Abnormal HDL Apolipoprotein A-I and A-II Kinetics in Hemodialysis Patients: A Stable Isotope Study

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Abstract. Low levels of HDL cholesterol and its major apoprotein constituents apoA-I and apoA-II are common in patients who have ESRD and are undergoing hemodialysis (HD), but the metabolic basis for the low HDL is poorly understood. This study aimed to investigate in vivo metabolism of apoA-I and apoA-II in five normotriglyceridemic ESRD-HD patients and compared it with five control subjects using endogenous stable isotope labeling methods coupled with a multicompartamental modeling. HDL cholesterol, apoA-I, and apoA-II levels were markedly decreased in the ESRD-HD patients by 39, 30, and 44%, respectively, in comparison with the control subjects. Fractional catabolic rate of apoA-I was found to be significantly increased by 59% to 0.360 ± 0.084/d in ESRD-HD patients as compared with control subjects of 0.227 ± 0.076/d (P = 0.028), whereas the production rates remained unchanged. Conversely, the apoA-II production rate significantly decreased by 31% to 1.50 ± 0.61 mg/kg per d in the ESRD-HD patients in comparison with control subjects of 2.17 ± 0.40 mg/kg per d (P = 0.047) with apoA-II fractional catabolic rate unchanged. These results revealed that the decreased levels of apoA-I are due solely to the increased rate of catabolism, whereas the reduced apoA-II levels are due primarily to the decreased rate of production in ESRD-HD patients. This differential regulation of apoA-I and apoA-II further supports the concept that apoA-I and apoA-II have distinct metabolic pathways.

Patients who have ESRD and undergo hemodialysis (HD) are at increased risk for coronary artery disease (CAD) (1,2), which is due at least in part to lipid abnormalities, typically called uremic dyslipidemia (3). One of the major lipid abnormalities of uremic dyslipidemia is a decreased level of HDL cholesterol (HDL-C) (4–6). In general, low HDL-C is often associated with elevated triglyceride (TG) levels, and this inverse relation is also observed in ESRD-HD patients (7), suggesting a possibility that hypertriglyceridemia but not uremia per se may be an underlying mechanism for the decreased levels of HDL. However, TG levels in ESRD-HD patients in Japan (5–7) are generally lower than counterparts in Western countries (4,8), and HDL-C in ESRD-HD patients is still lower than in control subjects with comparable TG levels (7), indicating that the decreased HDL-C is not due simply to hypertriglyceridemia in ESRD-HD patients.

Plasma concentrations of HDL-C and apoA-I, the major protein constituent of HDL, have been shown to be inversely associated with the incidence of CAD (9–11). Although the mechanism by which HDL may exert a direct protective effect against development of atherosclerosis is not yet well understood, HDL has been postulated to facilitate the efflux of cholesterol from peripheral tissues and transport it back to the liver in a process called reverse cholesterol transport (12). HDL metabolism is regulated by several enzymes, including hepatic triglyceride lipase (HTGL), lecithin:cholesterol acyltransferase (LCAT), and cholesteryl esters transfer protein (CETP). Previous studies reported decreased HTGL activity (13,14) and decreased LCAT activity (14,15) but inconsistent results in CETP activity (16,17) in ESRD-HD patients. Thus, it is important to assess whether and, if so, how these altered enzyme activities modulate synthesis and catabolism of HDL in vivo.

Unfortunately, little is known about the metabolic basis for the decreased HDL in ESRD-HD patients. To our knowledge, no HDL apolipoprotein kinetic studies in ESRD-HD patients have been reported to date. One kinetic study was reported by Fuh et al. (18) in which only apoA-I metabolism was studied in predialysis patients by using a conventional radiotracer technique. Because the majority of ESRD patients eventually need maintenance dialysis and the number of ESRD-HD patients has been increasing, a better understanding of the metabolic basis for the decreased levels of HDL in ESRD-HD patients is an urgent task and should provide a substantial clinical benefit. We therefore conducted an in vivo metabolism study of apoA-I and apoA-II in normotriglyceridemic ESRD-HD patients using endogenous stable isotope labeling.
were on medications that affect lipid metabolism. The study was

\[ \text{average hemodialysis was 7.9 yr. None of the study subjects, including} \]

\[ \text{disease in one patient (patient 5). The average duration of mainte-} \]

\[ \text{ration was chronic glomerulonephritis in four patients and polycystic kidney} \]

\[ \text{determination analysis.} \]

\[ \text{methods coupled with a multicompartmental modeling for data} \]

\[ \text{Materials and Methods} \]

\[ \text{Study Subjects} \]

\[ \text{Five ESRD-HD patients (three men and two women) and five} \]

\[ \text{control subjects (all men) were recruited for this study. Eligibility} \]

\[ \text{criteria of ESRD-HD patients included age between 40 and 70 yr,} \]

\[ \text{body mass index <25 kg/m², fasting plasma triglyceride concentra-} \]

\[ \text{tion <150 mg/dl, and no evidence of diabetes. The cause of ESRD} \]

\[ \text{was chronic glomerulonephritis in four patients and polycystic kidney} \]

\[ \text{disease in one patient (patient 5). The average duration of mainte-} \]

\[ \text{nance hemodialysis was 7.9 yr. None of the study subjects, including} \]

\[ \text{the ESRD-HD patients, had any history of familial hyperlipidemia or} \]

\[ \text{were on medications that affect lipid metabolism. The study was} \]

\[ \text{approved by the Ethics Committee of Jikei University School of} \]

\[ \text{Medicine. All study subjects gave their written informed consent to} \]

\[ \text{take part in this study.} \]

\[ \text{HDL Kinetic Study} \]

\[ \text{The study subjects were admitted to Jikei University Hospital} \]

\[ \text{(Shinbashi Hospital or Aoto Hospital) before the kinetic study. The} \]

\[ \text{stable-isotope turnover study was begun at 5 a.m. after 12-h fasting.} \]

\[ \text{Then, the subjects were fed hourly for 15 h, and each meal consisted} \]

\[ \text{of 1/15th of their required calories. In all ESRD patients, the study} \]

\[ \text{was performed 1 d after hemodialysis. Two plastic indwelling cath-} \]

\[ \text{eters were placed intravenously on contralateral arm veins: one cath-} \]

\[ \text{eter was used for the tracer infusion, and the other was used for} \]

\[ \text{the frequent blood sampling during the study. Three times deutermium} \]

\[ \text{labeled l-leucine (l-leucine-methyl-D3; Cambridge Isotope Labora-} \]

\[ \text{tories, Woburn, MA) was administered as a priming bolus of 1.0} \]

\[ \text{mg/kg at 8 a.m., immediately followed by a constant infusion of 1} \]

\[ \text{mg/kg per h for 12 h. Blood samples (15 ml) were drawn into tubes} \]

\[ \text{containing EDTA at a final concentration of 1 g/L before the tracer} \]

\[ \text{injection; after 10 min, then 1, 2, 3, 4, 5, 6, 8, 10, 12, 13, 14, 18, 24,} \]

\[ \text{36, and 48 h and an additional sampling at 72 h in all control subjects.} \]

\[ \text{Blood was kept on ice, and the plasma was immediately separated by} \]

\[ \text{centrifugation (3000 × g, 20 min at 4°C). NaN₃ and aprotinin were} \]

\[ \text{added to the plasma at final concentrations of 0.5 g/L and 200,000} \]

\[ \text{KIU/L, respectively.} \]

\[ \text{Isolation of Lipoproteins and Apolipoproteins} \]

\[ \text{HDL was isolated by an ultracentrifugation from 4 ml of plasma} \]

\[ \text{and proceeded for the analysis by a gas chromatography–mass spec-} \]

\[ \text{trometry (GC-MS) as previously reported (19). Briefly, isolated HDL} \]

\[ \text{(density 1.063 to 1.21 g/ml) was dialyzed against 10 mM ammonium} \]

\[ \text{bicarbonate, lyophilized, then delipidated. ApoA-I was isolated by} \]

\[ \text{preparative gradient SDS-PAGE (5 to 15%) (20), and apoA-II was} \]

\[ \text{isolated by preparative isoelectric focusing (pH 4 to 6) (21).} \]

\[ \text{Determination of Tracer/Tracee Ratio by GC-MS} \]

\[ \text{Samples were prepared for GC-MS analysis as reported previously} \]

\[ \text{(19–21). Briefly, apolipoprotein bands were cut from gels and dried} \]

\[ \text{overnight (90°C) and then were hydrolyzed in 6 N HCl (amino acid} \]

\[ \text{analysis grade; Wako Pure Chemical Industries, Osaka, Japan) at} \]

\[ \text{110°C for 24 h. The protein hydrolysates were lyophilized in a} \]

\[ \text{Speed-Vac evaporator (Savant Instrument, Farmingdale, NY). Free} \]

\[ \text{amino acids were purified from plasma or protein hydrolysates by} \]

\[ \text{cation exchange chromatography (AG-50W-X8; Bio-Rad Laborato-} \]

\[ \text{ries, Richmond, CA) and then derivatized to the N-heptafluorobutyl-} \]

\[ \text{isobutyl esters and analyzed by GC-MS on a 6890 gas chromatograph} \]

\[ \text{connected to a 5973 quadruple mass spectrometer (Hewlett Packard,} \]

\[ \text{Palo Alto, CA) in the chemical ionization mode, using methane as the} \]

\[ \text{reagent gas. Selective ion monitoring at 365 m/z (M + 2 isotopomer) for} \]

\[ \text{unlabeled leucine and 366 m/z (M + 3 isotopomer) for labeled} \]

\[ \text{leucine was used to determine the tracer/tracee (T/T) ratio by regres-} \]

\[ \text{sion analysis of standards of known T/T ratios (0 to 10%) as reported} \]

\[ \text{previously (22,23). Each sample was analyzed at least two times.} \]

\[ \text{Kinetic Modeling} \]

\[ \text{A multicompartmental model (Figure 1) was developed to deter-} \]

\[ \text{mine apoA-I and apoA-II kinetic parameters using an interactive} \]

\[ \text{computer program (SAAMII version 1.1; SAAM Institute, Seattle,} \]

\[ \text{WA) (24). In brief, compartment 1 represents the plasma amino acid} \]

\[ \text{pool, and plasma leucine T/T data are fitted by a three-exponential} \]

\[ \text{equation using SAAM II numerical module. The equations below} \]

\[ \text{describe the exponential function and consist of two parts, the first} \]

\[ \text{part representing T/T during the primed-constant infusion and the} \]

\[ \text{second part representing the T/T decay during the washout phase after} \]

\[ \text{the termination of infusion at 12 h.} \]

\[ \text{Plasma leucine T/T = swit * infusion + washout} \]

\[ \text{Infusion} = A0 + A1 * exp(−a1 * t) + A2 * exp(−a2 * t) + A3 * exp(−a3 * t) \]

\[ \text{Infusion12} = A0 + A1 * exp(−a1 * 12) + A2 * exp(−a2 * 12) + A3 * exp(−a3 * 12) \]

\[ \text{Washout} = (1 − swit) * infusion12 * (B1 * exp[−a1 *(t − 12)] + (1 − B1) * B2 * exp[−a2 *(t − 12)] + (1 − B1) * (1 − B2) * exp[−a3 *(t − 12)]) \]

\[ \text{At 12 h, “swit” is changed from 1 to 0 using a change condition} \]

\[ \text{function in SAAMII. Therefore, during the infusion period, “Plasma} \]

\[ \text{leucine T/T” equal to “infusion,” then to “washout” after 12 h.} \]

\[ \text{SAAMII can determine the best estimate of the parameters (A1, B1,} \]

\[ \text{and B2) to fit actual plasma T/T data, which are then implanted into the} \]

\[ \text{equation window in the multicompartmental model and used as a} \]

\[ \text{forcing function. The plasma leucine T/T curve of the mean of five} \]

\[ \text{control subjects is shown in Figure 2. Fitting curve precisely traces} \]

\[ \text{the observed T/T points, and fractional SD of most parameters reside} \]

\[ \text{within 20%. Compartment 2 accounts for a delay of HDL assembly} \]

\[ \text{and subsequent secretion. A single compartment (compartment 3) is} \]

\[ \text{figure 1. Multicompartmental model for HDL apolipoproteins. Com-} \]

\[ \text{partment 1 represents the plasma amino acid (leucine) pool, used as a} \]

\[ \text{forcing function. Compartment 2 accounts for delay for assembly} \]

\[ \text{and subsequent secretion of HDL apolipoproteins. HDL apolipoproteins} \]

\[ \text{apoA-I, apoA-II) comprise a single pool, compartment 3 (see the} \]

\[ \text{Materials and Methods for details).} \]
production rate (PR; mg/kg per d), a product of FCR and PV, was
considered to have no intravascular fluid expansion. We therefore used
patients in the present study all were normotensive, they were con-
nmotensive ESRD-HD patients are selected. Because the ESRD-HD
similar between ESRD-HD patients and control subjects when nor-
denotes blood volume. On the basis of the literature (29
* apolipoprotein concentration in plasma *(1
Analytical Methods
Materials and Methods for details).

allocated for the HDL apoA-I or apoA-II pool. Originally, an
extravascular pool was set up to allow the movement of HDL between
the plasma and extravascular pool. However, the exchange pathway
hit 0 after iteration and did not improve the overall fitting in all study
subjects. We therefore decided to eliminate this pathway. Plasma
concentrations of apoA-I and apoA-II were measured during the study
period to find that percentage changes of apoA-I and apoA-II were
within 5.6 and 4.4% in control subjects and 1.7 and 3.0% in
ESRD-HD patients, respectively, none of which was statistically
significant. Therefore, we assumed that steady-state conditions were
maintained throughout the study period, under which fractional cata-
bolic rate (FCR; /d) was equal to fractional synthetic rate (FSR).
Several studies (25,26) showed significantly increased plasma volume
(PV), whereas other studies showed similar to modestly increased PV
(a review by Mitch WE (27)). Overall, results of PV in ESRD-HD are
inconsistent, partially depending on the presence or absence of hy-
pertension, malnutrition, anemia, and methods used for PV determi-
nation (evans blue, iodinated131 I albumin, 51 Cr-labeled red blood
cell). Thus, we adjusted the PV by hematocrit (Hct) values using a
method recently reported by Mitra et al. (28). In brief, PV was
calculated by the formula PV = BV(1 - 0.86 * Hct), where BV
denotes blood volume. On the basis of the literature (29–32), BV is
similar between ESRD-HD patients and control subjects when nor-
motensive ESRD-HD patients are selected. Because the ESRD-HD
patients in the present study all were normotensive, they were con-
cidered to have no intravascular fluid expansion. We therefore used
7% as the percentage BV (% BV/body weight [BW]) in both
ESRD-HD patients and control subjects (29,33). Taken together, production rate (PR; mg/kg per d), a product of FCR and PV, was
calculated by the following formula:

\[
PR = FCR \times \frac{\text{apolipoprotein concentration in plasma}}{\text{PV/BW}} = FCR \times \frac{\text{apolipoprotein concentration in plasma}}{1 - 0.86 \times Hct} \times \frac{\text{PV/BW}}{\text{Hct}} = FCR \times \frac{\text{apolipoprotein concentration in plasma}}{1 - 0.86 \times Hct} \times 0.07
\]

Analytical Methods
The plasma total cholesterol and TG levels were determined by the
automated enzymatic technique using a Toshiba TBA-80FR auto-
analyzer (Toshiba, Tokyo, Japan). HDL-C was measured after hepa-
rin-manganese precipitation of plasma LDL-C was calculated using
the Friedewald formula. Plasma apoA-I and apoA-II concentrations
were quantified using immunoturbidimetric assays (34). CETP mass
was measured by a sandwich ELISA using two monoclonal antibod-
ies, JHC1 and JHC2, as reported previously (35). LCAT activity in
plasma was determined by a method using dimyristoyl phosphatid-
choline-cholesterol liposome as substrate (36). HTGL activity in
post-heparin plasma collected 10 min after intravenous injection of
heparin (30 U/kg body wt) was measured as the rate of radiolabeled
fatty acids liberation from [14C]triolein emulsion in gum arabic (13).

Statistical Analyses
Comparisons between groups were performed using Mann-Whit-
ney rank sum test. P < 0.05 was considered to be statistically
significant. All statistical procedures were performed using SPSS
software (version 9.1; SPSS, Chicago, IL).

Results
The plasma and lipoproteins lipid and apolipoprotein profiles are summarized in Table 1. The average total cholesterol
was significantly lower in the ESRD-HD patients (153 ± 11
mg/dl) than in control subjects (175 ± 8 mg/dl; P = 0.016
versus ESRD-HD patients), primarily as a result of a markedly
decreased HDL-C level (31.2 ± 5.3 mg/dl in ESRD-HD pa-
ients versus 51.1 ± 4.6 mg/dl in control subjects; P = 0.009).
In line with previous findings in the literature, LDL-C levels in
HD patients did not differ from those in control subjects. In
parallel to the decreased HDL-C, both apoA-I and apoA-II
levels in ESRD-HD patients significantly decreased by 30 and
44%, respectively, compared with control subjects. It should be
noted that the magnitude of decrease in apoA-II was somewhat
greater than that in apoA-I. As a result, the apoA-I/apoA-II ratio was nonsignificantly increased by 40% in the ESRD-HD
patients in comparison with the control subjects (6.74 ± 2.50
versus 4.83 ± 0.35; P = 0.17), thus indicating that the metab-
olism of HDL with apoA-I and apoA-II may be more
profoundly modulated than those with apoA-I but without apoA-II
in ESRD-HD patients. CETP masses were comparable be-
tween ESRD-HD patients and control subjects, and, although
not significant, LCAT activities were decreased by 30% in
ESRD-HD patients as compared with control subjects, a find-
ing consistent with previous studies (14,15). Likewise, HTGL
activities in three ESRD-HD patients were significantly
decreased by 33% in comparison with the control subjects in our
previous study (13).

The T/T ratio curves for apoA-I and apoA-II are shown in
Figure 3. In both subject groups and proteins, the calculated
curves generated by the multicompartmental model using
SAAMII yielded reasonable fittings. The mean T/T ratio of
apoA-I in the ESRD-HD patients reached a higher level and
thereafter decreased more rapidly than that in the control
subjects (Figure 3, top). In contrast, the T/T ratio of apoA-II in
the ESRD-HD patients increased to reach a somewhat higher
level, but the disappearance slope was almost identical to that
in the control subjects (Figure 3, bottom). Higher T/T peaks in
during the infusion period in ESRD-HD patients (9.32 ±
1.92%) than in control subjects (7.98 ± 1.77%).

![Figure 2. Tracer/tracer (T/T) ratio curve for plasma leucine in control subjects. Plasma leucine T/T curve is used as a forcing function in the multicompartmental model for apoA-I and apoA-II (Figure 1). All data points are given as the means of five control subjects and fitted by the three exponential model using SAAMII numerical model (see Materials and Methods for details).](image-url)
The kinetic parameters are summarized in Table 2. As expected by the T/T curve (Figure 3), the mean apoA-I FCR significantly increased by 59% to 0.360 ± 0.084/d in ESRD-HD patients as compared with control subjects of 0.227 ± 0.076/d (P = 0.028). PR of apoA-I were not different between groups, although the average PR in ESRD-HD groups was 36% higher than that in the control group (P = 0.117). As a result, the decreased apoA-I levels in ESRD-HD patients were primarily due to the increased rate of catabolism. We found a different metabolic alteration of apoA-II in the ESRD-HD patients. The apoA-II FCR were found to be similar between the two groups (0.196 ± 0.059/d in the ESRD-HD patients versus 0.186 ± 0.031/d in the control subjects), which is consistent with similar disappearance curves in Figure 3. In contrast, the apoA-II PR decreased significantly by 31% to 1.50 ± 0.61 g/kg per d in ESRD-HD patients as compared with control subjects of 2.17 ± 0.40 mg/kg per d (P = 0.047). Therefore, the decreased apoA-II levels in ESRD-HD patients were attributable to the decreased rates of production.

Discussion

In the present study, we demonstrate for the first time the metabolic basis for the two major HDL protein constituents,
Table 2. Kinetic parameters of ApoA-I and ApoA-II in ESRD-HD patients

<table>
<thead>
<tr>
<th>Subjects</th>
<th>ApoA-I</th>
<th></th>
<th>ApoA-II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (mg/dl)</td>
<td>FCR (/d)</td>
<td>PR (mg/kg per d)</td>
<td>Concentration (mg/dl)</td>
</tr>
<tr>
<td>ESRD-HD patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>98</td>
<td>0.358</td>
<td>19.35</td>
<td>15.9</td>
</tr>
<tr>
<td>2</td>
<td>89</td>
<td>0.302</td>
<td>14.30</td>
<td>7.7</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>0.297</td>
<td>13.97</td>
<td>18.9</td>
</tr>
<tr>
<td>4</td>
<td>89</td>
<td>0.342</td>
<td>15.39</td>
<td>14.1</td>
</tr>
<tr>
<td>5</td>
<td>87</td>
<td>0.503</td>
<td>22.72</td>
<td>17.8</td>
</tr>
<tr>
<td>mean ± SD</td>
<td>91 ± 5a</td>
<td>0.360 ± 0.084b</td>
<td>17.15 ± 3.78</td>
<td>14.9 ± 4.4c</td>
</tr>
<tr>
<td>Control subjects</td>
<td>(n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>129 ± 11</td>
<td>0.227 ± 0.076</td>
<td>12.59 ± 3.78</td>
<td>26.7 ± 1.9</td>
</tr>
</tbody>
</table>

a FCR, fractional catabolic rate; PR, production rate.
b P < 0.05.
c P < 0.01 compared with control subjects.

apoA-I and apoA-II, in ESRD-HD patients. The decreased apoA-I and apoA-II levels are entirely due to the increased rate of catabolism for the former and the decreased rate of production for the latter, respectively. We (37) and others (38,39) have previously shown the rate of catabolism to be the determinant of the plasma apoA-I level, whereas the rate of production is the determinant of the plasma apoA-II levels in normolipidemic humans. Therefore, apoA-I FCR and apoA-II PR as the primary regulators for their steady-state concentration is the determinant of the plasma apoA-II levels in ESRD-HD patients. Different metabolic regulations between apoA-I and apoA-II provided further evidence that HDL subclasses have different metabolic pathways depending on the presence or absence of apoA-II on HDL particles (37,40–42). Although this study is not able to identify the primary cause of the increased catabolism of apoA-I, several mechanisms might contribute to these findings. First, although statistically not significant, we found that LCAT activities were decreased by 30% in ESRD-HD patients as compared with control subjects, a finding consistent with previous studies (14,15). Recently, Miida et al. (43) showed that LCAT-dependent conversion of pre–β1-HDL (nascent HDL) into α-migrating HDL (mature HDL) was severely impaired in HD patients. This finding, together with in vivo kinetic data by Colvin et al. (44), who reported that pre–β1-HDL was a precursor to larger HDL particles in African green monkeys, indicates that LCAT, in concert with apoA-I, play a crucial role on the conversion of premature HDL into large cholesteryl esters-rich HDL particles. As a result, the depressed LCAT activity could lead more HDL particles to be removed directly from the circulation before nascent HDL matures, thus resulting in an increased overall apoA-I catabolism, as typically shown in patients with an inborn deficiency of LCAT (45). Second, an altered HDL composition associated with ESRD-HD (16) might lead to a dissociation of more apoA-I and subsequent transfer to TG-rich lipoproteins (chylomicron and VLDL), which are, in turn, removed at much faster rates than HDL and/or an altered affinity for cell-surface receptors including scavenger receptor class B type I. Plasma C-reactive protein levels were mildly elevated (0.3 to 0.8 mg/dl) in four ESRD-HD patients, suggesting an inflammation in the ESRD-HD patients. Inflammation has been shown to be associated with reduced levels of HDL (46). Under inflammatory conditions, serum amyloid A, an acute-phase reactant, is synthesized by the liver and displaces apoA-I from HDL, resulting in an accelerated apoA-I catabolism (47,48). Although we did not directly measure serum amyloid A concentrations, this can be a third reason. Beside LCAT, HTGL has been considered to be a factor modulating HDL. In this study, preliminary results of HTGL activities in three ESRD-HD patients were found to be decreased as compared with control subjects in our previous study (13). The decreased HTGL activity is supported by the study by Shoji et al. (14). Rashid et al. (49) reported that HTGL accelerates apoA-I catabolic rate in rabbit models. Therefore, the decreased HTGL activity can be a reason for delayed apoA-I catabolism, but our kinetic data show otherwise. Overall, it is indicated that alterations in other metabolic factors surpass the effect of the decreased HTGL activity in our ESRD-HD patients.

Of note is a nonsignificant increase in apoA-I PR in the ESRD-HD patients (36% increase versus control subjects). The exact mechanism remains unclear at present; collective data of in vivo albumin (25,26,50) and fibrinogen kinetics (25) demonstrated that these protein synthesis were increased in ESRD-HD patients. They speculated that increased protein synthesis might represent a compensatory response to decreased osmotic pressure caused by PV expansion. In this regard, increased apoA-I synthesis could be explained by the same mechanism. Why was apoA-II production markedly decreased in ESRD-HD patients in contrast to other proteins mentioned above? We could not identify the exact mechanism, either, because factors that regulate apoA-II PR per se have been poorly understood. This could be due to a different tissue response to uremia, as apoA-I is synthesized in the liver and
intestine, whereas apoA-II derives exclusively from the liver. Indeed, apoA-I and apoA-II show a different transcriptional response in an animal model (51). As reported previously, decreased apoA-II synthesis observed in this study is consistent with the concept that apoA-II synthesis is a primary determinant for the plasma apoA-II levels. It may be that apoA-II synthesis in ESRD-HD is still low with the compensatory action. This issue certainly deserves future studies.

The present observation contrasts with the previous observation by Fuh et al. (18), who found a delayed catabolism as well as a decreased rate of production of apoA-I in predialysis patients with chronic renal failure (CRF). Although the exact reason for this discrepancy is not clear, several differences do exist between the two studies. First, the patients in their study were not treated by HD, whereas our patients all underwent HD. Although most studies observed similar HDL-C levels between predialysis and ESRD-HD patients (4,5,8), HD may further exacerbate the already-existing abnormal HDL metabolism in CRF patients. On the basis of previous kinetic findings, decreased apoA-I always associates with increased rate of catabolism, which compares favorably to the present study but not their study. Second, there are some differences in lipid profile between the two studies. In this study, we carefully selected ESRD-HD patients who had decreased levels of HDL-C but normal TG levels to minimize potential confounding effect of hypertriglyceridemia on HDL metabolism. In contrast, CRF patients in their study obviously included hypertriglyceridemic patients. This finding, together with the fact that the apoA-I level in this study showed a greater reduction (−29.4%) than their counterpart (−19.5%), could lead to different alterations on HDL metabolism. A third reason could be different labeling methods used. They used conventional exogenous labeling with a radiotracer, whereas we used endogenous labeling with stable isotope amino acids. In their study, it is not clear whether the radiolabeled apoA-I is an autologous or a homologous protein, and, if the latter is the case, injected apoA-I is not the subject’s own apoA-I, which would in turn behave differently from endogenous apoA-I. In addition, because they used exogenous labeling, the tracer apoA-I may undergo a modification during the labeling procedure, which again causes a potential error in their study. Overall, a kinetic study using endogenous labeling techniques needs to be performed in predialysis patients to conclude their abnormality of HDL. Nonetheless, we believe that our data provide new and concrete information on apoA-I and apoA-II metabolism in ESRD-HD patients.

HDL are heterogeneous in apolipoprotein composition and comprise two major HDL subclasses: particles that contain apoA-I but not apoA-II (LpA-I) and those that contain both apoA-I and apoA-II (LpA-I:A-II) (52). We have previously shown that apoA-I on LpA-I is catabolized faster than apoA-I on LpA-I:A-II (41). Furthermore, we found the LpA-I levels to be inversely correlated with the rate of apoA-I catabolism, whereas the LpA-I:A-II levels closely correlated with the rate of apoA-II production in normolipemic subjects (37). These results thus indicate that apoA-I and apoA-II (i.e., LpA-I and LpA-I:A-II) have distinct metabolic pathways. Taken together, we propose that HDL metabolism in ESRD-HD patients is altered as follows. The decreased apoA-II production in ESRD-HD patients limits the ability of apoA-I to associate with apoA-II, thus altering HDL composition as LpA-I predominant. LpA-I is, in turn, catabolized at a faster rate than normal LpA-I, as evidenced by the increased rate of catabolism. The increased proportion of LpA-I relative to LpA-I:A-II is indeed consistent with previous observations by us (6) and others (53).

There are several limitations in the present study. Although not significant, age and gender were not strictly matched between control subjects and ESRD-HD patients. In general, catabolic rates of apoA-I and apoA-II are somewhat faster in men than in women (37). This finding, together with the fact that the ESRD-HD patients included two women, indicates that difference in apoA-I FCR would have been greater if some control subjects had been women. The effect of age is considered to be minimal relative to gender. Second, the sample size of the study subjects may not be large enough to draw a solid conclusion. This is certainly an inherent limitation of in vivo kinetic study, which involves full collaboration of study subjects as well as labor- and time-intensive procedures to determine kinetic parameters. In this study, we carefully selected ESRD-HD patients who had normal plasma TG concentrations to minimize the potential confounding effects of hypertriglyceridemia on HDL metabolism. Therefore, we believe that the present study reasonably represents the underlying metabolic defects of HDL in normotriglyceridemic ESRD-HD patients. Although further accelerated apoA-I FCR are likely to be expected, a caution should be exercised to apply our results to hypertriglyceridemic ESRD-HD patients, and this requires further study. Third, steady-state condition, which is a prerequisite for the kinetic study, may not be maintained in ESRD-HD patients who lack normal kidney function, but we found that apolipoprotein concentrations did not vary significantly during the study period, thus indicating that perturbation of lipoprotein metabolism is minimal during the study period. Finally, we did not directly measure PV by the tracer dilution method but instead used a formula to correct PV by using Hct to calculate PR. Although the resulted PV expansion in our ESRD-HD patients is comparable to a recent result obtained by the tracer dilution method (25), PR should be interpreted with caution.

In summary, the present study established that decreased levels of apoA-I are due solely to increased rates of catabolism, whereas reduced apoA-II levels are due to decreased rates of production in normotriglyceridemic ESRD-HD patients. This differential modulation of two major apolipoproteins within HDL should provide a basis to help develop a better strategy to ameliorate abnormal HDL metabolism and therefore prevent cardiovascular events in these high-risk patients.

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