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 multicomartmental 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...
methods coupled with a multicompartamental modeling for data analysis.

**Materials and Methods**

**Study Subjects**

Five ESRD-HD patients (three men and two women) and five control subjects (all men) were recruited for this study. Eligibility criteria of ESRD-HD patients included age between 40 and 70 yr, body mass index <25 kg/m², fasting plasma triglyceride concentration <150 mg/dL, and no evidence of diabetes. The cause of ESRD was chronic glomerulonephritis in four patients and polycystic kidney disease in one patient (patient 5). The average duration of maintenance hemodialysis was 7.9 yr. None of the study subjects, including the ESRD-HD patients, had any history of familial hyperlipidemia or were on medications that affect lipid metabolism. The study was approved by the Ethics Committee of Jikei University School of Medicine. All study subjects gave their written informed consent to take part in this study.

**HDL Kinetic Study**

The study subjects were admitted to Jikei University Hospital (Shinbashi Hospital or Aoto Hospital) before the kinetic study. The stable-isotope turnover study was begun at 5 a.m. after 12-h fasting. Then, the subjects were fed hourly for 15 h, and each meal consisted of 1/15th of their required calories. In all ESRD patients, the study was performed 1 d after hemodialysis. Two plastic indwelling catheters were placed intravenously on contralateral arm veins: one catheter was used for the tracer infusion, and the other was used for the frequent blood sampling during the study. The three times deuterium-labeled l-leucine (l-leucine-methyl-D3; Cambridge Isotope Laboratories, Woburn, MA) was administered as a priming bolus of 1.0 mg/kg at 8 a.m., immediately followed by a constant infusion of 1 mg/kg per h for 12 h. Blood samples (15 ml) were drawn into tubes containing EDTA at a final concentration of 1 g/L before the tracer injection; after 10 min, then 1, 2, 3, 4, 5, 6, 8, 10, 12, 13, 14, 18, 24, 36, and 48 h and an additional sampling at 72 h in all control subjects. Blood was kept on ice, and the plasma was immediately separated by centrifugation (3000 × g, 20 min at 4°C). NaN₃ and aprotinin were added to the plasma at final concentrations of 0.5 g/L and 200,000 KIU/L, respectively.

**Isolation of Lipoproteins and Apolipoproteins**

HDL was isolated by an ultracentrifugation from 4 ml of plasma and proceeded for the analysis by a gas chromatography–mass spectrometry (GC-MS) as previously reported (19). Briefly, isolated HDL (density 1.063 to 1.21 g/ml) was dialyzed against 10 mM ammonium bicarbonate, lyophilized, then delipidated. ApoA-I was isolated by preparative gradient SDS-PAGE (5 to 15%) (20), and apoA-II was isolated by preparative isoelectric focusing (pH 4 to 6) (21).

**Determination of Tracer/Tracee Ratio by GC-MS**

Samples were prepared for GC-MS analysis as reported previously (19–21). Briefly, apolipoprotein bands were cut from gels and dried overnight (90°C) and then were hydrolyzed in 6 N HCl (amino acid analysis grade; Wako Pure Chemical Industries, Osaka, Japan) at 110°C for 24 h. The protein hydrolysates were lyophilized in a Speed-Vac evaporator (Savant Instrument, Farmingdale, NY). Free amino acids were purified from plasma or protein hydrolysates by cation exchange chromatography (AG-50W-X8; Bio-Rad Laboratories, Richmond, CA) and then derivatized to the N-heptafluorobutyl iso-butyl esters and analyzed by GC-MS on a 6890 gas chromatograph connected to a 5973 quadruple mass spectrometer (Hewlett Packard, Palo Alto, CA) in the chemical ionization mode, using methane as the reagent gas. Selective ion monitoring at 365 m/z (M + 2 isotopomer) for unlabeled leucine and 366 m/z (M + 3 isotopomer) for labeled leucine was used to determine the tracer/tracee (T/T) ratio by regression analysis of standards of known T/T ratios (0 to 10%) as reported previously (22,23). Each sample was analyzed at least two times.

**Kinetic Modeling**

A multicompartamental model (Figure 1) was developed to determine apoA-I and apoA-II kinetic parameters using an interactive computer program (SAAMII version 1.1: SAAM Institute, Seattle, WA) (24). In brief, compartment 1 represents the plasma amino acid pool, and plasma leucine T/T data are fitted by a three-exponential equation using SAAM II numerical module. The equations below describe the exponential function and consist of two parts, the first part representing T/T during the primed-constant infusion and the second part representing the T/T decay during the washout phase after the termination of infusion at 12 h.

1. Plasma leucine T/T = swit * infusion + washout
2. Infusion = A0 + A1 * exp(-a1 * t) + A2 * exp(-a2 * t) + A3 * exp(-a3 * t)
3. Infusion 12 = A0 + A1 * exp(-a1 * 12) + A2 * exp(-a2 * 12) + A3 * exp(-a3 * 12)

At 12 h, “swit” is changed from 1 to 0 using a change condition function in SAAMII. Therefore, during the infusion period, “Plasma leucine T/T” equal to “infusion,” then to “washout” after 12 h. SAAMII can determine the best estimate of the parameters (Ai, Bi, and ai) to fit actual plasma T/T data, which are then implanted into the equation window in the multicompartmental model and used as a forcing function. The plasma leucine T/T curve of the mean of five control subjects is shown in Figure 2. Fitting curve precisely traces the observed T/T points, and fractional SD of most parameters reside within 20%. Compartment 2 accounts for a delay of HDL assembly and subsequent secretion. A single compartment (compartment 3) is
calculated by the following formula: production rate (PR; mg/kg per d), a product of FCR and PV, was 7% as the percentage BV (% BV/body weight [BW]) in both considered to have no intravascular fluid expansion. We therefore used patients in the present study all were normotensive, they were con-
motensive ESRD-HD patients are selected. Because the ESRD-HD denotes blood volume. On the basis of the literature (29–32), BV is
 inconsistently, partially depending on the presence or absence of hy-
pertension, malnutrition, anemia, and methods used for PV determi-
nation (evans blue, iodinated 131I albumin. 51Cr-labeled red blood
cell). Thus, we adjusted the PV by hematocrit (Hct) values using a
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nation (a review by Mitch WE (27)). Overall, results of PV in ESRD-HD are
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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-
sidered 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:

$$PR = FCR \times \text{apolipoprotein concentration in plasma} \times \frac{PV}{BW} = FCR \times \text{apolipoprotein concentration in plasma} \times \frac{(1 - 0.86 \times Hct) \times BV}{BW} = FCR \times \text{apolipoprotein concentration in plasma} \times \frac{(1 - 0.86 \times Hct) \times 0.07}{0.07}$$

### 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 signifi-
cantly 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-
tients 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 pro-
foundly 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
finding 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 ±
0.35; \( P = 0.17 \)), thus indicating that the metab-
olism of HDL with apoA-I and apoA-II may be more pro-
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finding 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 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,
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 concentrations are applicable to hypoalphalipoproteinemia associated with ESRD-HD patients. Different metabolic regulation 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).--migrating HDL (mature 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

Table 2. Kinetic parameters of ApoA-I and ApoA-II in ESRD-HD patients

| Subjects | ApoA-I | | ApoA-II | |
|----------|--------|----------|--------|
|          | Concentration (mg/dl) | FCR (/d) | PR (mg/kg per d) |
| ESRD-HD patients | | | |
| 1 | 98 | 0.358 | 19.35 |
| 2 | 89 | 0.302 | 14.30 |
| 3 | 90 | 0.297 | 13.97 |
| 4 | 89 | 0.342 | 15.39 |
| 5 | 87 | 0.503 | 22.72 |
| mean ± SD | 91 ± 5 | 0.360 ± 0.084 | 17.15 ± 3.78 |
| Control subjects (n = 5) | | | |
| mean ± SD | 129 ± 11 | 0.227 ± 0.076 | 12.59 ± 3.78 |

*FCR, fractional catabolic rate; PR, production rate.

b P < 0.05.

a > P < 0.01 compared with control subjects.
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 normolipidemic 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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References


44. Colvin PL, Moriguchi E, Barrett PH, Parks JS, Rudel LL: Small HDL particles containing two apoA-I molecules are precursors in vivo to medium and large HDL particles containing three and four apoA-I molecules in nonhuman primates. J Lipid Res 40: 1782–1792, 1999


