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Impaired metabolism of high density lipoprotein in uremic patients

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Impaired metabolism of high density lipoprotein in uremic patients. We measured lipoproteins, apolipoproteins, lipoprotein lipase (LPL), hepatic triglyceride lipase (HTGL), lecithin: cholesterol acyltransferase (LCAT) and parameters of calcium metabolism to evaluate the roles of these enzymes and hypertriglyceridemia for impaired high-density lipoprotein (HDL) metabolism in chronic renal failure, and to examine the impact of altered calcium homeostasis on the lipoprotein-regulating enzymes. The subjects were 25 healthy volunteers and 66 uremic patients, 24 treated with hemodialysis (HD) and 42 with continuous ambulatory peritoneal dialysis (CAPD). Lipoprotein analysis revealed: (1) reduction in HDL cholesterol especially in HDL_2 subfraction; (2) increase in HDL triglyceride; and (3) decreased ratio of HDL_2 cholesterol to HDL_3 cholesterol in both HD and CAPD patients. Simple regression analysis showed: (1) a positive correlation between VLDL triglyceride and triglyceride/cholesterol ratio of HDL; (2) positive correlations of LPL level in post-heparin plasma to cholesterol concentrations in HDL_2, HDL_3 and total HDL, and to apolipoproteins A-I and A-II; and (3) inverse correlations of HTGL to HDL_2 cholesterol and to the ratio of HDL_2 cholesterol/HDL_3 cholesterol. Multiple regression analysis of HDL cholesterol indicated positive association with LPL and inverse correlation with VLDL triglyceride. Four variables including LPL, HTGL, LCAT and VLDL triglyceride explained 51.5% of the variation of HDL cholesterol. HDL_2 cholesterol was associated positively with LPL and negatively with VLDL triglyceride in the model. HDL_3 cholesterol was associated positively with LPL, HTGL and LCAT and inversely with VLDL triglyceride. Stepwise multiple regression analysis indicated that independent predictors of HTGL were gender, parathyroid hormone levels by a mid-portion assay, ionized calcium and age, and that those of LCAT were ionized calcium and age. These results suggest that elevated VLDL and alterations in the enzyme levels contributed to deranged HDL metabolism in uremic patients, and that changes in the enzyme levels were associated with impaired calcium homeostasis.

Patients with chronic renal failure are at an increased risk for atherosclerosis [1], and metabolic abnormalities associated with uremia may stimulate the atherogenesis. Impaired lipoprotein metabolism is often observed in uremic patients [2–4] and one of the major abnormalities is decreased high density lipoprotein (HDL) cholesterol [5, 6]. Although low HDL cholesterol is an independent risk factor for atherosclerosis [7, 8], the mechanism of HDL abnormality in uremia is not fully understood. Plasma lipoproteins are metabolized and regulated by several enzymes including lipoprotein lipase (LPL), hepatic triglyceride lipase (HTGL), and lecithin:cholesterol acyltransferase (LCAT). Previous studies revealed that uremic patients have reduced [9–12] or normal [13, 14] activity of LPL and remarkably low HTGL activity [9–14] in post-heparin plasma. LPL and HTGL have potential effects on HDL metabolism as shown in non-uremic subjects [15–17]. LPL generates precursor of HDL during lipolysis of triglyceride-rich lipoproteins, and HTGL promotes the conversion of HDL_2 to HDL_3. Uremic patients on hemodialysis have low activity of LCAT which esterifies cholesterol and incorporates cholesteryl ester into HDL core [18, 19]. Furthermore, inverse correlation between serum triglyceride and HDL cholesterol levels suggests the interaction of HDL with triglyceride-rich lipoproteins [20]. Thus it is possible that hypertriglyceridemia and alterations of these three enzymes affect HDL metabolism in chronic renal failure, and it is important to determine the mechanisms of changes in these enzymes in uremia. Deranged calcium regulation is another metabolic consequence of uremia. Uremic patients have impaired activation of vitamin D by the kidney, hypocalcemia, hyperphosphatemia, and secondary hyperparathyroidism [21, 22]. Since calcium has diverse effects on biological functions, altered calcium homeostasis may result in other metabolic disturbances. Recent studies by us [23, **Note added in proof**] and others [24, 25] have shown the relationship between calcium and lipoprotein metabolism experimentally. Akmal et al [26] have shown in uremic dogs that excess parathyroid hormone suppressed post-heparin lipolytic activity. These experimental data raise the hypothesis that impaired calcium regulation affects HDL metabolism in uremic patients by altering the levels of the enzymes which are involved in lipoprotein metabolism. The purpose of this study is to evaluate the roles of the enzymes and hypertriglyceridemia for derangement of HDL metabolism in uremic patients, and to examine the possible effects of deranged calcium metabolism on alterations in lipoprotein-regulating enzymes in uremic patients.

Methods

Subjects

The 93 Japanese subjects consisted of 25 healthy volunteers, 24 uremic patients treated on HD and 44 patients on CAPD. Diabetic subjects were excluded. Mean ages (± SE) of each

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group were 49.2 ± 2.9, 50.8 ± 1.8 and 50.5 ± 1.7 years, respectively, and these are not statistically different. Genders (male/female) were 14/11, 12/12 and 24/18, respectively, and were not different statistically. Body mass indices (23.5 ± 0.6, 23.2 ± 0.8, 21.9 ± 0.4 kg/m^2, respectively) were also comparable among the three groups. HD patients received 12 to 15 hours of HD every week using bicarbonate dialysate containing 3.5 mEq/liter calcium (Kindaly AF-1P, Fuso, Japan). CAPD patients used three or two bags of 1 to 1.5 liter intraperitoneal dialysate containing 1.5% glucose (Dianeal 1.5, Baxter, Round Lake, Illinois, USA) and one or two bags of 1 to 1.5 liter dialysate containing 2.5% glucose (Dianeal 2.5, Baxter) every day. 1α-hydroxyvitamin D_3 was administered to 17 HD patients (average 0.47 μg/day) and 40 CAPD patients (average 0.42 μg/day). Four HD and one CAPD patients received 0.25 μg/day of 1,25-dihydroxyvitamin D_3. Calcium carbonate was prescribed for 10 HD (average 2.6 g/day) and 20 CAPD patients (average 2.6 g/day). Also, four HD and two CAPD patients took aluminum hydroxide gel (average 1.6 and 2.3 g/day, respectively). No patient received lipid lowering drugs.

Blood sampling

All blood samples were obtained after a more than 12 hour fast. Fresh blood collected in a heparinized syringe was used for ionized calcium measurement. Serum was obtained by centrifugation at 1500 g for 20 minutes at room temperature and used for the measurement of lipoproteins, apolipoproteins, parathyroid hormone, total calcium and phosphorus. Plasma for LCAT activity was prepared from blood collected in a tube containing sodium EDTA which was immediately cooled in iced water after sampling. For LPL and HTGL measurements, blood was collected 10 minutes after intravenous injection of sodium heparin (30 U/kg body wt) in a tube containing sodium EDTA and was immediately cooled in iced water. Post-heparin plasma was obtained by centrifugation at 1,500 g for 10 minutes at 4°C and was stored below −40°C until LPL and HTGL assays were performed.

Analysis of lipoproteins and apolipoproteins

Serum lipoproteins were analyzed by one-step preparative ultracentrafugation described by Bronzert and Brewer [27] with modification. Lipoproteins were fractioned into five classes according to density: very low density lipoprotein (VLDL, d < 1.006), intermediate density lipoprotein (IDL, d = 1.006 to 1.019), low density lipoprotein (LDL, d = 1.019 to 1.063), high density lipoprotein-2 (HDL_2, d = 1.063 to 1.125), and high density lipoprotein-3 (HDL_3, d > 1.125). Serum of each donor was transferred in four tubes (0.20 ml per tube, 0.5PC tube, Hitachi), and added with the same volume of KBr solution to adjust solvent density to 1.006, 1.019, and 1.063 and 1.125 g/ml, respectively. They were spun at 100,000 rpm (436,000 × g) for three hours at 4°C in a Hitachi RP100 AT2 rotor. Cholesterol and triglyceride concentrations were measured by enzymatical methods (Cholesterol HR, Wako, Osaka; Clinimate TG-2, Daiichi, Tokyo) in whole serum, the d < 1.006 fraction (VLDL), the d < 1.019 fraction (VLDL + IDL), the d < 1.063 fraction (VLDL + IDL + LDL), the d > 1.063 fraction (HDL_2 + HDL_3), and the d > 1.125 fraction (HDL_3). Lipid content in each lipoprotein fraction was calculated by subtraction.

Serum concentrations of apolipoprotein (apo) A-I and A-II were measured by immunoturbidimetry (ApoAuto II, Daiichi, Japan).

Enzyme assay

Plasma LCAT activity was measured by the method described by Nagasaki and Akanuma [28]. For LPL and HTGL, we measured both lipolytic activities and immunoreactive protein masses in post-heparin plasma. LPL and HTGL activities were measured using [^{3}H]-triolein emulsified with gum arabic as described elsewhere [4]. Immunoreactive LPL and HTGL masses were measured by sandwich-enzyme immunoassay (EIA) systems developed by Ikeda et al [29] using monoclonal antibodies to each enzyme.

Parathyroid hormone and other measurements

Parathyroid hormone (PTH) was measured by two assay methods. One was radioimmunoassay using antibodies recognizing the mid- and C-terminal portion of the peptide hormone (PTH-HS, Yamasa Shoyu, Japan) [30]. The other was a two-site immunoradiometric assay using two different antibodies specific to N- and C-terminus, respectively, of the hormone molecule (Allegro Intact PTH, Nichols Institute Diagnostics, California, USA) [31]. Plasma ionized calcium was measured by a calcium-selective ion electrode method (Horiba, Japan). Total calcium and phosphorus concentrations were measured by routine laboratory methods.

Statistics

Difference among means was evaluated by one way analysis of variance (ANOVA) with multiple comparison (Scheffe F-test). Correlational studies were done by either simple linear regression or multiple regression analysis. P values less than 0.05 were taken as significant. In stepwise multiple regression analysis, F-to-enter value was set at 4.0.

Results

Lipoproteins and apolipoproteins

In HD patients, serum cholesterol concentration was not different from the normal value. However, its distribution in lipoprotein fractions was altered (Table 1). Cholesterol levels in IDL were significantly increased, whereas LDL cholesterol was not elevated. VLDL cholesterol increased with marginal significance (P = 0.056). HDL cholesterol was significantly lower in HD patients than in healthy subjects. Although cholesterol levels in both HDL_2 and HDL_3 subfractions were decreased in HD patients, the reduction was more evident in HDL_2 subfraction. The ratio of HDL_2 cholesterol to HDL_3 cholesterol was significantly lower in HD patients than in healthy subjects. In CAPD patients, serum cholesterol level was significantly increased when compared with either that in healthy subjects or that in HD patients. Changes in cholesterol distribution among lipoprotein fractions were similar to that in HD patients, and CAPD patients had increased levels of VLDL and IDL cholesterol and decreased HDL cholesterol. IDL cholesterol in CAPD patients was four times as high as the normal value. Changes in cholesterol concentration in HDL subfractions were similar to those in HD patients. Both HD and CAPD patients showed a significant elevation in serum triglyceride concentration. They had significantly increased triglyceride levels in VLDL, IDL

Table 1. Analysis of lipoproteins and apolipoproteins

	Control	HD	CAPD	Significance by ANOVA
Cholesterol *mg/dl*				
Serum	187.6 ± 8.4	185.5 ± 9.3	223.4 ± 8.7[b,c]	0.0045
VLDL	23.0 ± 1.5	43.9 ± 7.2	54.8 ± 4.6[c]	0.0002
IDL	5.3 ± 1.1	13.6 ± 1.4[a]	22.4 ± 1.5[b,c]	0.0001
LDL	103.6 ± 6.9	93.4 ± 8.8	109.4 ± 7.4	NS
HDL	55.6 ± 2.6	34.6 ± 2.8[a]	36.9 ± 1.7[b]	0.0001
HDL_2	37.8 ± 2.3	21.8 ± 2.4[a]	21.6 ± 1.4[b]	0.0001
HDL_3	17.8 ± 0.5	12.8 ± 0.6[a]	15.3 ± 0.6[b,c]	0.0001
Triglyceride *mg/dl*				
Serum	68.2 ± 7.9	188.9 ± 32.7[a]	209.6 ± 18.1[b]	0.0001
VLDL	30.2 ± 4.5	121.6 ± 30.0[a]	124.2 ± 16.2[b]	0.0026
IDL	5.7 ± 0.8	13.6 ± 1.4[a]	18.9 ± 1.4[b,c]	0.0001
LDL	19.6 ± 2.8	36.1 ± 3.4[a]	47.6 ± 3.2[b]	0.0001
HDL	12.7 ± 0.9	16.1 ± 1.6	18.9 ± 0.8[b]	0.0003
Apolipoproteins *mg/dl*				
apo A-I	121.7 ± 4.4	106.9 ± 5.1	116.6 ± 4.0	NS
apo A-II	28.9 ± 1.1	25.8 ± 1.0	25.7 ± 1.0	NS
Ratios				
HDL_2-C/HDL_3-C *mg/mg*	2.13 ± 0.12	1.68 ± 0.15	1.44 ± 0.10[b]	0.0008
HDL-TG/HDL-C *mg/mg*	0.237 ± 0.020	0.531 ± 0.073	0.551 ± 0.033[b]	0.0001
HDL-C/(A-I + A-II) *mol/mol*	23.0 ± 1.2	17.4 ± 0.8[a]	17.3 ± 0.6[b]	0.0002
HDL-TG/(A-I + A-II) *mol/mol*	2.38 ± 0.21	3.81 ± 0.47[a]	3.93 ± 0.16[b]	0.0041

Difference among the three groups were evaluated by one way analysis of variance (ANOVA).

Abbreviations are: apo, apolipoprotein; HDL-C, HDL cholesterol; HDL_2-C, HDL_2 cholesterol; HDL_3-C, HDL_3 cholesterol; HDL-TG, HDL triglyceride. Data are mean ± SE.

Significant difference ($P < 0.05$) assessed by multiple comparison (Scheffe F-test) between control and HD[a], between control and CAPD[b], and between HD and CAPD[c].

and LDL. Increase in HDL triglyceride was significant in CAPD patients.

There was no significant difference in either apo A-I or A-II among the three groups. Triglyceride/cholesterol ratio in HDL fraction was significantly higher in both HD and CAPD patients than in the control subjects, indicating the alteration of HDL lipid composition in the uremic patients. Compositional change of HDL was also significant when assessed by molar ratios of HDL cholesterol/(apo A-I + apo A-II) and HDL triglyceride/(apo A-I + apo A-II). These results indicate cholesterol depletion and triglyceride enrichment of HDL in the uremic patients.

LPL, HTGL and LCAT

We first examined the relationship between immunoreactive LPL mass and LPL activity as shown in Figure 1, because a previous study suggested the presence of inhibitor(s) of LPL activity in uremic plasma [32] whereas others could not confirm it [9, 13]. We found a linear relationship between immunoreactive LPL mass and LPL activity either in the healthy subjects, HD or CAPD patients. There was no significant difference in the slope of the fitting lines among the three groups. This was also the case with HTGL. These results indicate that changes in post-heparin LPL and HTGL activities in uremic patients are due to changes in enzyme protein concentrations. Therefore, we employed the values of immunoreactive protein concentrations for LPL and HTGL in the study below.

Table 2 summarizes the results of comparison among groups and between genders in the levels of LPL, HTGL and LCAT. LPL level in HD patients tended to be lower than that in the healthy subjects, although the difference was not significant. CAPD patients had a mean LPL which was not different from the normal value. There was no significant difference in LPL protein concentration between genders. HTGL protein levels were significantly lower in both HD and CAPD patients than those of the healthy subjects. Significant difference was observed in HTGL protein levels between male and female in each of the three groups. Men had 1.7 to 2.5 times higher HTGL protein mass than women. LCAT activity in HD patients was significantly lower than the control value, whereas CAPD patients had almost normal LCAT activity. No consistent difference was seen in LCAT activities between genders.

Calcium, phosphorus and PTH

There was no significant difference in serum total calcium concentrations among the three subject groups. However, HD and CAPD patients had significantly lower ionized calcium levels than healthy group, as shown in Table 3. Both uremic groups also showed hyperphosphatemia. They had extremely high immunoreactive PTH levels measured by either the mid-portion assay or the sandwich assay.

Simple regression analysis of HDL metabolism

By simple regression analysis in the total subjects, LPL correlated positively to HDL cholesterol and apo A-I (Fig. 2). LPL also correlated significantly to HDL_2 cholesterol ($r = 0.392$, $P = 0.001$), HDL_3 cholesterol ($r = 0.320$, $P = 0.01$) and apo A-II ($r = 0.331$, $P = 0.005$). HTGL correlated inversely to HDL_2 cholesterol and to the ratio of HDL_2 cholesterol/HDL_3 cholesterol in uremic patients (Fig. 3). In these relations, the correlation coefficients became improved when analyzing the subjects with HTGL less than 700 ng/ml ($r = -0.563$, $P = 0.0002$ and $r = -0.559$, $P = 0.0002$, respectively), suggesting HTGL deficiency becomes more critical in that range. There

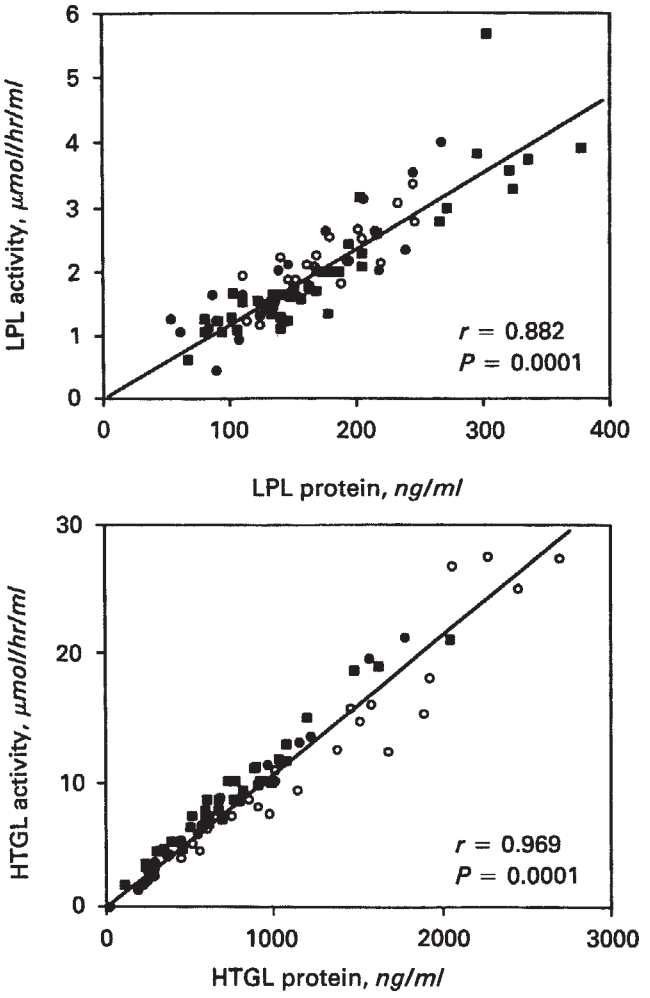

Fig. 1. *Correlation between enzyme protein concentration and lipolytic activity in LPL and HTGL.* Open circle (○), closed circle (●) and closed square (■) indicate healthy subjects, HD and CAPD patients, respectively.

Table 2. Comparison of LPL, HTGL and LCAT levels between genders and among groups

	Control	HD	CAPD	ANOVA
LPL				
Male	175.6 ± 10.0	148.8 ± 18.7	159.3 ± 13.7	NS
Female	173.8 ± 13.5	141.4 ± 15.8	202.1 ± 21.1	NS
Total	174.8 ± 8.0	145.1 ± 12.0	177.0 ± 12.2	NS
HTGL				
Male	1565 ± 175	792 ± 154[a]	865 ± 86[b]	0.0003
Female	870 ± 142	322 ± 70[a]	515 ± 55[b]	0.0007
Total	1259 ± 134	557 ± 96[a]	720 ± 61[b]	0.0001
LCAT				
Male	80.9 ± 8.3	41.4 ± 8.1[a]	69.5 ± 5.1[c]	0.0057
Female	62.2 ± 16.1	52.3 ± 8.0	85.3 ± 5.8[c]	0.0112
Total	74.0 ± 5.4	47.1 ± 5.7[a]	76.0 ± 4.0[c]	0.0002

Difference among the three groups were evaluated by one way analysis of variance (ANOVA).

Difference in HTGL between genders was significant ($P < 0.05$) in each group. There was no significant gender difference either in LPL or in LCAT in each group. Data are mean ± SE.

Significant difference ($P < 0.05$) assessed by multiple comparison (Scheffe F-test) between control and HD[a], between control and CAPD[b], and between HD and CAPD[c].

Table 3. Calcium and related parameters in the subjects

	Control	HD	CAPD	ANOVA
Ca *mEq/liter*	4.56 ± 0.03	4.62 ± 0.10	4.61 ± 0.06	NS
Phosphorus *mg/dl*	3.26 ± 0.09	6.17 ± 0.36[a]	6.37 ± 0.20[b]	0.0001
Ca^{++} *mmol/liter*	1.17 ± 0.01	0.97 ± 0.03[a]	1.07 ± 0.02[b,c]	0.0001
m-PTH *pg/ml*	243 ± 31	21067 ± 4559[a]	20100 ± 3584[b]	0.006
Intact PTH *pg/ml*	34 ± 3	267 ± 51[a]	253 ± 33[b]	0.0001

Difference among the three groups were evaluated by one way analysis of variance (ANOVA).

In the comparison of m- and intact PTH, log-transformed values gave similar statistical results. Data are mean ± SE.

Significant difference ($P < 0.05$) assessed by multiple comparison (Scheffe F-test) between control and HD[a], between control and CAPD[b], and between HD and CAPD[c].

was no significant correlation between HTGL and HDL_3 cholesterol, apo A-I or apo A-II. There was no demonstrable correlation of LCAT activity to any parameter of HDL metabolism when assessed by simple regression analysis. There was an inverse correlation between HDL cholesterol and serum triglyceride ($r = -0.588$, $P = 0.0001$). The inverse correlation remained significant when analyzed in each of the three groups separately. An inverse correlation was also found between HDL cholesterol and VLDL triglyceride ($r = -0.560$, $P = 0.0001$). HDL triglyceride correlated positively to VLDL triglyceride ($r = 0.490$, $P = 0.0001$). We found a significant positive correlation between VLDL triglyceride and the triglyceride/cholesterol ratio in HDL fraction ($r = 0.777$, $P = 0.0001$).

Multiple regression analysis of factors affecting HDL metabolism

Based on the above results, data were analyzed by multiple regression method. In this model, HDL-, HDL_2- or HDL_3-cholesterol was an dependent variable and independent variables consisted of LPL, HTGL, LCAT and VLDL triglyceride (Table 4). VLDL triglyceride was log-transformed to fit the linear model. There were independent associations of HDL cholesterol with LPL and VLDL triglyceride levels. HDL_2 cholesterol also had independent relationships with LPL and VLDL triglyceride. HDL_3 cholesterol showed significant relationship with LPL, HTGL, LCAT and VLDL triglyceride. HDL triglyceride was significantly related with LPL and VLDL triglyceride. HDL triglyceride also showed inverse association with HTGL with borderline significance ($P = 0.0524$). In the model, the four independent variables explained 51.5%, 46.9%, 38.6% and 34.6% of the variations of HDL-, HDL_2-, and HDL_3-cholesterol and HDL triglyceride levels, respectively.

Relationship between calcium metabolism and lipoprotein-regulating enzymes

Possible correlations were examined between parameters of calcium metabolism and the lipoprotein-regulating enzyme levels by simple regression analysis. For HTGL, male and female

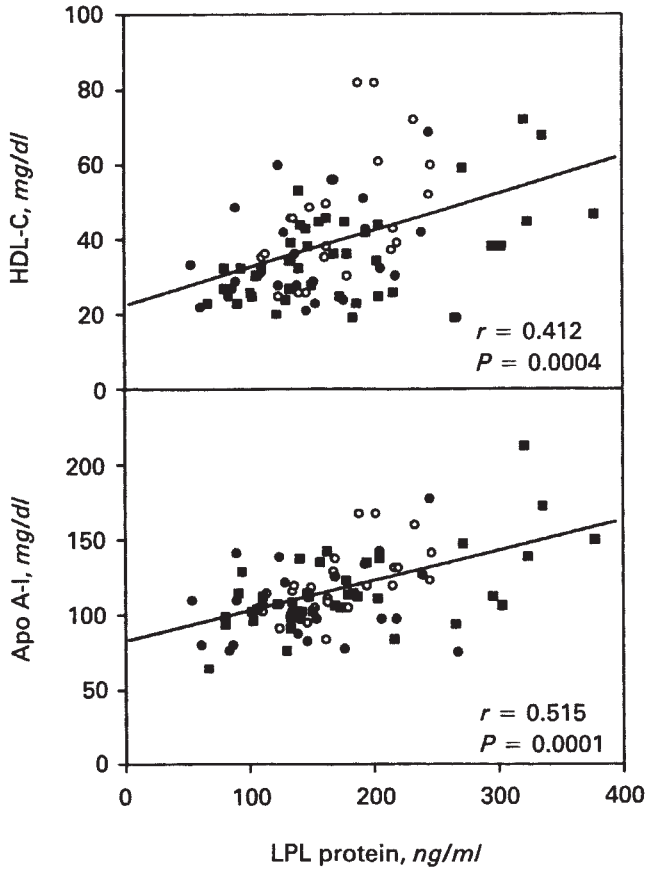

Fig. 2. *Correlation between LPL and HDL parameters.* Open circle (○), closed circle (●) and closed square (■) indicate healthy subjects, HD and CAPD patients, respectively. HDL-C, HDL cholesterol; apo A-I, apolipoprotein A-I.

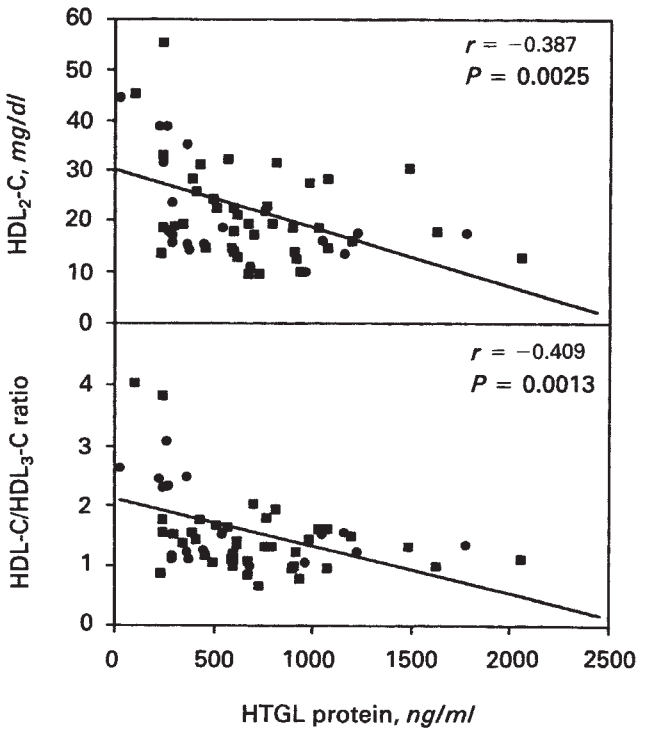

Fig. 3. *Correlation between HTGL and HDL metabolism.* Closed circle (●) and closed square (■) indicate HD and CAPD patients, respectively. $HDLC_2$, HDL_2-C cholesterol: $HDLC_3$-C, HDL_3 cholesterol.

subjects were analyzed separately because HTGL levels were significantly different between genders. LPL showed no significant correlation with total calcium, phosphorus, ionized calcium, mid-portion PTH or intact PTH level. HTGL and LCAT also showed no correlation to total calcium, phosphorus, mid-portion PTH or intact PTH level. However, we found a significant positive correlation between ionized calcium and HTGL level ($r = 0.344$, $P = 0.0143$ for male; $r = 0.318$, $P = 0.0486$ for female). There was also a positive correlation between ionized calcium and LCAT activity ($r = 0.249$, $P = 0.0365$).

Furthermore, we examined the independent factors affecting the three enzymes using stepwise multiple regression analysis among the variables including age, gender, total and ionized calcium, phosphorus, log-transformed m- and intact PTH levels (Table 5). No variable was entered as a significant predictor of LPL. Gender, log(m-PTH), ionized calcium and age were indicated as independent predictors of HTGL. Ionized calcium and age were entered as independent predictors of LCAT.

Table 4. Multiple regression analysis of factors affecting HDL lipids in total subjects

Variables				
Dependent	Independent	β	P value	R^2
HDL-C	LPL	0.291	0.0018	
	HTGL	0.124	NS	
	LCAT	0.068	NS	
	log(VLDL-TG)	−0.597	0.0001	0.515 ($P = 0.0001$)
HDL_2-C	LPL	0.284	0.0035	
	HTGL	0.084	NS	
	LCAT	−0.004	NS	
	log(VLDL-TG)	−0.573	0.0001	0.469 ($P = 0.0001$)
HDL_3-C	LPL	0.204	0.0467	
	HTGL	0.206	0.0399	
	LCAT	0.271	0.0083	
	log(VLDL-TG)	−0.445	0.0001	0.386 ($P = 0.0001$)
HDL-TG	LPL	0.323	0.0028	
	HTGL	−0.200	0.0524	
	LCAT	0.037	NS	
	log(VLDL-TG)	0.524	0.0001	0.346 ($P = 0.0001$)

Abbreviations are: β, standard regression coefficient; R^2, multiple coefficient of determination; HDL-C, HDL cholesterol; HDL_2-C, HDL_2 cholesterol; HDL_3-C, HDL_3 cholesterol; HDL-TG, HDL triglyceride; VLDL-TG, VLDL triglyceride.

Discussion

The purpose of this study was to evaluate the roles of LPL, HTGL, LCAT and hypertriglyceridemia for deranged HDL metabolism in uremic patients, and to examine the possible impact of altered calcium metabolism on these lipoprotein-regulating enzymes. The HD and CAPD patients had decreased HDL cholesterol levels, altered HDL subfraction distribution, and compositional changes in HDL fraction. Univariate and multivariate regression analyses indicated the contributions of

Table 5. Stepwise multiple regression analysis of factors affecting HTGL and LCAT

	Step	Variable entered	β	F value	R^2
HTGL	1	Gender	−0.483	27.4	
	2	log (m-PTH)	−0.22	5.5	
	3	Ca^{++}	0.231	6.1	
	4	Age	−0.198	4.6	0.370
LCAT	1	Ca^{++}	0.256	4.9	
	2	Age	−0.237	4.2	0.116

Significant predictors of HTGL and LCAT were explored among the parameters including gender, age, total calcium, Ca^{++}, phosphorus, log (m-PTH) and log (intact PTH) by stepwise multiple regression analysis. Dummy variables were used for genders (male = 1, female = 2). F value to enter was set at 4.0 at each step. Final results were given in the Table. β, standard regression coefficient; R^2, multiple coefficient of determination.

LPL, HTGL, LCAT and VLDL triglyceride levels to HDL metabolism. The multiple regression model including the three enzymes and log-transformed VLDL triglyceride value as independent variables explained 51.5% of the variation of HDL cholesterol in the study subjects. Stepwise multiple regression analyses revealed significant positive association of ionized calcium to HTGL and LCAT and inverse relation between PTH and HTGL. These results suggest that HDL abnormality in uremia is largely attributable to alterations in the enzymes and VLDL level, and that suppression of HTGL and LCAT is associated with impaired calcium metabolism.

It is well known that low HDL cholesterol is often associated with hypertriglyceridemia [20]. We confirmed the inverse correlation between HDL cholesterol and triglyceride levels in serum and VLDL. There is a hypothesis that increased triglyceride and decreased HDL are the results of lowered LPL activity [33] based on the fact that HDL precursor "nascent HDL" is generated from surface remnant of triglyceride-rich lipoproteins during hydrolysis of core triglyceride by LPL [34, 35]. We confirmed significant positive correlations of LPL to HDL cholesterol, HDL_2 cholesterol, HDL_3 cholesterol and apolipoproteins A-I and A-II. Also, multiple regression analysis indicated that LPL was an independent factor affecting HDL metabolism. These data are compatible with the view that a considerable amount of HDL is derived from LPL action. However, mean LPL level was not significantly decreased in either HD or CAPD patients who had marked reduction in HDL cholesterol. In previous studies, low HDL cholesterol was repeatedly found although LPL was not always decreased in uremia [13, 14]. Thus, although LPL is one of the important factors determining HDL cholesterol level, it seems difficult to explain low HDL cholesterol levels in uremia by changes in LPL level only.

In the HD and CAPD patients, HDL cholesterol was decreased whereas HDL triglyceride was increased, indicating compositional change of HDL lipids. VLDL triglyceride correlated positively to HDL triglyceride and inversely to HDL cholesterol. Triglyceride/cholesterol ratio of HDL fraction was increased in the uremic patients, and the ratio correlated positively to VLDL triglyceride concentrations, indicating that accumulation of VLDL was associated with triglyceride enrichment and cholesterol depletion of HDL. This reciprocal relationship between HDL triglyceride and HDL cholesterol in the presence of hypertriglyceridemia was probably due to enhanced lipid transfer reaction. Lipid transfer reaction between HDL and less dense lipoproteins, especially VLDL, is mediated by cholesteryl ester transfer protein (CETP) [36]. CETP mediates transfer of triglycerides from VLDL to HDL, and cholesteryl ester transfer in the opposite direction, resulting in enrichment of HDL with triglyceride and depletion of HDL cholesterol. Elevated level of VLDL, in other words, hypertriglyceridemia, stimulates this process [37]. Thus it is probable that hypertriglyceridemia led to compositional change of HDL lipids and subsequent reduction in HDL-cholesterol. Since HDL_2 is a main donor of cholesteryl ester, cholesterol reduction was more evident in HDL_2 than HDL_3 subfraction in the patients. Also, enhanced lipid transfer may account for the accumulation of cholesterol-rich remnant lipoprotein in uremia.

A recent study in non-uremic subjects by Brinton, Eisenberg and Breslow [38] has shown that compositional change of HDL expressed by the decreased ratio of HDL cholesterol/(apo A-I + apo A-II) was associated with increased fractional catabolic rate of HDL apolipoproteins and reduced HDL cholesterol level. They speculated that low ratio of HDL cholesterol/(apo A-I + apo A-II) was due to enhanced lipid transfer. The uremic patients in the present study had a significantly decreased ratio of HDL cholesterol/(apo A-I + apo A-II) and increased ratio of HDL triglyceride/(apo A-I + apo A-II). These data were in line with the above report by Brinton et al, and raised the possibility that enhanced lipid transfer could reduce HDL cholesterol not only by changing composition of HDL but also by stimulating the catabolism of HDL. Clearly, in vivo kinetic study in uremic patients is needed to evaluate the latter possibility.

Normally, enrichment of HDL_2 with triglyceride is followed by depletion of the lipid by HTGL, converting HDL_2 to HDL_3 [35, 39]. In univariate analysis in HD and CAPD patients, HTGL inversely correlated to HDL_2 cholesterol and to the ratio of HDL_2 cholesterol/HDL_3 cholesterol. By multivariate regression analysis in the total subjects, HTGL correlated positively to HDL_3 cholesterol and inversely to HDL triglyceride with borderline significance, although the relationship between HTGL and HDL_2 cholesterol became insignificant. These results are in agreement with a previous study in the general population [17] and suggest that decreased HTGL in uremia was associated with impaired conversion of HDL_2 to HDL_3. This relationship between HTGL and HDL subfractions may predict that the lowered HTGL is followed by increased HDL_2 cholesterol levels, whereas HDL_2 cholesterol was decreased in the patients. This apparent discrepancy may be explained by the result of multiple regression analysis indicating that VLDL triglyceride was the most powerful predictor of HDL_2 cholesterol. The positive effect of low HTGL on HDL_2 cholesterol was overcome by the negative effect of high VLDL.

LCAT is the key enzyme which keeps the chemical gradient of cholesterol from cells to plasma [40]. At the surface of HDL, LCAT catalyzes the esterification of free cholesterol and incorporates cholesteryl ester into HDL core. By LCAT action HDL particles become "mature" from nascent HDL with discoidal shape to spherical HDL_3 and finally HDL_2 [35]. Previous studies showed decreased LCAT activity in HD patients [18, 19], and one may speculate that decreased HDL_2 cholesterol is related to low LCAT activity. In the present study, both HD and CAPD patients had low HDL_2 cholesterol, whereas LCAT

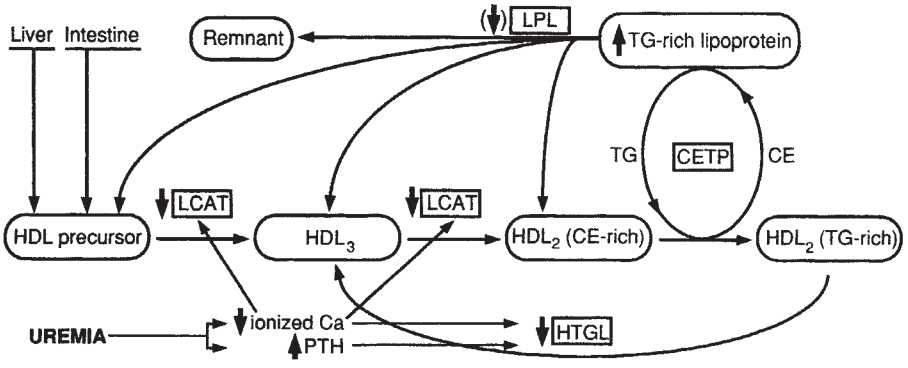

Fig. 4. *Proposed alterations in HDL metabolism in uremic patients.* HDL precursor is derived from secretion by the liver and intestine and from hydrolysis of triglyceride-rich lipoproteins by LPL. The precursor undergoes conversion to HDL_3 and finally HDL_2 by the action of LCAT. CETP mediates the exchange of cholesteryl ester of HDL_2 for triglyceride of triglyceride-rich lipoproteins, resulting in triglyceride-enrichment and cholesterol-depletion of HDL. HDL triglyceride can be removed by HTGL and this promotes re-conversion of HDL_2 to HDL_3. In uremia, patients with reduced LPL have decreased HDL formation and subsequent reduction in HDL_3-, HDL_2- and total HDL-cholesterol. Decreased LCAT (especially in HD patients) is followed by the lowered HDL_3 formation. Elevated VLDL triglyceride promotes the CETP-mediated lipid transfer and HDL becomes triglyceride-enriched and cholesterol-depleted. Remarkable decrease in HTGL accounts for impaired re-conversion of HDL_2 to HDL_3. Decreased LCAT is associated with low ionized calcium. Decreased HTGL is also associated with low plasma ionized calcium and elevated parathyroid hormone levels, both of which are the consequences of uremia.

was decreased only in HD patients. The latter finding is similar to the result by Dieplinger, Schoenfeld and Fielding [41]. Multivariate regression analysis did not indicate significant association of LCAT and HDL_2 cholesterol level. The enzyme, however, showed a significant positive relationship with HDL_3 cholesterol. These data suggest that LCAT plays an important role in the formation of HDL_3, but its influence on HDL_2 metabolism becomes less when compared with the larger effects of VLDL and LPL.

In this work, 51.5% of variation in HDL cholesterol was explained by VLDL triglyceride, LPL, HTGL and LCAT. The remaining portion was unexplained by the four variables. This part would be comprised of variations in secretion of HDL precursor by the liver and intestine, HDL receptor activity, apolipoprotein loss into peritoneal dialysate in CAPD patients [42] and CETP activity. CETP activity is reported to be reduced in uremia [41, 43]. A recent work in nephrotic syndrome has shown the increased CETP mass and negative correlation between CETP and HDL [44]. The effect of CETP on plasma concentration and composition of HDL has been characterized in patients heterozygous and homozygous for CETP deficiency [45]. Thus, the inclusion of CETP assay in our study could improve the result by multiple regression analyses.

It is not known at present why the levels of the lipoprotein-regulating enzymes are affected in uremia. LPL activity in post-heparin plasma was reported to be reduced [9–12] or normal [13, 14]. A previous study suggested the inhibitor of LPL in uremic plasma [32], whereas others could not confirm it [9, 13]. We measured LPL by both activity assay and EIA, because possible discrepancy between the two assay methods was expected if an inhibitory substance interfered with the activity assay system. However, we could not find the discrepancy. The result indicates that changes in LPL activity measured by the method we used were attributable to the changes in LPL protein concentrations in post-heparin plasma. We, however, did not rule out impaired in vivo action of LPL due to altered susceptibility of substrate lipoproteins to the enzyme. It is possible that decreased ratio of apo C-II/C-III [46] is related to impaired catabolism of triglyceride-rich lipoprotein in uremia.

Akmal et al recently demonstrated in uremic dogs that excess PTH caused hypertriglyceridemia by suppressing LPL activity in post-heparin plasma [26]. However, the relationship between LPL and PTH was not confirmed in this patient study. Despite marked reduction of LPL in uremic dogs [26], reported post-heparin LPL activities are not always low in uremic patients [4, 13, 14]. Change in LPL in uremic patients was not significant also in the present study, while they had an extremely high level in circulating PTH. Thus, the effect of PTH on LPL seems to be less in humans than in the dog. Alternatively, the effect of PTH was hidden by some factors having greater influence on LPL, such as insulin resistance or hyperinsulinemia [47], which were variable among patients.

Decrease in HTGL activity is a consistent finding in uremia [9–14], although the cause is not clear. Association of decreased HTGL with reduced glomerular filtration ratio was reported in chronic renal failure patients without dialysis therapy [48]. Since HTGL is a hepatic enzyme, impaired renal function itself does not account for the suppression of the enzyme. It is likely that some metabolic alteration due to renal failure affects liver function. Using multivariate analysis, we examined possible relationship between low HTGL and impaired calcium metabolism. The result indicated that HTGL level was independently associated with ionized calcium and PTH concentrations, and also with age and gender. The age- and gender-related alterations in HTGL are in line with previous reports [46, 50]. We also found a significant relationship between ionized calcium and LCAT. These data support the hypothesis that impaired

homeostasis of calcium results in lipoprotein abnormalities through impairment of the enzymes.

In conclusion, HDL metabolism in uremia is affected by LPL, HTGL, LCAT and VLDL levels in different ways. Impaired calcium metabolism seems to be partly responsible for the alterations in the enzyme. Proposed alterations in HDL metabolism in uremia are presented in Figure 4. Since decreased HDL cholesterol is an independent risk for atherosclerosis, normalization of HDL metabolism may improve the prognosis of the patients. It appears important to reduce VLDL triglyceride and to restore the enzymes for amelioration of HDL metabolism; also improvement of calcium homeostasis may give a favorable effect on lipoprotein metabolism.

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Note added in proof

NISHIZAWA Y, MIKI T, OKUI Y, MATSUSHITA Y, INOUE T, MORII H: Deranged metabolism of lipids in patients with chronic renal failure: Possible role of secondary hyperparathyroidism. *Jpn J Med* 25:40–45, 1986

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