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Endothelial Lipase Activity Predicts High-Density Lipoprotein Catabolism in Hemodialysis

Novel Phospholipase Assay in Postheparin Human Plasma

Veronica Miksztowicz, Mary G. McCoy, Laura Schreier, Leonardo Cacciagiú, Alicia Elbert, Ana Inés Gonzalez, Jeffrey Billheimer, Patrick Eacho, Daniel J. Rader, Gabriela Berg

Objective—A novel phospholipase assay was used to measure for the first time the behavior of endothelial and hepatic phospholipase activities in postheparin human plasma of hemodialyzed patients and its relationship with atherogenic and antiatherogenic lipoprotein levels.

Methods and Results—Endothelial and hepatic phospholipase activity was assessed in a total SN1-specific phospholipase assay, using (1-decanoylthio-1-deoxy-2-decanoyl-sn-glycero-3-phosphoryl) ethylene glycol as the substrate. Hemodialyzed patients presented lower values of total and hepatic phospholipase activity than controls: 4.4 (1.9–9.0) versus 7.5 (3.6–18.0) and 2.6 (0.7–6.2) versus 6.6 (1.3–15.2) μmol of fatty acid released per milliliter of postheparin plasma per hour, respectively ($P < 0.001$); however, endothelial lipase (EL) phospholipase activity was increased in patients: 1.7 (0.8–3.0) versus 1.1 (0.1–2.7) μmol of fatty acid released per milliliter of postheparin plasma per hour ($P = 0.008$). EL was negatively associated with high-density lipoprotein (HDL)-cholesterol ($r = -0.427$; $P = 0.001$), and apolipoprotein A-I levels, total phospholipase, and hepatic lipase activity were directly associated with low-density lipoprotein-cholesterol and apolipoprotein B. The association of EL and HDL-cholesterol remained significant when adjusting for waist circumference ($\beta = -0.26$; $P = 0.05$), and the effect of hepatic lipase on low-density lipoprotein-cholesterol continued after adjusting for age ($\beta = 0.46$; $P = 0.001$).

Conclusion—Our results support the hypothesis that EL is the predominant enzyme responsible for lipolytic catabolism of HDLs in hemodialyzed patients and resolve the apparent paradox observed between low hepatic lipase activity and decreased HDL-cholesterol levels observed in these patients. In addition, the ability to assess total hepatic lipase and EL phospholipase activity in plasma will increase our knowledge of the mechanisms involved in controlling HDL levels and cardiovascular risk in hemodialyzed patients, as well as other populations with low levels of HDL-cholesterol. (*Arterioscler Thromb Vasc Biol.* 2012;32:3033-3040.)

Key Words: chronic kidney disease ■ endothelial lipase ■ hepatic lipase ■ high-density lipoprotein-cholesterol ■ phospholipase activity

Chronic kidney disease (CKD) is widely accepted as a risk factor for cardiovascular disease and mortality.^{1–3} Patients undergoing hemodialysis (HD) present higher prevalence of dyslipidemias,⁴ accelerated atherosclerosis, and higher risk of death because of cardiovascular disease than the general population.^{1,5}

Lipoprotein abnormalities associated with CKD are characterized by hypertriglyceridemia (with high levels of very-low-density lipoprotein and the presence of remnant lipoproteins), low levels of low-density lipoproteins (LDL), and low levels of high-density lipoproteins (HDL).^{6,7} In addition to changes in the concentration, the lipoproteins are often modified, such as LDL enriched in triglycerides (TG)

and small-dense LDL.^{6,8} This pattern is, in part, a consequence of the altered metabolism of these lipoproteins associated with the insulin-resistant (IR) state frequently linked to CKD.⁹ Currently, it is known that IR develops simultaneously with the decrease of glomerular filtration, even in the early stages of CKD.⁹ However, independent mechanisms of IR may also be involved in the lipoprotein metabolism in patients with CKD.

The enzymes endothelial lipase (EL) and hepatic lipase (HL) are primarily responsible for the metabolism of lipoproteins. Both enzymes are members of an extracellular lipase family, which also includes lipoprotein lipase (LPL).¹⁰ The 3 enzymes are heparin-binding lipases anchored to the

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endothelial surface and mediate the hydrolysis of TG and phospholipids (PL) at the SN1 position within circulating lipoproteins, with resultant structural changes leading to their removal from the plasma. Although the 3 lipases have both TG and PL lipase activity, LPL is predominantly a TG lipase, EL hydrolyzes principally PL, and HL has an intermediate TG and PL lipase activity. Both HL and EL are involved in HDL metabolism,¹¹ and HL is also responsible for the metabolism of apolipoprotein B (apoB)-containing lipoproteins (especially intermediate-density lipoprotein and LDL).¹² Higher concentrations of EL or HL are associated with lower levels of high-density lipoprotein-cholesterol (HDL-C).^{13,14}

Recently, the inverse relationship between HL and HDL-C levels was shown to be primarily in the HDL₂ subfraction, confirming the involvement of HL in converting lipid-rich HDL₂ to smaller, more dense HDL₃.¹⁰ HL has been previously evaluated in HD patients,^{6,15} and it has been demonstrated that both HL mass and HL TG lipase activity are reduced in HD patients, although the HL phospholipase activity was not determined.⁶

The overexpression of EL results in reduced HDL-C and apolipoprotein A-I (apoA-I) in mice¹⁶ and is inversely correlated with HDL-C in humans.¹⁷ In the only study of EL in HD patients, Fujii et al¹⁸ found that serum EL concentration was significantly higher and HDL-C levels were lower in CKD patients with low serum albumin and high C-reactive protein (CRP) levels, than in those without these abnormalities. Although EL mass has been determined in some studies, to our knowledge EL activity has not been analyzed. EL is a complex glycoprotein that is active as a homodimer. N-glycosylation has been shown to affect EL-mediated PL hydrolysis.¹⁹ EL is also inactivated upon cleavage by proprotein convertases.²⁰ In addition, ANGPTL3 has been shown to inactivate plasma EL.²¹ In addition, HD patients present uremic toxin molecules in plasma, such as low-molecular-weight advanced glycation end products and acrolein, which have been reported to modify enzymatic activities.²² Furthermore, previous studies have shown that uremic plasma has LPL and HL activity inhibitors,^{23,24} so it is important to evaluate lipolytic enzyme activities directly related to lipoprotein levels.

Our aim was to use a novel phospholipase assay to assess for the first time the relationship of EL and HL phospholipase activities in postheparin human plasma of HD patients and its relationship with atherogenic and antiatherogenic lipoprotein levels.

Materials and Methods

Subjects

Thirty-six patients with end-stage renal disease receiving maintenance HD, who were referred to the dialysis center Kidney Disease Center and Arterial Hypertension (CEREHA), Buenos Aires, Argentina, were selected consecutively for the present study. Six experienced type 2 diabetes mellitus, 3 had glomerulopathies, 6 had polycystic kidney disease, 2 had nephrosclerosis secondary to hypertension, 1 had obstructive nephropathy, 2 had tubulointerstitial nephropathy, 1 had lupus nephritis, and 15 were of unknown cause. In parallel, 34 subjects recruited among hospital employee volunteers were selected as controls. The following exclusion criteria were considered for both groups: liver dysfunction, thyroid disorders, or acute infectious

diseases. None of the subjects received corticosteroids, immunosuppressive agents, or drugs known to influence lipid metabolism, such as statins or fibrates. In no case did alcohol consumption surpass 15 g/day. Patients and controls did not follow any regular exercise training program. Patients were treated with antihypertensive drugs (angiotensin receptor blockers or calcium channel blockers). Diabetic patients were receiving insulin once or twice daily (dose range, 10–35 IU), and none of them were treated with oral hypoglycemic agents. Patients followed a standardized diet for HD treatment, containing 1.2 g proteins/kg body weight per day and 35 cal/kg per day, appropriately administered with phosphate binders and vitamins. Controls followed a varied diet, with calorie intake according to individual body weight. Patients were dialyzed with conventional low-flux HD treatment, for at least 4 hours, 3 times per week, using bicarbonate-containing dialysis fluid. The blood flow, dialysate flow rate, dialyzer model, and treatment time were tailored to individual patients to achieve the target equilibrated urea KT/V (K=clearance in mL/min, T=length of dialysis duration in minutes, and V=patient urea distribution volume in mL) of 1.25.

The weight and height of each participant were measured, and body mass index was calculated to evaluate obesity degree. Waist circumference was taken midway between the lateral lower rib margin and the superior anterior iliac crest in a standing position, always by the same investigator.

Written informed consent was obtained from all the participants included in the study. The study had the approval of the Ethics Committees from the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

Samples

After a 12-hour overnight fast, blood samples were drawn. In the patients' group, blood was obtained in red-top plastic tubes (BD-Vacutainer) with clot activator for serum and in K3-EDTA tubes (BD-Vacutainer) for plasma, after the longest interdialysis interval, before the initiation of HD. Serum or plasma was kept at 4°C within 48 hours for the evaluation of glucose, albumin, lipids, and lipoproteins or stored at -70°C for further determination of insulin, adiponectin, and high-sensitivity CRP (hs-CRP).

To measure total, endothelial, and hepatic phospholipase activity, heparin (60 IU/kg body weight) was administered intravenously. After 10 minutes, blood from the contralateral arm was collected in tubes on ice. Postheparin plasma (PHP) was obtained by centrifugation at 1500 g at 4°C for 10 minutes and kept at -70°C.

Measurements

Total cholesterol, TG, and fasting glucose were measured using commercial enzymatic kits (Roche Diagnostics, Mannheim, Germany) in a Cobas C-501 autoanalyzer, coefficient of variation (CV) intraassay <1.9%, CV interassay <2.4%, and averaging CV values of these parameters. HDL-C and LDL-cholesterol (LDL-C) were determined by standardized selective precipitation methods, using phosphotungstic acid/MgCl₂ and polyvinylsulfate as precipitating reagents, respectively.^{25,26} CV intraassay <2.0%, and CV interassay <3.0%. Serum hs-CRP, apoA-I, and apoB-100 were determined by immunoturbidimetry (Roche Diagnostics, Mannheim, Germany), CV intraassay <1.9%, and CV interassay <2.5% for the 3 parameters. Insulin was measured with Immulite/Immulite 1000 Insulin (Siemens, USA), CV intraassay <2.6%, and CV interassay <3.9%. To estimate IR, the homeostasis model assessment for insulin resistance (HOMA-IR) index was calculated as fasting insulin (μU/mL)×fasting glucose (mmol/L)/22.5. TG/HDL-C index was also used as a surrogate marker of IR. Sera levels of adiponectin were determined by monoclonal antibody-based ELISA (R&D Systems, USA).

Lipases Activity

Total SN1-specific phospholipase activity was determined using (1-decanoylthio-1-deoxy-2-decanoyl-sn-glycero-3-phosphoryl) ethylene glycol (ThioPEG) as the substrate (Figure 1A).²⁷ Hydrolysis

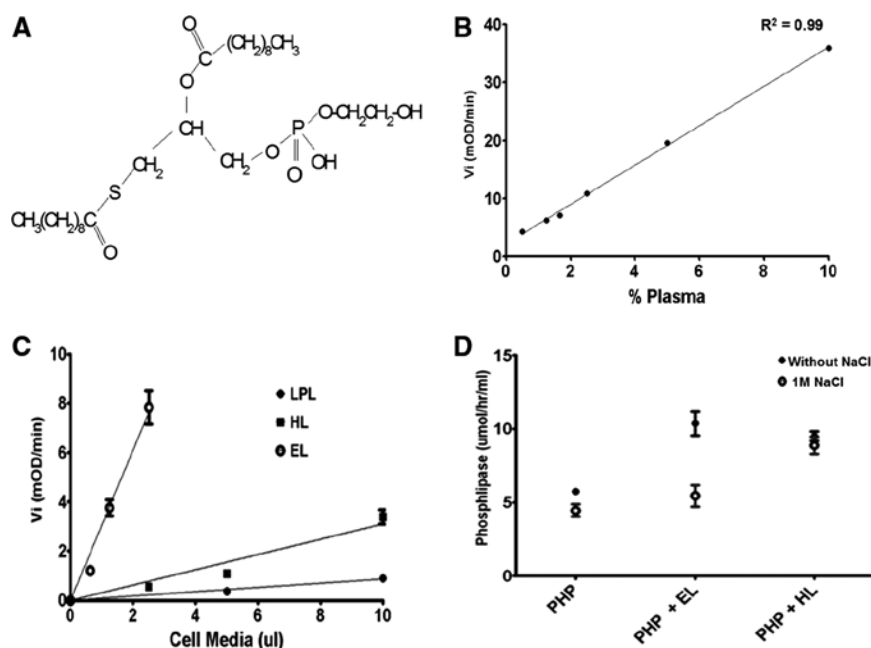


Figure 1. **A**, 1-decanoylthio-1-deoxy-2-decanoyl-sn-glycero-3-phosphoryl ethylene glycol (ThioPEG) structure. **B**, Linearity of SN1 phospholipase activity with different concentrations of postheparin plasma (PHP). **C**, SN1 phospholipase activity of endothelial lipase (EL), hepatic lipase (HL), and lipoprotein lipase (LPL): cell culture medium from COS cells overexpressing EL, HL, or LPL was used as enzyme source. **D**, Inhibition of plasma phospholipase activity of EL, but not HL, by high salt. EL or HL medium from COS cells overexpressing the enzyme was added to PHP with media from nontransfected cells serving as a control. Closed circles indicate no addition of salt, and open circles indicate addition of 1 mol/L salt to assay.

of ThioPEG at the SN1 position produces a thiol that reacts with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) to form a mixed disulfide and the nitro-5-thiobenzoate anion. The later absorbs at 412 nm. The phospholipase activity is directly proportional to the initial velocity of the hydrolysis reaction at 37°C occurring in the wells of a 96-well plate. Initial velocity is determined by following the appearance of absorbance at 412 nm over time, for the linear portion of the curve.²⁷

Briefly, an emulsion of 4.09 mmol/L ThioPEG (Avanti) in 100 mmol/L HEPES, pH 8.3, and 7 mmol/L Triton X-100 was prepared by sonication with a Bransom Sonifier. A solution of 271 mmol/L DTNB (Sigma) in dimethylsulfoxide was prepared by vortexing. A 1:1 mol mixture of ThioPEG to DTNB was prepared by adding the DTNB solution to the ThioPEG emulsion, resulting in a chromogenic substrate solution containing 4.03 mmol/L ThioPEG and 4.03 mmol/L DTNB.

Total SN1 phospholipase activity was measured by adding 20 μ L of a one-tenth dilution of PHP and 80 μ L of chromogenic substrate to the wells of a 96-well plate and following the absorbance at 412 nm for 30 minutes in a Molecular Devices SpectraMax 250 microplate reader. The total SN1 phospholipase activity was linear between 0.5% and 10% plasma (Figure 1B).

To determine the HL phospholipase activity, the PHP dilutions were preincubated in ice for 15 minutes with 1 mol/L NaCl to inhibit the EL activity. Then, the chromogenic substrate solution was added, and the plate was read at 412 nm as described previously. Finally, EL activity is calculated as the difference between total phospholipase activity and hepatic phospholipase activity. The intraassay CV for total lipase, HL, and EL were 3.8%, 3.1%, and 15.7%, respectively. The interassay CV for total lipase, HL, and EL were 3.8%, 5.3% and 28.8%, respectively. The EL CV is higher because it is the difference between total lipase and HL. In some cases, medium from monkey kidney fibroblast cell line (COS) cells overexpressing EL, HL, or LPL was used as a source of lipase.¹¹

All enzyme activities are calculated using the molar adsorption coefficient value for the nitro-5-thiobenzoate anion, the depth of solution, and the volume of the enzyme source. Results are expressed in μ mol of fatty acid released per milliliter of PHP per hour.

In addition, HL TG activity in PHP was determined by measuring the oleic acid produced by the enzyme-catalyzed hydrolysis of an emulsion containing [³H]-triolein (Amersham TRA 191; Amersham, Buckinghamshire, United Kingdom), according to the Nilsson Ehle method²⁸ modified.²⁹ The assay mixture containing labeled and unlabeled triolein (Sigma T-7140; 1.3 μ mol/mL

of glyceryl trioleate with a specific activity of 10×10^6 cpm/ μ mol) was mixed with 0.11 μ mol/mL of L-lysophosphatidylcholine (Sigma L-4129) and 0.2% BSA (Sigma A-6003) in 0.2 mol/L buffer (Tris-HCl pH 8.8 with NaCl 0.15 mol/L). This mixture was incubated with PHP in saline solution (1:10) and 1 mol/L NaCl for 30 minutes at 30°C. After incubation, the reaction was stopped, and released fatty acids were isolated by extraction with a carbonate-borate buffer, pH 10.5. The [³H]-oleic acid was quantified by counting with a Liquid Scintillation Analyzer (Packard TRI-CARB 2100; Packard Instruments, Meridian, CT). HL activity was calculated from the difference in counts per minute between the blank and the sample. Results were expressed as μ mol of fatty acid released per milliliter of PHP per hour. CV intraassay was 4%, and CV interassay was 9%. As a result of the complexity of this assay, the CV is considered to be quite satisfactory.

Statistical Analysis

Data are presented as mean \pm SD or median (range) according to normal or skewed distribution, respectively. Differences between groups were tested using the unpaired Student *t* test, χ^2 test, or the Mann-Whitney *U* test, according to the data distribution. Pearson or Spearman analysis, for parametric or nonparametric variables, was used to determine correlations between parameters. To verify the difference between groups of EL activity or HL activity as phospholipase, we performed an ANCOVA, controlling for necessary confounders such as waist circumference and age, respectively. Stepwise and multiple linear regression analyses were used to identify independent correlates of plasma EL activity and HL PL activity. Previously, each variable was examined for normal distribution, and abnormally distributed variables were log-transformed. The SPSS 19.0 software package (Chicago, IL) was used for statistical analysis. A *P* < 0.05 was considered significant.

Results

Characteristics of the Study Population

The clinical and biochemical characteristics of the patients and controls are shown in Table 1. In CKD group, 17 patients were women and 19 men, whereas in the control group, 18 were women and 16 were men. Patients with CKD were older (*P* < 0.001) and presented higher waist circumference

Table 1. Clinical and Biochemical Characteristics of Control and CKD Groups

	Controls (n=34)	CKD (n=36)	P Value
Age, y	36±15	49±11	0.001
Sex, w/m*	18/16	17/19	NS
BMI, kg/m ²	23.5±4.2	24.5±4.6	NS
Waist circumference, cm	83.3±14.8	93.8±16.1	0.026
Total-C, mmol/L	4.8±0.9	4.7±1.0	NS
Triglyceride, mmol/L	1.0 (0.4–2.9)	1.9 (0.5–5.8)	0.001
HDL-C, mmol/L	1.5±0.4	1.1±0.3	0.001
LDL-C, mmol/L	2.9±1.1	2.5±0.8	NS
ApoA-I, g/L	1.7±0.4	1.2±0.2	0.001
ApoB-100, g/L	0.9±0.3	0.8±0.2	NS
Albumin, g/L	37 (31–46)	37 (29–42)	NS
Serum creatinine, μmol/L	84.9±3.5	930.1±46.9	0.01
Serum urea, mmol/L	5.7±0.2	27.1±1.4	0.01
Hemodialysis period, y	...	4.83±0.87	...

Data are expressed as mean±SD or median (range) for skewed distributed data. None of the patients or controls were receiving fibrates or statin treatment. w indicates women; m, men; BMI, body mass index; CKD, chronic kidney disease; Total-C, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; apoA-I, apolipoprotein A-I; apoB, apolipoprotein B.

* χ^2 test.

($P=0.026$) than controls. Regarding lipid and lipoprotein profile, patients presented higher TG and lower HDL-C and apoA-I levels ($P<0.001$). No differences were observed in total cholesterol, LDL-C, apoB, and albumin levels.

In reference to IR and inflammatory state markers, insulin ($P<0.001$), HOMA-IR index ($P<0.003$), TG/HDL-C ($P<0.001$), hs-CRP ($P=0.022$), and adiponectin ($P=0.015$) levels were higher in patients compared with controls (Table 2); meanwhile, CKD patients presented lower glucose levels ($P=0.015$) than controls.

After adjusting for age, differences in TG, HDL-C, and apoA-I ($F=12.5$, $F=20.5$, and $F=55.1$, respectively; $P<0.001$), as well as differences in insulin ($F=10.1$; $P=0.002$), HOMA-IR ($F=6.7$; $P=0.012$), TG/HDL-C ($F=10.6$; $P=0.002$), and hs-CRP ($F=7.0$; $P=0.01$) remained significant. When considering waist circumference, only differences in TG ($F=4.7$; $P<0.05$), apoA-I ($F=15.2$; $P<0.001$), glucose

Table 2. Insulin Resistance and Inflammatory Markers in Control and CKD Groups

	Control (n=34)	CKD (n=36)	P Value
Glucose, mmol/L	4.9 (3.9–5.1)	4.7 (2.7–12.9)	0.015
Insulin, pmol/L	32.0 (14.0–116.8)	62.6 (16.7–581.0)	0.001
HOMA-IR	1.0 (0.3–4.9)	1.7 (0.4–19.8)	0.003
hs-CRP, mg/L	1.3 (0.1–11.7)	2.6 (0.1–64.6)	0.022
TG/HDL-C	1.7 (0.7–11.4)	4.1 (0.8–18.1)	0.001
Adiponectin, μg/mL	11.2 (2.6–28.2)	18.2 (4.3–3.3)	0.015

Data are expressed as median (range) for skewed distributed data. HOMA-IR indicates homeostasis model assessment for insulin resistance index; hs-CRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein-cholesterol; TG, triglycerides; CKD, chronic kidney disease.

($F=10.8$; $P<0.002$), and adiponectin ($F=21.1$; $P<0.001$) remained significant.

EL, HL, and Total Phospholipase Activities

Using ThioPEG, the SN1 phospholipase activity was assessed in COS cells overexpressing EL, HL, and LPL (Figure 1C). The activity was linear with respect to the amount of media added, with EL>HL>LPL. The TG lipase activity of the 3 preparations were comparable (data not shown), illustrating that EL is primarily a phospholipase and that LPL has little phospholipase activity in agreement with previous report.¹¹ The phospholipase activity of PHP is linear over a 10-fold range of plasma concentration (Figure 1B). Preheparin plasma contains negligible SN1 phospholipase activity (0.3 ± 0.4 mOD/min), demonstrating that the phospholipase activity is caused by the heparin-binding phospholipases, HL and EL. The phospholipase activity caused by EL and HL can be differentiated by the inhibition of EL, but not HL, by high salt. This is shown in Figure 1D, where the increase in phospholipase activity of PHP obtained by adding EL was inhibited by 1 mol/L NaCl, whereas a similar increase obtained by adding HL was not affected. Patients with CKD presented lower values of total SN1 phospholipase activity than controls: 4.4 (1.9–9.0) versus 7.5 (3.6–18.0) μmol of fatty acid released per milliliter of PHP per hour, $P<0.001$ (Figure 2A). Lower values of HL phospholipase activity than controls were also observed: 2.6 (0.7–6.2) versus 6.6 (1.3–15.2) μmol of fatty acid released per milliliter of PHP per hour, $P<0.001$ (Figure 2B). However, EL phospholipase activity was increased in CKD group: 1.7 (0.8–3.0) versus 1.1 (0.1–2.7) μmol of fatty acid released per milliliter of PHP per hour, $P=0.008$ (Figure 2C).

As shown in Table 3, EL was negatively associated with HDL-C and apoA-I levels, whereas no association was observed between these parameters and HL or total lipase activity, highlighting the role of EL on HDL-C behavior in CKD patients. On the contrary, total phospholipase activity and HL activity were directly associated with LDL-C and apoB. Finally, total phospholipase activity and HL phospholipase activity were negatively associated with age and adiponectin; meanwhile, EL activity positively correlated with waist circumference, insulin levels, and HOMA-IR index; surprisingly, no correlation was observed with hs-CRP.

We previously reported a decrease in HL TG activity in these patients, as well as an inverse correlation with intermediate density lipoprotein-cholesterol and LDL-TG.⁶ In this study, HL phospholipase activity and HL triglyceride activity correlated significantly ($r=0.563$; $P=0.0001$), confirming that both enzymatic activities of HL are decreased in these patients.

Effect of Age and Waist Circumference on the Enzyme Activities and Lipoprotein Behavior

Because total phospholipase and HL phospholipase activity were associated with age, an ANCOVA was undertaken; differences in both activities persisted significantly between groups, even after adjusting for age ($F=16.1$ and 22.5 , respectively; $P<0.001$).

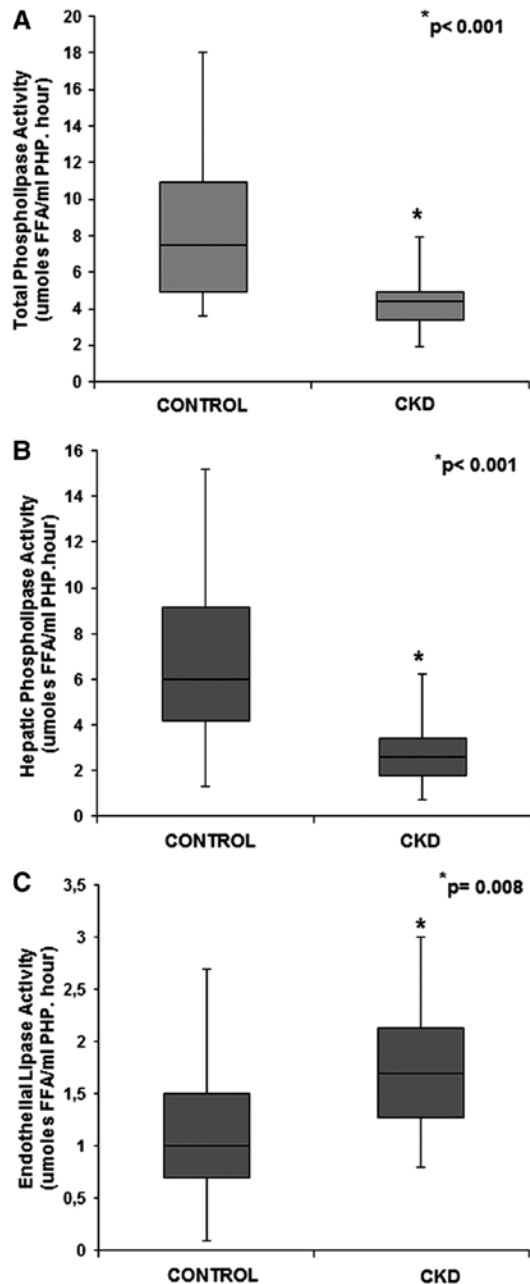


Figure 2. A, Total phospholipase activity. B, Hepatic phospholipase activity. C, Endothelial phospholipase activity in control and chronic kidney disease (CKD) groups. FFA indicates free fatty acids.

Similarly, given the association observed between EL activity and waist circumference, we also performed an ANCOVA adding this confounder in the model; the difference in EL activity between CKD and control group was lost ($F=0.83$; $P=0.367$).

Multivariate regression analyses were performed to distinguish the contribution of different variables to the variance of EL and HL activities (Table 4). In reference to EL, the model included markers of IR; EL activity was independently associated with waist circumference ($P=0.019$). Regarding HL, age and adiponectin were included in the first model; HL was significantly related to both variables ($P=0.021$ and $P=0.042$,

respectively). However, when waist circumference (model 2), as well as hs-CRP (model 3), was included in the model, only age remained associated with HL activity.

Given that both enzymes remained associated with waist circumference and age, respectively, the impact of the enzymes activities on HDL-C and LDL-C was adjusted by these confounders. The association of EL and HDL-C remained significant, when adjusting for waist circumference ($\beta=-0.26$; $P=0.05$); in turn, the effect of HL on LDL-C continued to be associated even after adjusting for age ($\beta=0.46$; $P=0.001$).

Discussion

In the present study, we used a novel enzyme assay to assess for the first time the role of EL and HL phospholipase activity in the lipoprotein abnormalities associated with CKD. This plasma assay takes advantage of the specificity of HL, EL, and LPL as SN1 lipases; the negligible phospholipase activity of LPL; and the inhibition of EL, but not HL, by high salt. The increased EL phospholipase activity observed in CKD subjects is in agreement with increased mass of EL reported by Fujii et al¹⁸ in CKD patients with low serum albumin or high CRP.

In the present study of HD patients, we showed that plasma EL activity is increased and is associated with lower HDL-C and apoA-I concentrations. On the other hand, HL phospholipase activity that has also been shown to be inversely associated with HDL-C is decreased in these patients, suggesting that EL activity is an important contributor to lipolytic catabolism of HDL-C in CKD. LPL, which is primarily a TG lipase, has also been shown to be decreased in CKD.³⁰

It is well established that cardiovascular disease is currently the primary cause of morbidity and mortality in patients with CKD under HD.³¹ We previously reported that CKD patients show unfavorable alterations in the plasma lipid-lipoprotein profile, consisting of high TG and intermediate-density lipoprotein-cholesterol, low HDL-C, and decreased HL activity (as TG lipase) associated with modified LDL depleted in cholesterol and enriched in TG.⁶

Similarly, it is well known that plasma HDL-C levels are inversely associated with the risk of cardiovascular disease, as was first demonstrated by the Framingham study.³² This consistent inverse association denotes that the antiatherogenic function of this lipoprotein would be strongly linked to its concentration. Given the controversy between the lower HDL-C levels and the lower HL activity in CKD, other factors should be responsible for the HDL-C catabolism in these patients.

Previous studies have shown that overexpression of EL results in a dramatic decrease in HDL-C and apoA-I levels in mice.¹⁶ More recently, Maugeais et al¹⁴ have reported that the hydrolysis of HDL-C induced by EL overexpression results in depletion of PL and cholesterol, leading to the production of smaller HDL particles. Furthermore, several studies in humans demonstrated a significant negative relationship between serum EL concentration and HDL-C levels.^{33,34}

Regarding the lower activity of HL (as TG lipase) previously found in CKD patients,⁶ Klin et al³⁵ have provided evidence that in CKD there is a downregulation of mRNA of the enzyme and impairment of HL production, activity, and

Table 3. Spearman Correlation Coefficients with Total Phospholipase Activity, Hepatic Phospholipase Activity, and Endothelial Lipase Activity

	Total Phospholipase Activity	HL Activity	EL Activity
Age	−0.355 (0.003)	−0.372 (0.002)	0.055 (NS)
BMI	0.002 (NS)	0.015 (NS)	0.057 (NS)
Waist circumference	−0.002 (NS)	−0.090 (NS)	0.287 (0.050)
Total-C	0.232 (NS)	0.255 (NS)	−0.03 (NS)
Triglyceride	−0.043 (NS)	−0.139 (NS)	0.230 (NS)
HDL-C	−0.063 (NS)	0.07 (NS)	−0.427 (0.001)
LDL-C	0.302 (0.012)	0.257 (0.034)	0.051 (NS)
ApoA-I	0.109 (NS)	0.236 (NS)	−0.371 (0.002)
ApoB-100	0.337 (0.005)	0.288 (0.016)	0.108 (NS)
Glucose	0.268 (0.028)	0.251 (0.041)	0.008 (NS)
Insulin	−0.035 (NS)	−0.155 (NS)	0.362 (0.003)
HOMA-IR	0.019 (NS)	−0.08 (NS)	0.27 (0.024)
TG/HDL-C	0.027 (NS)	−0.097 (NS)	0.350 (0.003)
hsCRP	−0.078 (NS)	−0.154 (NS)	0.052 (NS)
Adiponectin	−0.408 (0.006)	−0.468 (0.001)	−0.032 (NS)

Values are expressed as r (P values). BMI indicates body mass index; total-C, total cholesterol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; HOMA-IR, homeostasis model assessment for insulin resistance index; hs-CRP, high-sensitivity C-reactive protein; HL, hepatic lipase; EL, endothelial lipase.

release. However, other investigators have suggested that low HL activity is associated with metabolic alterations caused by renal failure, such as impaired calcium metabolism,³⁶ or with the presence of plasma inhibitors.²³ Evaluating HL as phospholipase, we observed a decrease in plasma activity, which was directly associated with LDL-C and apoB and inversely associated with age and adiponectin. After adjusting the results for age, the differences between groups, as well as its association with adiponectin, remained significant. These results suggest that this cytokine could be involved in the HL

activity regulation, in accordance with previous studies in diabetic patients.³⁷ Meanwhile, the lack of association of HL with hs-CRP is in accordance with previous reports on acute inflammation.³⁸

Given the high prevalence of IR in patients with end-stage renal disease, it is important to consider the impact of this factor on the metabolic alterations previously described. In this study, we found direct associations between EL and surrogate markers of IR, such as waist circumference and HOMA index, as it has been previously described in patients with metabolic syndrome.³³ After performing an ANCOVA, the difference in EL activity observed between CKD patients and controls was lost when waist circumference was considered, reflecting the contribution of IR in the control of this enzyme. Nevertheless, the inverse association concerning EL activity and HDL-C and apoA-I remained significant after adjusting for abdominal obesity. This result suggests that the impact of EL on HDL-C in CKD patients goes beyond the IR state of these patients. It should be taken into account that the IR state in CKD patients is multifactorial and still remains to be elucidated.³⁹

Data in humans have provided support for the concept that EL is related to the degree of obesity. Badellino et al³⁴ showed that EL concentrations in preheparin plasma and PHP are positively correlated with measures of adiposity, such as body mass index and waist circumference in healthy individuals. They attributed part of the association between EL and obesity to the presence of an inflammatory state. Several studies, both in culture⁴⁰ and humans³³ have reported direct association between EL and markers of inflammation, even independent of the degree of abdominal obesity. Fujii et al¹⁸ found that serum EL levels were significantly correlated with tumor necrosis factor- α and hs-CRP in CKD patients; it is suggested that serum EL levels may be elevated in many dialysis patients

Table 4. Multivariate Regression Analyses Showing the Independent Contributions of Different Variables to Endothelial Lipase Activity and Hepatic Lipase Activity

Model	Independent Variables	β	P Value
Dependent variable: Endothelial lipase activity			
Model 1	Waist circumference	0.369	0.019
	HOMA-IR	0.135	0.375
Dependent variable: Hepatic lipase activity			
Model 1	Age	−0.365	0.021
	Adiponectin	−0.319	0.042
Model 2	Age	−0.408	0.017
	Adiponectin	−0.299	0.061
	Waist circumference	0.119	0.439
Model 3	Age	−0.423	0.015
	Adiponectin	−0.285	0.079
	Waist circumference	0.154	0.348
	CRP	−0.101	0.511

EL activity, HOMA-IR, HL activity, age, adiponectin, and CRP were \log_{10} -transformed before analysis. β is the standardized coefficient. CRP indicates C-reactive protein; HOMA-IR, homeostasis model assessment for insulin resistance index.

because most of them have chronic inflammation. In our study, surprisingly, EL activity was not associated with hs-CRP concentrations, although CKD patients presented higher hs-CRP levels than controls. Given this controversy, it must be stressed that previous data have been based on measures of plasma EL concentration, not activity, and we cannot rule out the possibility that EL activity could be regulated by more complex inflammatory pathways in HD patients, which deserves further investigation.

Our findings extend previous reports about factors that can modulate HDL-C levels in CKD patients, such as a decrease in apoA-I/apoA-II, lecithin cholesterol acyl transferase, and ATP-binding cassette sub-family A member 1 expression, among others.⁴ Furthermore, recently, it has been suggested that not only HDL-C levels but also the lipoprotein functionality may be altered in these patients.⁴¹ Our results support the concept that EL is an important enzyme responsible for HDL-C lipolytic catabolism in CKD patients and resolve the paradox previously observed between the low HL activity and the decreased HDL-C levels observed in these patients. In addition, the ability to assess total, HL, and EL phospholipase activity in plasma will increase our knowledge of the mechanisms involved in HDL-C behavior and cardiovascular risk in CKD, as well as other patient populations with low HDL-C. Overall, EL would be an interesting potential target for therapeutic inhibition as a novel strategy to raise HDL-C and reduce cardiovascular risk in CKD patients.

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Disclosures

None.

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