples were used to determine FFA concentrations in triplicate using an enzymatic assay. LPL activity was expressed as the amount of FFA released from VLDL during the incubation period. Values are mean $\pm$ s.d. $P<0.05$ Wilcoxon test.

protein size but also on electrostatic interactions between negative charges of dialysis membranes and positively charged amino-acid residues.[25,27] As compared with other apoCs, apoC-I displays the lowest molecular weight (6500 Da) and the highest isoelectric point (pHi, 8.5) due to the presence of a cluster of lysine residues in its C-terminal region.[28] Therefore, it is plausible that the higher propensity of apoC-I to be cleared during HD is linked to these unique physicochemical properties. In support of this view, Ishikawa *et al.* have recently identified apolipoprotein C-I as a major protein adsorbed on polysulfone membranes during HD. This would suggest that adsorption on the dialysis membrane, rather that direct clearance is the primary mechanism accounting for the apo C-I decrease during HD.[29]

Interestingly, the extent of apoC-I loss during HD seems to be dependent on its localization among circulating lipoproteins. Indeed, plasma apoC-I was significantly decreased (by approximately 30%) only in the VLDL fraction after HD, whereas in the meantime HDL-apoC-I levels remained constant. Although the molecular mechanism accounting for the difference in VLDL-bound and HDL-bound apoC-I to undergo filtration during HD is unclear, it might relate in some way to marked differences in half-life and stability of VLDL and HDL classes in the blood stream. Indeed, and as assessed earlier through kinetic studies, mean residence time of HDL in human plasma (a few days) is considerably longer than mean residence time of VLDL (a few hours).[30,31] High turnover of VLDL is due to fast TG hydrolysis, which leads sequentially from VLDL to intermediate density lipoprotein, and then to LDL. Interestingly, rapid VLDL lipolysis and associated lipid core shrinkage produce, among other events, the release of loosely bound, surface components, among them apoprotein Cs.[32] In the end, the dissociated, lipoprotein-free apoC-I is likely to be more prone to filtration through dialysis membranes than HDL-bound apoC-I. In addition, it must be emphasized that the extent of apoC-I release from the VLDL surface might well have been increased by heparin injection during HD. Indeed, heparin injection is known to disrupt the anchoring of LPL at the vessel wall, thus increasing lipolysis activity and, as a consequence, apoC-I release from the VLDL surface.

Recently, apoC-I was identified as a physiological inhibitor of CETP through its ability to modify the electrostatic charge properties of circulating HDL.[33] In this study, HDL apoC-I content did not differ before and after HD, and this was accompanied with no change in CETP mass and activity in total plasma when corrected for hemoconcentration. In contrast, post-dialysis VLDL was shown to contain fewer apoC-I molecules than pre-dialysis VLDL, but again in the absence of changes in CETP activity (Figure 6). The latter point might well reflect the inability of physiological levels of apoC-I to produce significant changes in the electrostatic charge properties of VLDL, which are very large, lipid-rich/protein-poor, and slow-migrating lipoproteins as compared with HDL. In other words, apoC-I would be a potent inhibitor of CETP when bound to HDL (as shown in earlier studies from our group[20,33]), but with no inhibitory potential when bound to the large, lipid-rich VLDL. In direct and strong support of the latter view, we observed through *in vitro* manipulations that VLDL-bound apoC-I had the ability to inhibit CETP activity only at high levels, which went beyond the range of apoC-I to VLDL ratio that is observed in humans. This study indicates than HD would actually produce a selective decrease only in the inactive form of apoC-I (i.e. VLDL-bound apoC-I), and not in the inhibitory, HDL-bound apoC-I fraction, thus resulting in no change in plasma CETP activity.

In addition to its role in CETP activity modulation, apoC-I was found in previous studies to constitute a significant inhibitor of LPL activity.[22] *In vitro* enrichment of VLDL-like particles with apoC-I induces a concentration-dependent inhibition of the LPL-mediated FFA release.[22] In addition, and in support of the physiological relevance of this function, recent studies demonstrated that the hyperlipidemia observed in apoC-I transgenic mice was mainly due to an apoC-I-mediated inhibition of LPL activity.[22] Interestingly, a significant increase in the ability of VLDL to act as substrate for LPL was observed in this study after HD, and the mean 35% increase in LPL activity after HD was of similar magnitude than the concomitant 40% decrease in apoC-I content of VLDL. Beyond earlier studies, which were conducted either *in vitro* or *in vivo* in apoC-I-KO or apoC-I transgenic mouse models, this study brings the first, direct evidence for apoC-I abnormalities in a population of patients undergoing HD. Fewer amounts of apoC-I in VLDL, as observed in the patients with chronic renal failure immediately after HD, would secondarily lead to a significant rise in the ability of these particles to act as substrate for LPL. Finally, apoC-I loss might contribute to face the reduction in LPL activity and the accumulation of TG-rich lipoproteins

that are major components of chronic renal failure.[34] In addition, it can be postulated that regular apoC-I clearance by HD might contribute to the reduction in triglyceridemia that was reported to occur in dialyzed patients as compared with undialyzed patients.[35] The impact of decrease in VLDL-apoC-I levels on TG metabolism and cardiovascular disease in high-risk patients with chronic renal failure will deserve further attention in longitudinal studies of larger populations of patients.

MATERIALS AND METHODS

Patients

Patients (14 females and 14 males; 61.2 ± 14.4 years old) were recruited at the Hemodialysis Center of the University Hospital of Dijon (CHU Dijon, France). Four patients (two females and two males, 59.5 ± 12 years old) were recruited at the Department of Nephrology, Dialysis, and Renal Transplantation, CHU Saint Jacques, Besançon. A written informed consent was obtained from each subject before the study. All the subjects were suffering from chronic renal failure, and they underwent 4-h sessions of membrane HD two or three times a week. The mean time since the beginning of HD was 5.5 ± 4.8 years. Twenty-five percent of the patients were diabetics and received insulin or oral antidiabetic drugs. Nine patients received hypolipidemic drugs. Venous blood samples were collected just before, and immediately after HD. Plasma was centrifugated at low speed and stored at -80°C until analysis. All the patients were heparanized. Dialysis was conducted by using an FX8 membrane (Fresenius Medical Care, Bad Homburg Germany; ultrafiltration coefficient = 12) ($n = 10$), an FX60s membrane (Fresenius Medical Care, ultrafiltration coefficient = 40) ($n = 4$), or a Nephral 300 or 400 ST membrane (Hospal, Lyon, France; ultrafiltration coefficient = 40 or 50) ($n = 22$). No differences were observed between these groups, with similar evolution of lipid and apoprotein concentrations before and after dialysis. Therefore, results combining data from these groups are presented in this paper.

Biochemical and lipid analysis

Urea, Creatinine, and total protein concentrations were measured on a Vitros 950 analyzer using colorimetric or enzymatic assays following the procedures provided by the manufacturer (Ortho-clinical diagnostic, Issy les moulineaux, France). Total cholesterol, HDL cholesterol, and TGs were measured by enzymatic methods on a Dimension X-Pand analyzer (Dade Behring, Paris, France) Apolipoprotein B, A-I, C-II, and C-III concentrations were measured by commercial immunoturbidimetric assays following the procedure provided by the manufacturers (apo A-I and apo B: Dade-Behring, apoC-II, and ApoC-III: Diasys, Bouffemont, France).

Cholesteryl ester transfer protein mass concentration and activity

CETP mass levels were determined by a specific immunoassay with TP2 anti-CETP monoclonal antibodies.[36] Purified CETP was obtained from human plasma by using a sequential chromatography procedure.[20] Cholesteryl ester transfer activity was determined as the rate of transfer of fluorescent 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)-cholesteryl esters from labeled liposome donors (phospholipids, 5 μmol/l) to VLDL acceptors (range, 10–500 μmol/l) in the presence of purified CETP (8 μg) in a final volume of 200 μl. Incubations were conducted for 3 h at 37°C in the absence or in the presence of purified apoC-I (concentration, 0.5 μmol/l).[33] ApoC-I to TG ratios were calculated by taking into account both the amount of purified apoC-I added to the incubation media (0.5 μmol/l), and the VLDL-associated apoC-I (0.012 mol apoC-I/mole TG). Percentage of CETP inhibition was calculated as the ratio of transfer rates in the presence of apoC-I to corresponding transfer rates with no apoC-I added. Transfer rate values were determined from the linear, initial portion of the time course transfer curves.

Determination of apoC-I concentration by ELISA

Competitive ELISA for apoC-I was set up according to the general procedure used in our laboratory to quantify apoA-IV.[36] Polyclonal antibodies to human apoC-I were raised in a rabbit injected four times with 250 μg of human apoC-I that had been purified to homogeneity as described previously.[20] The absorbance was measured at 490 nm. Eight dilutions (apoC-I concentrations from about 0.0024 to 27.4 mg/l) were used to draw a calibration curve for each plate.

Preparation of VLDL by ultracentrifugation

VLDL were ultracentrifugally isolated from patient plasmas as the $d < 1.006$ fraction, with two 1.40-h, 120 000-r.p.m. spins in a 70.Ti rotor in an L90-K ultracentrifuge (Beckman, Palo Alto, CA, USA). Apo B content of the VLDL was determined by an immunoturbidimetic assay on a Dimension X-Pand analyzer.

LPL activity

The ability of VLDL to act as a substrate for LPL was determined as described previously.[37] Briefly, VLDL (60 nmol TGs/assay) were diluted in 0.1 M Tris buffer (pH 8.5), containing 1% (w/v) fatty acid-free bovine serum albumin (final volume 100 μl). The incubation was started by adding 0.84 μl LPL (purified from bovine milk, Sigma, Saint Louis, MI, USA) in 10 μl Tris-buffer (0.1 M Tris, pH 8.5) to 100 μl VLDL-TG, followed by incubation at 37°C. The reaction was stopped after 6 min by the addition of Triton X-100 (1% (v/v), final concentration), vortexing and cooling on ice. A blank sample was obtained in parallel by adding Triton before the addition of LPL and it was maintained on ice. Finally, FFA concentrations were determined in triplicate using an enzymatic assay (NEFA-C Wako, Osaka, Japan).

Apolipoprotein analysis by polyacrylamide gradient gel electrophoresis

VLDL apolipoproteins were delipidated with ethanol/ether (3:2), diluted in the sample buffer containing a reducing agent and applied on 4–12% NuPage Bis Tris Novex sodium dodecyl sulfate-polyacrylamide gels as recommended by the manufacturer (Invitrogen, Cergy Pontoise, France). Proteins were stained with Coomassie Brilliant Blue G-250, and the apparent molecular weights of individual protein bands were determined by reference to protein standards (Mark12, Invitrogen).

Western blot analysis of apolipoprotein C-I in VLDL

Samples were applied on 4–12% NuPage Bis Tris Novex sodium dodecyl sulfate-polyacrylamide gels as described above and were further transferred to a nitrocellulose membrane. The resulting blots were blocked overnight at 4°C with 10% low fat dried milk in Tris-buffered saline containing 0.1% Tween, and washed with

Tris-buffered saline-Tween. The blots were incubated successively with polyclonal anti-apoC-I antibodies and with peroxidase-conjugated anti-goat antibodies (Sigma) as described previously. Blots were finally developed using an ECL kit[20] (GE Healthcare, Uppsala, Sweden).

Statistical analyses

Wilcoxon test was used to determine the significance between the data means.

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