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Preβ1-high-density lipoprotein metabolism is delayed in patients with chronic kidney disease not on hemodialysis

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KEYWORDS:
ABCA1;
CAPD;
Cholesterol efflux capacity;
CKD;
Lecithin–cholesterol acyltransferase;
Prebeta-HDL;
Phospholipid-transfer protein

BACKGROUND: Preβ1-high-density lipoprotein (HDL) is a lipid-poor cholesterol acceptor that is converted to lipid-rich HDL by lecithin–cholesterol acyltransferase (LCAT). In patients receiving hemodialysis, preβ1-HDL metabolism is hampered even if HDL cholesterol is normal. Hemodialysis may affect preβ1-HDL metabolism by releasing lipases from the vascular wall due to heparin.

OBJECTIVES: We investigated whether preβ1-HDL metabolism is delayed in patients with chronic kidney disease (CKD) who are not receiving hemodialysis.

METHODS: We examined 44 patients with Stage 3 or higher CKD and 22 healthy volunteers (Control group). The patients with CKD were divided into those without renal replacement therapy (CKD group, n = 22) and those undergoing continuous ambulatory peritoneal dialysis (CAPD group, n = 22). Plasma preβ1-HDL concentrations were determined by immunoassay. During incubation at 37°C, we used 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) to inhibit LCAT activity and defined the conversion halftime of preβ1-HDL ($CHT_{pre\beta1}$) as the time required for the difference in preβ1-HDL concentration in the presence and absence of 5,5-DTNB to reach half the baseline concentration.

RESULTS: The absolute and relative preβ1-HDL concentrations were higher, and $CHT_{pre\beta1}$ was longer in the CKD and CAPD groups than in the Control group. Preβ1-HDL concentration was significantly correlated with $CHT_{pre\beta1}$ but not with LCAT activity in patients with CKD and CAPD.

CONCLUSION: Preβ1-HDL metabolism is delayed in patients with CKD who are not on hemodialysis. This preβ1-HDL metabolic delay may progress as renal function declines.

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Introduction

The incidence of chronic kidney disease (CKD) is growing worldwide. The cardiovascular disease (CVD) risk increases progressively as kidney function declines, peaking in patients undergoing chronic hemodialysis. CVD accounts for 30% of all deaths among dialysis patients.[1] In the general population, low-density lipoprotein cholesterol (LDL-C) is strongly associated with the development of atherosclerosis.[2] Furthermore, intense LDL-C–lowering therapy with statins appreciably improves cardiovascular outcomes.[3] However, statin therapy yields less impressive results in patients on hemodialysis.[4–6]

In addition to multiple proatherogenic factors, including uremic toxins, inflammation, and oxidative stress, CKD patients have an impaired antiatherogenic system. High-density lipoprotein (HDL) is the only antiatherogenic lipoprotein. The protein components of HDL contribute to a number of favorable effects and are distributed unevenly across several HDL subfractions with different functions. Although HDL cholesterol (HDL-C) is consistently correlated with the incidence of CVD,[7,8] the recent failure of HDL-C–raising agents strongly implies that HDL function is more important than HDL-C.[9–11]

Preβ1-HDL is a lipid-poor HDL subfraction composed of small amounts of free cholesterol, phospholipids, and apolipoprotein AI (ApoAI). Among the HDL subfractions, preβ1-HDL removes free cholesterol most efficiently from lipid-loaded macrophages when ATP-binding cassette transporter A1 (ABCA1) is upregulated on the cell surface.[12,13] Once free cholesterol on preβ1-HDL has been esterified by lecithin–cholesterol acyltransferase (LCAT), preβ1-HDL is converted to lipid-rich large spherical HDL with α-mobility.[14] Unlike its antiatherogenic potential, the preβ1-HDL concentration is significantly elevated in patients with coronary artery disease,[15–17] hyperlipidemia, and type 2 diabetes.[18–22] We previously reported that preβ1-HDL concentrations increased markedly in hemodialysis patients because of impaired LCAT-dependent preβ1-HDL conversion.[23] Such impaired preβ1-HDL catabolism was observed not only in patients with low HDL-C level but also in those with normal HDL-C level. It is possible that preβ1-HDL is not a precursor of all large HDL particles.[24] However, hemodialysis patients receive repetitive intravenous heparin injections. Heparin releases lipases from vascular endothelial cells that increase preβ1-HDL through HDL remodeling.[20] Therefore, it remains unclear whether preβ1-HDL concentration is altered in CKD patients who do not receive hemodialysis.

To clarify whether preβ1-HDL metabolism is delayed in CKD patients not on hemodialysis, we examined plasma preβ1-HDL concentrations and conversion time between preβ1-HDL and α-migrating HDL in patients with Stage 3 or higher CKD and compared these parameters with those of healthy volunteers. We included some patients on continuous ambulatory peritoneal dialysis (CAPD) as study subjects because CAPD does not require heparin.

Materials and methods

Patients and controls

We examined 44 patients whose estimated glomerular filtration rate (eGFR) was <60 mL/min/1.73 m^2. Half of the patients did not receive any renal replacement therapy (CKD group), whereas the other half received CAPD (CAPD group). Neither the CKD nor CAPD groups had regular heparin injections. We enrolled 22 healthy volunteers as a Control group. We excluded patients who were undergoing hemodialysis or who had an acute coronary syndrome, acute stroke, systemic infection, including acute pyelonephritis, and malignancies or who had been taking lipid-lowering agents and/or nonsteroidal anti-inflammatory drugs.

All participants provided written informed consent before inclusion in the study. This study was conducted in accordance with the ethical principles stated in the latest version of the Declaration of Helsinki.[25] This protocol was approved by the institutional review boards or ethics committees of all participating institutions.

Blood samples

Venous blood was drawn into vacuum blood collection tubes supplemented with K_2-EDTA after at least a 12-hour fast for preβ1-HDL measurement. Plain vacuum blood collection tubes were used for the other biochemical laboratory tests. To block the conversion of preβ1-HDL to α-HDL after the blood was drawn, the K_2-EDTA-containing tubes were immediately plunged into ice water. Plasma was recovered after 15 min of centrifugation at 0°C, 1500 × *g*, kept at 0°C in ice water, and used for incubation experiments within 6 hours.

Serum was obtained after centrifugation at room temperature. The plasma was used for the incubation experiment the same day.

Analytical methods

Lipids, apolipoproteins, and LCAT activity

We measured total cholesterol and triglyceride (TG) concentrations enzymatically. LDL-C and HDL-C concentrations were determined using commercial homogeneous assays (Choletest-LDL; Sekisui Medical, Tokyo, Japan; MetaboLead HDL-C; Kyowa Medex, Tokyo, Japan). Both reagents have satisfactory analytical performance, and the measured values were in good agreement with those assessed by reference methods of the Centers for Disease Control and Prevention.[26] Apolipoprotein concentrations were measured by a turbidimetric immunoassay. LCAT activity was determined by the endogenous substrate method, which measures the rate of free cholesterol reduction during incubation at 37°C.[15]

Conversion kinetics between preβ1-HDL and α-migrating HDL

The conversion time of preβ1-HDL was determined using freshly isolated plasma as described previously.[27,28] In this experiment, we used 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) to inhibit LCAT activity. Briefly, plasma aliquots were incubated in Eppendorf tubes at 37°C in the presence or absence of DTNB (at a final concentration of 2 mM) dissolved in 0.1 M phosphate buffer (pH 7.4). The LCAT reaction was stopped after 0, 30, 60, 90, and 120 min by immersing the Eppendorf tubes in ice water. To inhibit changes in the preβ1-HDL concentration during storage, all plasma samples were mixed with a 20-fold volume of 50% sucrose and stored at −80°C until measurement.[29]

Preβ1-HDL concentrations were determined by sandwich enzyme immunoassays using a specific monoclonal antibody against preβ1-HDL (mAb55201).[29] Two-dimensional (2D) gel electrophoresis confirmed that mAb55201 reacts only with preβ1-HDL.[30] Furthermore, the preβ1-HDL concentration determined by this assay was correlated with that determined by native 2D gel electrophoresis.[29] The thawed plasma samples were diluted with 1% bovine serum albumin–phosphate-buffered saline. The samples or standards (human ApoAI) were added to a 96-well plastic plate coated with mAb55201 and incubated at room temperature for 60 min. The trapped preβ1-HDL was coupled with a goat anti-human ApoAI polyclonal antibody labeled with horseradish peroxidase at room temperature for 60 min. The absorbance was measured colorimetrically at 492 nm. The preβ1-HDL concentration was expressed as the ApoAI concentration.

To determine the conversion kinetics of preβ1-HDL, the data were plotted on a semi-log graph with a linear scale on the x-axis (min) and a logarithmic scale on the y-axis (preβ1-HDL concentration). We defined the conversion halftime of preβ1-HDL ($CHT_{pre\beta1}$) as the incubation time for the difference in preβ1-HDL concentration in the presence and absence of DTNB to reach half the level of its baseline concentration (Fig. 1).[27] We consider that DTNB-sensitive conversion of preβ1-HDL is mediated mostly by the LCAT reaction. $CHT_{pre\beta1}$ is not affected by phospholipid-transfer protein (PLTP)-induced preβ1-HDL formation.

When plasma is incubated at 37°C for 24 h in the presence of DTNB, preβ1-HDL increases exponentially, accompanied by the emergence of large α-HDL (Miida T, unpublished data). Either PLTP or PLTP activator induces the same changes in HDL subfractions.[31] As a marker for PLTP-induced HDL remodeling, we defined the slope of the regression line of the semi-log graph in the presence of DTNB: Slope $(DTNB)_{pre\beta1}$ (Fig. 1).

Statistical analysis

All data are presented as the mean ± standard deviation or median (interquartile range), unless otherwise stated. Statistical analyses were performed using the R language for Statistical Computing (version 3.4.3; Vienna, Austria).

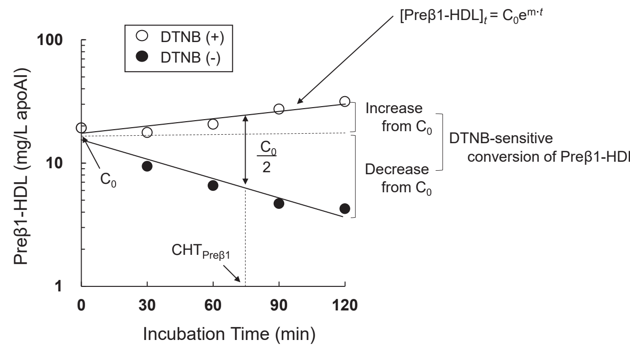

Figure 1 Markers for conversion between preβ1-HDL and α-HDL. Fresh plasma was incubated at 37°C for up to 120 min in the presence (circles) or absence (dots) of DTNB. Preβ1-HDL concentrations were measured every 30 min via immunoassay and plotted against time on a semi-log graph. $CHT_{pre\beta1}$ and Slope $(DTNB)_{pre\beta1}$ were determined as described in the Materials and Methods section. C_0, baseline preβ1-HDL concentration; m, slope of the regression line in the presence of DTNB = Slope $(DTNB)_{pre\beta1}$; $CHT_{pre\beta1}$, conversion halftime of preβ1-HDL; DTNB, 5, 5-dithio-bis (2-nitrobenzoic acid); LCAT, lecithin–cholesterol acyltransferase; t, time. $[\text{pre}\beta1\text{-HDL}]_t = C_0 \times e^{mt}$, $\text{LN } [\text{pre}\beta1\text{-HDL}]_t = \text{LN } (C_0 \times e^{mt})$, $\text{LN } [\text{pre}\beta1\text{-HDL}]_t = \text{LN } C_0 + \text{LN } e^{mt}$, $\text{LN } [\text{pre}\beta1\text{-HDL}]_t = b + mt$: $b = \text{LN } C_0$, $m = \text{Slope } (DTNB)_{pre\beta1}$.

Pearson's chi-square test was used for the analysis of discontinuous variables. For continuous variables, the Mann–Whitney *U* test was used to compare 2 groups. One-way analysis of variance or the Kruskal–Wallis test, with *post hoc* tests applied (Tukey–Kramer test for Gaussian distributions and Steel–Dwass test for non-Gaussian distributions), was used to compare multiple groups. Spearman's rank correlation coefficient was used to assess the strength of associations between 2 non-Gaussian continuous variables. In all analyses, $P < .05$ was taken to indicate statistical significance.

Results

Comparison of clinical features

The clinical characteristics of the Control, CKD, and CAPD groups were similar (Table 1). About two-thirds of the CKD group were classified as Stage 3. In the CKD group, about 60% of renal impairment was caused by an unknown etiology, whereas diabetic nephropathy and glomerulonephritis were 2 common causes of renal impairment in the CAPD group. Although we excluded CKD and CAPD patients treated with lipid-lowering agents, most patients in the CKD and CAPD groups had been treated with antihypertensive agents (Table 1).

Biochemical parameters for renal function, inflammation, lipids, and apolipoprotein

Serum creatinine concentrations increased with the development of CKD, whereas the eGFR decreased significantly.

Table 1 Clinical characteristics of the study subjects

Group	Control (n = 22)	CKD (n = 22) Subtotal Stages 3–5	Stage 3 (n = 14)	Stages 4 and 5 (n = 8)	CAPD (n = 22)	*P* value
Age (y)	57.8 ± 11.0	65.3 ± 13.3	63.3 ± 16.9	66.5 ± 11.3	58.6 ± 9.9	.065
Male (%)	59.1 (13)	86.3 (19)	85.7 (12)	87.5 (7)	54.5 (12)	.053
Duration of dialysis (mo)	N/A	N/A	N/A	N/A	21.2 ± 13.4	N/A
Primary causes of renal failure						
Diabetic nephropathy (%)	N/A	9.1 (2)	7.1 (1)	12.5 (1)	31.8 (7)	.140
Glomerulonephritis (%)	N/A	9.1 (2)	7.1 (1)	12.5 (1)	36.4 (8)	.072
Nephrosclerosis (%)	N/A	18.2 (4)	28.5 (4)	0 (0)	9.1 (2)	.660
Others (%)	N/A	4.5 (1)	0 (0)	12.5 (1)	0 (0)	1.000
Unknown (%)	N/A	59.1 (13)	57.1 (8)	62.5 (5)	22.7 (5)	.032
Antihypertensive agents (%)	0 (0)	77.3 (17)	78.6 (11)	75.0 (6)	90.9 (20)	<.001
ACEI or ARB (%)	0 (0)	59.1 (13)	50.0 (7)	75.0 (6)	86.4 (19)	<.001
CCB (%)	0 (0)	31.8 (7)	35.7 (5)	25.0 (2)	72.7 (16)	<.001
Lipid-lowering agents (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker, CCB, calcium-channel blocker; CAPD, continuous ambulatory peritoneal dialysis; CKD, chronic kidney disease; N/A, not applicable.

The data are shown as the mean ± SD, or percentage (n).

P values were calculated using analysis of variance for continuous variables and Pearson's chi-square test for categorical comparison (ie, Control, CKD [Subtotal or Stage 3 and Stages 4 and 5] and CAPD groups).

The CKD group was divided into 2 subgroups: Stage 3 ($30 \leq$ eGFR < 60 mL/min/1.73 m^2) and Stages 4 and 5 (eGFR < 30 mL/min/1.73 m^2) groups.

Albumin concentrations were markedly lower in the CAPD group than in the other groups (Table 2). C-reactive protein (CRP) levels did not differ among the groups. We failed to find a clear relationship between serum amyloid A (another inflammatory marker) and CKD development.

The lipoprotein parameters showed characteristic changes in the CKD and CAPD groups (Table 2). In the CKD and CAPD groups, the median LDL-C concentration decreased below 100 mg/dL, which was lower than that in the Control group, whereas the median TG concentration showed no differences among the 3 groups. The median ApoB concentrations of the groups were similar. On the other hand, their HDL-related parameters (HDL-C, ApoAI, and ApoAII) were lower to a similar degree compared with the Control group. Furthermore, the CKD group was classified into 2 subgroups, according to CKD stage, to assess the effects of the development of renal dysfunction on these biological parameters: Stage 3 ($30 \leq$ eGFR < 60 mL/min/1.73 m^2, n = 14) and Stages 4 and 5 (eGFR < 30 mL/min/1.73 m^2, n = 8) CKD groups. The median LDL-C concentration in the Stages 4 and 5 CKD group was similar to that in the CAPD group, whereas the median TG concentration tended to be lower than that in the CAPD group. The mean HDL-C concentration decreased with progression of renal dysfunction.

Preβ1-HDL concentration, $CHT_{pre\beta1}$, LCAT activity, and slope $(DTNB)_{pre\beta1}$

Parameters related to preβ1-HDL metabolism were abnormal in patients with renal dysfunction. The absolute and relative preβ1-HDL concentrations were significantly higher in the CKD group than the Control group. These findings were more evident in the CAPD group (Table 3). Furthermore, the absolute preβ1-HDL concentration was significantly higher in the Stages 4 and 5 CKD group than in the Stage 3 CKD group ($P < .05$). We found a similar tendency in $CHT_{pre\beta1}$. Interestingly, although the preβ1-HDL concentration of the Stage 3 CKD group was not significantly different from that of the Control, $CHT_{pre\beta1}$ was significantly longer in the Stage 3 CKD group than the Control ($P < .05$). Although LCAT activity decreased significantly in the CKD and CAPD groups compared with the Control group, their absolute changes were much less than those in preβ1-HDL concentration and $CHT_{pre\beta1}$.

Slope $(DTNB)_{pre\beta1}$ differed markedly among the groups. Slope $(DTNB)_{pre\beta1}$ gradually and significantly decreased with declining renal function. The median values in the Stages 4 and 5 CKD and CAPD groups were half or lower than that in the Control group (Table 3).

Effects of TG, $CHT_{pre\beta1}$, and LCAT activity on preβ1-HDL concentrations

In the individual groups (the Control, CKD, and CAPD groups), we analyzed the correlations among TG concentration, $CHT_{pre\beta1}$, LCAT activity, and the absolute preβ1-HDL concentration. The preβ1-HDL concentration was not significantly correlated with the TG concentration in any group (Fig. 2A–C). In comparison, the preβ1-HDL concentration was significantly correlated with $CHT_{pre\beta1}$ in the CKD and CAPD groups (Fig. 3B and C). In some CAPD patients, the preβ1-HDL concentrations were

Table 2 Renal function, inflammation, lipid, and apolipoprotein data

Group	Control (n = 22)	CKD (n = 22) Subtotal Stages 3–5	CKD (n = 22) Stage 3 (n = 14)	CKD (n = 22) Stages 4 and 5 (n = 8)	CAPD (n = 22)	*P* value
Serum creatinine (mg/dL)	0.73 (0.64–0.86)	1.28 (1.07–3.33)[*]	1.13 (1.03–1.23)[*]	3.88 (3.33–4.70)[*,‡]	N/A	<.001
eGFR (mL/min/1.73 m^2)	81.4 (70.3–89.9)	39.9 (15.5–53.6)[*]	52.8 (41.3–55.8)[*]	12.9 (10.8–15.5)[*,‡]	N/A	<.001
Serum albumin (g/dL)	4.3 (4.2–4.5)	4.2 (3.9–4.4)	4.2 (4.1–4.4)	4.1 (3.8–4.3)	3.2 (2.9–3.5)[*,†,‡,§]	<.001
CRP (mg/dL)	0.04 (0.02–0.08)	0.10 (0.04–0.20)	0.10 (0.04–0.19)	0.15 (0.06–0.36)	0.09 (0.04–0.18)	.063
SAA (mg/L)	5.7 (3.7–8.1)	3.6 (2.0–6.4)	2.9 (1.8–4.7)	8.3 (3.2–24.1)	5.7 (2.4–13.5)	.478
Lipids						
TC (mg/dL)	218 ± 49	183 ± 36[*]	189 ± 41	173 ± 24	190 ± 43	.023
TG (mg/dL)	103 (73–143)	106 (77–177)	122 (82–176)	99 (68–205)	133 (102–224)	.130
LDL-C (mg/dL)	122 (100–138)	96 (80–127)	101 (91–142)	86 (71–104)	82 (65–102)[*]	.002
HDL-C (mg/dL)	60 ± 17	45 ± 14[*]	47 ± 13	42 ± 15[*]	43 ± 13[*]	<.001
Apolipoproteins						
ApoAI (mg/dL)	153.8 ± 30.4	121.2 ± 21.3[*]	120.7 ± 18.4[*]	122.0 ± 27.0[*]	126.6 ± 25.7[*]	<.001
ApoAII (mg/dL)	30.3 ± 5.5	24.1 ± 3.8[*]	24.8 ± 4.3[*]	22.9 ± 2.4[*]	24.4 ± 3.9[*]	<.001
ApoB (mg/dL)	94.0 (80.6–108.0)	90.9 (77.1–105.6)	90.9 (81.3–111.5)	91.0 (73.9–100.5)	90.2 (76.3–102.0)	.722
ApoCII (mg/dL)	3.8 (2.6–5.0)	4.2 (2.7–5.2)	4.4 (2.7–5.2)	3.2 (2.7–5.6)	3.6 (2.4–5.5)	.840
ApoCIII (mg/dL)	9.2 (8.1–10.5)	9.8 (8.3–11.1)	9.2 (7.9–10.4)	10.2 (9.5–13.3)	12.1 (9.3–16.0)	.043
ApoE (mg/dL)	4.2 (3.4–5.5)	3.4 (2.9–4.5)	3.5 (3.3–4.5)	2.9 (2.8–4.4)	4.2 (3.8–5.2)	.064

Apo, apolipoprotein; CAPD, continuous ambulatory peritoneal dialysis; CKD, chronic kidney disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; N/A, not applicable; SAA, serum amyloid A; TC, total cholesterol; TG, triglycerides.

The data are shown as the median (interquartile range) or mean ± SD.

P values were calculated using the Mann–Whitney *U* test for serum creatinine and eGFR (ie, Control and CKD [Subtotal or Stage 3 and Stages 4 and 5] groups).

P values were calculated using analysis of variance for TC, HDL, ApoAI, and ApoAII and the Kruskal–Wallis was used for other parameters, followed by the Tukey–Kramer and Steel–Dwass *post hoc* tests among 3 or 4 groups, as appropriate. (ie, Control, CKD [Subtotal or Stage 3 and Stages 4 and 5], and CAPD groups).

The data for the CKD group are presented for the Stage 3 and Stages 4 and 5 subgroups as shown in Table 1.

*$P < .05$ vs Control group.

†$P < .05$ vs CKD Subtotal group.

‡$P < .05$ vs CKD Stage 3 group.

§$P < .05$ vs CKD Stages 4 and 5 group.

Table 3 Parameters of preβ1-HDL and LCAT activity

Group	Control (n = 22)	Subtotal Stages 3–5	CKD (n = 22) Stage 3 (n = 14)	Stages 4 and 5 (n = 8)	CAPD (n = 22)	*P* value
Preβ1-HDL (mg/L apoAI)	19.7 (15.4–24.9)	27.6 (20.6–35.0)*	23.9 (19.5–28.2)	37.0 (33.8–41.3)*,‡	43.1 (32.3–56.6)*,†,‡	<.001
Preβ1-HDL (% apoAI)	1.3 (1.0–1.4)	2.2 (1.8–2.8)*	2.0 (1.7–2.3)	3.0 (2.4–4.2)*	3.2 (2.4–4.2)*,†,‡	<.001
$CHT_{pre\beta1}$ (min)	33.1 (29.0–38.1)	50.8 (42.6–76.8)*	44.0 (38.1–51.4)*	76.1 (73.6–100.8)*,‡	74.7 (62.0–93.1)*,†,‡	<.001
LCAT (ΔFC nmol/mL/h 37°C)	96.5 (84.7–103.0)	68.9 (59.4–84.8)*	68.9 (59.2–91.5)	69.8 (59.8–79.5)*	71.7 (58.5–90.0)*	.001
Slope $(DTNB)_{pre\beta1}$ ($\times 10^{-3}$)	4.2 (3.4–6.0)	2.3 (1.4–3.7)*	3.0 (1.5–4.2)	2.1 (1.5–2.2)*	1.6 (1.0–2.4)*	<.001

CAPD, continuous ambulatory peritoneal dialysis; $CHT_{pre\beta1}$, conversion halftime of preβ1-HDL; CKD, chronic kidney disease; LCAT, lecithin-cholesterol acyltransferase; Slope $(DTNB)_{pre\beta1}$, slope of semi-log graph of preβ1-HDL concentration in the presence of DTNB against incubation time.
The data are shown as the median (interquartile range).
P values were calculated using the Kruskal–Wallis test followed by the Steel–Dwass post hoc test among 3 or 4 groups (ie, Control, CKD [Subtotal or Stage 3 and Stages 4 and 5], and CAPD groups).
The data of the CKD group are presented in the Stage 3 and the Stages 4 and 5 subgroups as shown in Table 1.
$^*P < .05$ vs Control group.
$^†P < .05$ vs CKD Subtotal group.
$^‡P < .05$ vs CKD Stage 3 group.

significantly higher than those expected from their $CHT_{pre\beta1}$ values (Fig. 3C). We did not find a significant correlation between LCAT activity and the preβ1-HDL concentration (Fig. 4A–C) or between LCAT activity and $CHT_{pre\beta1}$ in any group (Supplementary Fig. 1A–C).

Discussion

This study showed that preβ1-HDL metabolism is delayed in CKD patients not on hemodialysis. $CHT_{pre\beta1}$ was 1.5- and 2.3-fold longer in the CKD and CAPD groups than in the Control group, respectively ($P < .001$). Furthermore, the preβ1-HDL concentration increased with $CHT_{pre\beta1}$ as CKD reached more advanced stages (Table 3).

Although cholesterol efflux capacity (CEC) predicts CVD risk in non-CKD patients much better than HDL-C, CEC does not have sufficient predictive ability in CKD patients. A lower CEC is associated with higher CVD morbidity and mortality in subjects with normal kidney function, independent of HDL-C concentration.[32,33] A recent meta-analysis confirmed these observations.[34] In contrast, CEC was not associated with CVD in patients with CKD on and not on hemodialysis.[35–37] The relative risk of incident CVD for a 1 standard deviation increase in CEC was 0.92 (95% CI 0.83–1.02) in hemodialysis patients.[35] CEC measured in Stages 3 to 5 CKD patients' serum was greater than that in control subjects, whereas the CEC measured with isolated HDL was similar to that in control subjects.[38]

As preβ1-HDL was markedly higher in patients with advanced-stage CKD on CAPD (Table 3) and hemodialysis,[23] CEC may increase in these patients. Cellular cholesterol efflux is comprised ABCA1-dependent, ABCG1-dependent, and scavenger receptor BI–dependent pathways.[39] Free or lipid-poor ApoAI is the acceptor of the ABCA1-dependent pathway, whereas lipid-rich spherical HDL is the acceptor of the other pathways. About two-thirds of cholesterol efflux is proportional to preβ1-HDL concentration.[40] The preβ1-HDL concentration gradually increased from Stage 3 to Stages 4 and 5 in CKD patients (Table 3). Furthermore, CAPD patients had 2.2-fold higher preβ1-HDL concentrations than control subjects (Table 3). Kuchta et al. reported the same tendency in CKD Stages 3a, 3b, and 4 patients.[41] Many reports showed that preβ1-HDL increases in patients with CVD risk factors, such as hypercholesterolemia,[18,19,42] hypertriglyceridemia,[20,43] type 2 diabetes,[21,22] and coronary artery disease.[15–17,44,45] In type 2 diabetic patients, the preβ1-HDL concentration was positively correlated with the maximum intima-media thickness and plaque score.[21] However, their preβ1-HDL concentration was only 21%–70% higher than that of the controls, which was much lower than those in the Stages 4 and 5 CKD and CAPD groups.[15,18–22] Therefore, the effect of high preβ1-HDL concentrations on CEC measurement is much less in these patients than in CKD patients.

Previous studies have indicated that plasma preβ1-HDL concentration can increase because of either enhanced

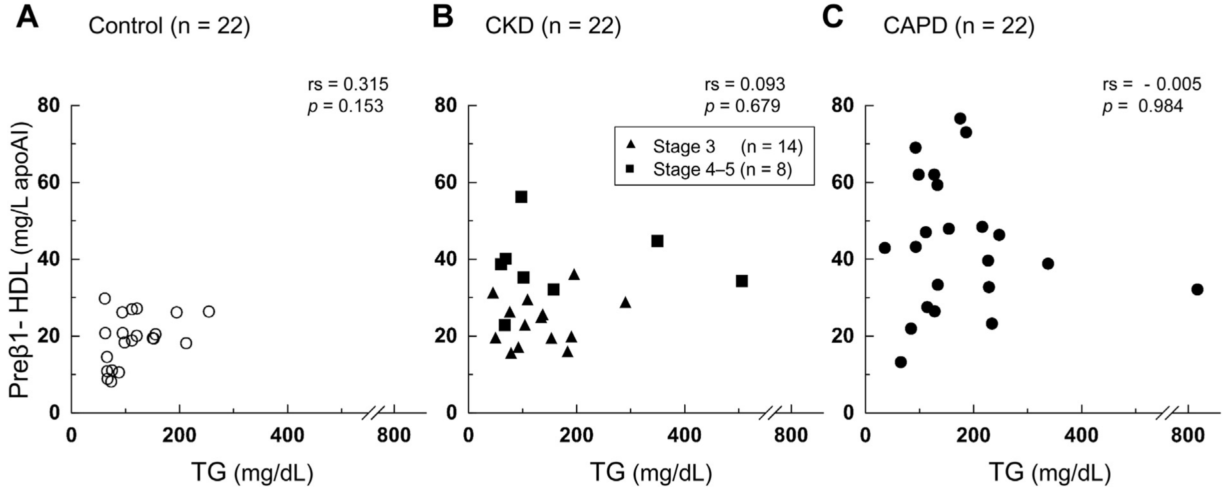

Figure 2 Correlation between TG and preβ1-HDL concentrations in the Control (A), CKD (B), and CAPD (C) groups. TG, triglycerides; CKD, chronic kidney disease; CAPD, continuous ambulatory peritoneal dialysis. Spearman's rank correlation coefficient was used to assess correlations between 2 variables.

production or impaired catabolism. ABCA1 interacts with free ApoAI and promotes preβ1-HDL biogenesis.[46] Hepatic lipase, endothelial lipase, cholesteryl ester transfer protein (CETP), and PLTP promote preβ1-HDL formation.[20,47] Preβ1-HDL can be generated by serum amyloid A–induced HDL remodeling.[48] In one study, CETP and PLTP activities did not change in end-stage renal disease.[49] In other studies, PLTP activity was increased in CAPD and hemodialysis patients, although PLTP activity was measured using a commercial fluorometric kit,[50,51], which showed conflicting results to the gold-standard radiometric assay.[52,53] Therefore, we quantified the velocity of increase in preβ1-HDL concentration in the presence of DTNB because PLTP promotes preβ1-HDL formation.[31] Slope $(DTNB)_{pre\beta1}$ was significantly lower in the CKD and CAPD groups than the Control group (Table 3), suggesting that PLTP-mediated conversion is impaired in these patients. This hypothesis should be examined in a future study.

We speculate that $CHT_{pre\beta1}$ is determined mainly by α-LCAT activity and reflects HDL function not related to cholesterol efflux. LCAT activity is associated with HDL (α-LCAT activity) as well as very-low-density lipoprotein and LDL (β-LCAT activity).[54] Patients with fish-eye disease lack α-LCAT activity but have preserved β-LCAT activity.[55] Although total LCAT activity is near normal, plasma preβ1-HDL is not converted into large HDL at 37°C. In the present study, the median LCAT activity (including α- and β-LCAT activity) was only 29% and

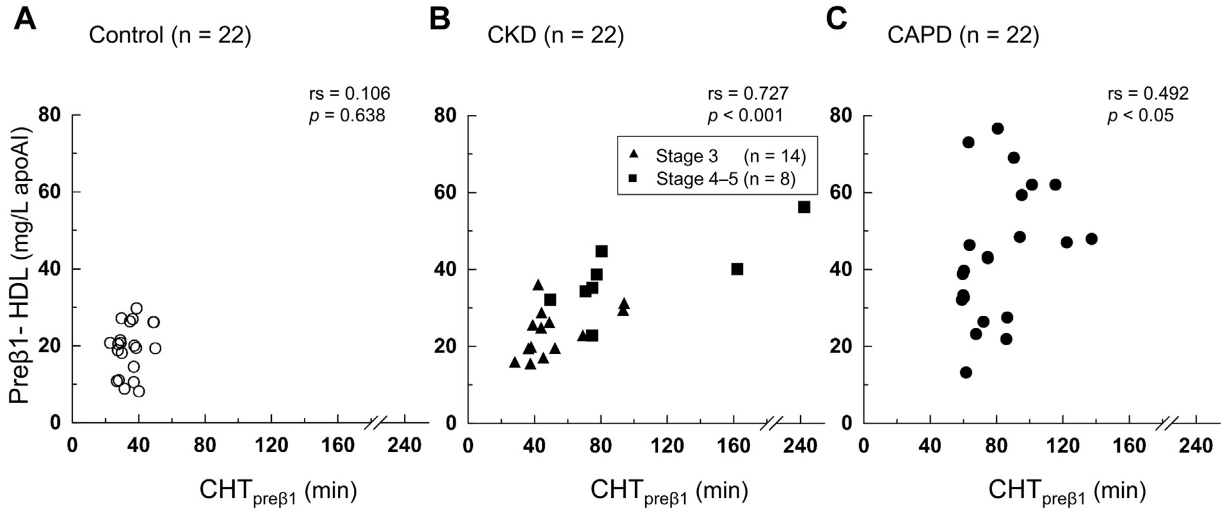

Figure 3 Correlation between $CHT_{pre\beta1}$ and preβ1-HDL concentration in the Control (A), CKD (B), and CAPD (C) groups. $CHT_{pre\beta1}$, conversion halftime of preβ1-HDL; CKD, chronic kidney disease; CAPD, continuous ambulatory peritoneal dialysis; DTNB, 5, 5-dithio-bis (2-nitrobenzoic acid). Fresh plasma was obtained from Control and CKD patients and incubated with or without DTNB for up to 120 min. The preβ1-HDL concentration was measured every 30 min via immunoassay. $CHT_{pre\beta1}$ was determined as the time taken for half of the baseline preβ1-HDL to be converted into mature spherical HDL by LCAT. Spearman's rank correlation coefficient was used as described in Figure 2.

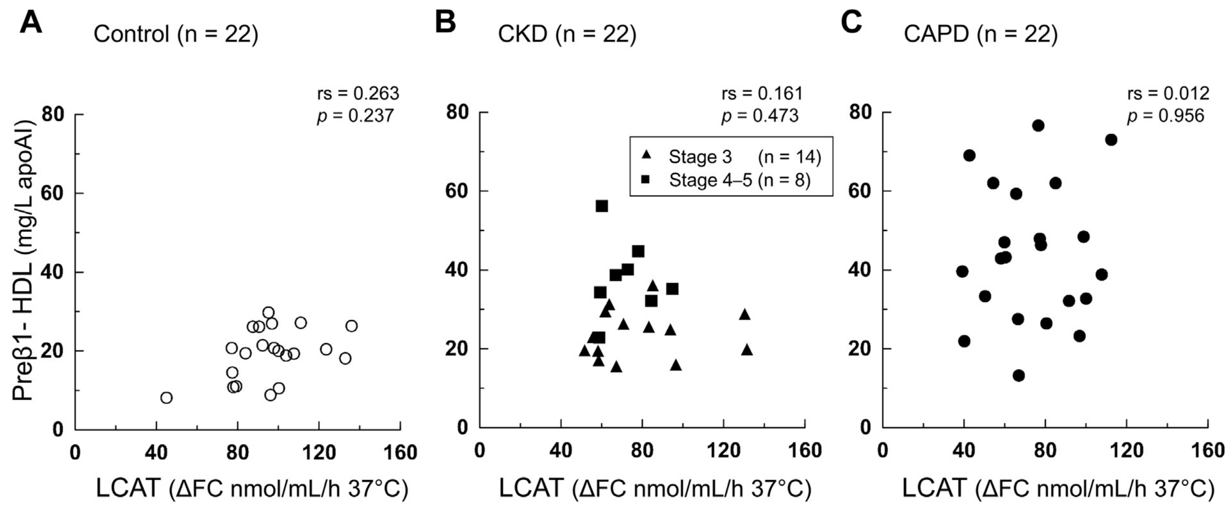

Figure 4 Correlation between LCAT activity and preβ1-HDL concentration in the Control (A), CKD (B), and CAPD (C) groups. LCAT, lecithin–cholesterol acyltransferase; CKD, chronic kidney disease; CAPD, continuous ambulatory peritoneal dialysis. Spearman's rank correlation coefficient was used as described in Figure 2.

26% lower in CKD and CAPD patients than in control subjects, respectively. However, $CHT_{pre\beta1}$ was markedly prolonged in CKD and CAPD patients (Table 3). Kuchta et al. measured the concentrations of preβ1-HDL and HDL containing ApoAI but not ApoAII (designated as LpAI) in Stages 3 and 4 CKD patients not on hemodialysis.[41] The preβ1-HDL concentration increased significantly, but LpAI tended to decrease as the CKD stage progressed. LpAI concentration reflects large HDL subfractions, but not small HDL subfractions, including preβ1-HDL.[56] The high preβ1-HDL/LpAI ratio in CKD patients was likely because of impaired LCAT-dependent preβ1-HDL conversion to the large HDL subfractions. It remains to be determined why LCAT-dependent preβ1-HDL conversion is more severely impaired than expected based on the LCAT activity level in CKD patients.

$CHT_{pre\beta1}$ may be used as a biomarker of HDL dysfunction in CKD patients. In our previous studies, $CHT_{pre\beta1}$ did not differ between men and women, younger and older subjects,[27] normolipidemic and hyperlipidemic subjects,[57] or smokers and nonsmokers.[57] $CHT_{pre\beta1}$ did not change in probucol-treated patients whose preβ1-HDL concentration increased significantly.[28] Furthermore, $CHT_{pre\beta1}$ is not affected by PLTP-induced preβ1-HDL formation (Fig. 1). As the preβ1-HDL concentration was strongly correlated with $CHT_{pre\beta1}$ (Fig. 3B and C), it could be used instead of $CHT_{pre\beta1}$ in CKD patients.

Some limitations of our study should be acknowledged. First, the number of CKD patients was relatively small. Second, the present study was a cross-sectional case–control study and could not clarify cause-and-effect relationships. Third, we used DTNB to inhibit LCAT activity. Although DTNB is a nonspecific sulfhydryl inhibitor, it is commonly used as an LCAT inhibitor in lipoprotein research. However, it can react with the sulfhydryl groups of other enzymes and might interfere with lipoprotein metabolism in an unexpected way. Fourth, we did not examine the association between preβ1-HDL concentration and $CHT_{pre\beta1}$ with atherosclerosis. A prospective longitudinal study is required to answer these questions.

In conclusion, our findings indicate that preβ1-HDL metabolism is delayed in CKD patients not on hemodialysis. Such a metabolic delay of preβ1-HDL is likely to progress as renal function declines. We speculate that the preβ1-HDL concentration could be used as a clinical marker for HDL dysfunction in CKD patients.

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Author's contributions: All authors confirm that they have contributed to the intellectual content of this article and have met the following 3 requirements: (1) significant contributions to the study conception and design, data acquisition, or analysis and interpretation of the data; (2) drafting or revising the article for intellectual content; and (3) final approval of the published article.

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Disclosure

The authors have no conflicts of interest to disclose.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jacl.2020.07.011.

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