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Lipoprotein particle abnormalities and the impaired lipolysis in renal insufficiency

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Background. Increased concentrations of very low- (VLDL) and intermediate-density (IDL) lipoproteins in chronic renal failure (CRF) are thought to result from a defect(s) in degradation of plasma triglyceride (TG)-rich lipoproteins. The purpose of this study was to identify lipoprotein abnormalities associated with the reduced lipolytic rate constant, k_1, of combined VLDL and IDL substrate from renal patients and asymptomatic controls.

Methods. The VLDL + IDL were isolated from 18 predialytic patients (CRF-I), 8 patients on hemodialysis (CRF-II) and 10 asymptomatic controls. The lipolytic rate constant (k_1) of VLDL + IDL was measured by an assay using bovine milk lipoprotein lipase and determination of TG before and after incubation by gas chromatography (GC). Neutral lipids were measured by GC and apolipoproteins by electroimmunoassays; the apolipoprotein-defined TG-rich lipoproteins including Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E were determined by immunoaffinity chromatography.

Results. The k_1 values of VLDL + IDL were significantly ($P < 0.001$) lower in CRF-I and CRF-II patients (0.0341 and 0.0352 min^{-1}, respectively) than controls (0.0515 min^{-1}). The levels of apolipoproteins B, C-III and E, and TG-rich Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E particles normalized to 100 mg TG per VLDL + IDL were significantly higher in both groups of CRF patients than in controls. All three TG-rich lipoproteins were characterized by significantly increased percent contents of free (FC) and esterified (CE) cholesterol and a decreased percentage of TG. The k_1 values of the combined CRF-I and CRF-II patient groups showed significant negative correlations ($P < 0.001$) with FC ($r = -0.81$) and CE ($r = -0.63$) and a positive correlation with TG ($r = 0.72$). Among lipoprotein particles, only Lp-A-II:B:C:D:E levels showed a significant negative correlation with k_1 values ($r = -0.47$, $P < 0.03$).

Conclusions. This study shows that the abnormal neutral lipid composition of all three TG-rich lipoprotein particles and increased concentrations of Lp-A-II:B:C:D:E particles represent the main factors affecting the in vitro lipolytic rates of VLDL + IDL substrate in both the CRF patients before dialysis and patients on hemodialysis.

Key words: chronic renal failure, lipids, apolipoproteins, triglycerides, VLDL, uremia, hemodialysis.

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Chronic renal failure (CRF) is characterized by specific compositional and metabolic abnormalities of plasma lipoproteins [1–6]. One of these abnormalities is the increased concentration of very low-density (VLDL) and intermediate-density (IDL) lipoproteins considered to result from a defect in degradation rather than formation of triglyceride (TG)-rich lipoproteins [6–9]. Impaired degradation of TG-rich lipoproteins in patients with CRF may be due either to a deficient lipolytic system [6–8, 10–13] and/or an inadequate substrate composition [6, 14–16]. Arnadottir et al have observed that VLDL particles from patients on hemodialysis were lipolyzed to a lesser extent than VLDL particles from healthy controls, and suggested that increased relative contents of cholesterol and phospholipids, and, in particular, increased levels of apolipoprotein (apo) C-III bound to apoB-containing lipoproteins may represent the main compositional abnormalities interfering with the lipolytic degradation of uremic TG-rich lipoproteins [14–16].

Triglyceride-rich lipoproteins of very low and intermediate densities represent a mixture of particles heterogeneous with respect to chemical, physical and metabolic properties [17–19]. The heterogeneous character of TG-rich lipoproteins has been attributed to the presence of several, discrete lipoprotein families of particles characterized by similar density properties but distinct qualitative apolipoprotein composition [20]. Three major TG-rich lipoprotein families identified as lipoprotein (Lp) B:C, Lp-B:C:E and Lp-A-II:B:C:D:E differ from one another not only chemically, but also with respect to metabolic properties. It has already been shown, perti-

nent to this study, that these TG-rich lipoprotein families differ in their relative affinities for lipoprotein lipase (LPL) in that the affinity of Lp-A-II:B:C:D:E particles is significantly lower than those of Lp-B:C and Lp-B:C:E particles [21]. To further explore the compositional abnormalities that may be interfering with the normal lipolytic degradation and consequent accumulation of TG-rich lipoproteins in renal insufficiency, we have conducted a kinetic and compositional study of TG-rich lipoproteins isolated from predialytic CRF patients, patients on hemodialysis and healthy control subjects. The specific aim of this study was to determine and compare the lipolytic rate constants, k_1, and lipid, apolipoprotein and lipoprotein family profiles of TG-rich lipoproteins (VLDL + LDL, $d < 1.019$ g/mL) between renal patients and healthy controls.

METHODS

The study was conducted in accordance with the ethical principles described by the Declaration of Helsinki and was approved by the Sahlgrenska University Hospital Ethics Committee.

Subjects

Twenty-six adult, non-diabetic patients with CRF were investigated. There were 18 predialytic patients (CRF-I; 11 men and 7 women; mean age 50 ± 13 years) with a reduced glomerular filtration rate (GFR) ranging from 5 to 59 mL/min/1.73 m^2 body surface area (BSA; mean GFR 30.8 ± 14.6 mL/min/1.75 m^2 BSA). The second group of 8 patients (CRF-II; 3 men and 5 women; mean age 61 ± 14 years) with end-stage renal failure was treated with chronic hemodialysis. Ten healthy, asymptomatic subjects (7 men and 3 women; mean age 46 ± 13 years) served as controls.

The CRF patients were treated with antihypertensive drugs, phosphate-binding drugs and vitamins as appropriate. Patients treated with corticosteroids, immunosuppressive agents or lipid-lowering drugs were excluded from the study.

The CRF-II patients were dialyzed with conventional low-flux hemodialysis treatment generally for four to five hours three times weekly using bicarbonate and glucose containing dialysis fluid. Low-molecular-weight heparin (LMWH) was used as anticoagulant.

Blood samples were drawn after a 12-hour overnight fast into ethylenediaminetetraacetate (EDTA)-containing tubes. In CRF-II patients, blood was drawn immediately before hemodialysis treatment and administration of LMWH. Protease inhibitor, ε-amino caproic acid (εACA), was added to a final concentration of 10 mmol/L, and plasma samples were shipped from Göteborg to Oklahoma City by overnight mail. Upon arrival, the LPL inhibitor, diisopropyl fluorophosphate (DFP), was added to a final concentration of 1 mmol/L. A freshly prepared antioxidant, butylated hydroxytoluene, was added to a final concentration of 0.005% [22]. Plasma was blown gently with a stream of argon over the surface to prevent the contact with air, and the tube was covered air-tight with double-layered parafilm.

Isolation of lipoproteins

The VLDL and IDL were isolated from individual plasma samples by density gradient ultracentrifugation according to a modified procedure of Lee and Downs [23]. The surface of the quick-seal polyallomer centrifuge tubes was purged with argon, and 17 mL of pre-chilled NaCl solution (d = 1.021 g/mL) containing 3 mmol/L EDTA and 10 mmol/L εACA were placed to the bottom of the tube. Plasma was then delivered to the bottom of the tube and layered underneath the salt solution. Tubes sealed with heat were centrifuged in a 50.2 Ti rotor at 43,000 rpm for 21 hours at 5°C. Lipoproteins floating to the top of the tube were collected under a stream of argon. The density of the top-layer solution was measured to be 1.019 g/mL indicating that the samples collected encompassed VLDL and IDL. The VLDL + IDL fractions from both patients and controls were kept under argon and used within two days of isolation.

Isolation of bovine milk LPL

Bovine milk lipoprotein lipase was first enriched from bovine skim milk by the affinity coflotation with added Intralipid, followed by acetone-ether treatment and lyophylization [24]. On the day of experiment, the LPL was isolated as described previously [25] with minor modifications. The lyophilized LPL-acetone-powder (40 mg) was stirred in (4 mL) 50 mmol/L NH_4OH–NH_4Cl buffer, pH 8.5, containing 0.1% Triton N-101 (Sigma). The solution was centrifuged to remove the insoluble portion. The clear solution was applied to a heparin-Sepharose mini-column calibrated to contain 1 mL of wet gel. The column was washed sequentially with 4 mL of 50 mmol/L NH_4OH–NH_4Cl buffered 0.3 mol/L NaCl and 4 mL of buffered 0.72 mol/L NaCl and then eluted with 3 mL of buffered 2 mol/L NaCl, pH 8.5. The activity of the isolated LPL was measured with artificial substrate *p*-nitrophenyl acetate and monitored at 418 nm. The average enzyme activity was measured to be 0.231 ± 0.050 μmol/min/mL throughout all experiments.

Lipolysis of triglyceride-rich lipoproteins

Freshly isolated VLDL + IDL fractions were incubated at 37°C under argon with freshly prepared bovine milk LPL at a substrate:enzyme ratio of 50:1 (vol:vol). The substrate was adjusted to a constant TG concentration of 100 mg/dL in 50 mmol/L ammonium buffer, pH 8.5. Fatty acid-free bovine albumin (108 mg/mL) was included as a fatty acid acceptor in a total volume of 5 mL

of lipolytic mixture. Aliquots were taken out from the incubation mixture at time intervals 5, 10, 15, 30 and 60 minutes, and immediately extracted with n-heptane-isopropanol (3:7, vol:vol) for TG analyses. A mixture containing no LPL served as a 0-time point. The VLDL + IDL fractions from patients and controls were incubated simultaneously with the same freshly isolated LPL preparation.

The TG levels in VLDL + IDL fractions before and after incubation with LPL at various time intervals were determined in duplicate samples by gas chromatography (GC) according to the method of Kuksis et al [26].

The pseudo-first order rate (k_1) of lipolysis of VLDL + IDL fractions was calculated from the decreasing TG concentrations at various time intervals using a computer software, Graphic Pad, with the exponential regression equation. The TG value at 0-time (1 mg/mL) was taken as 100%.

Isolation and measurement of lipoprotein families

The TG-rich lipoprotein families Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E were isolated by immunoaffinity chromatography of VLDL + IDL fractions on anti-apoA-II, anti-apoC-III and anti-apoE immunosorbers and their concentrations were expressed in terms of apoB contents. Anti-apoA-II, anti-apoC-III and anti-apoE immunosorbers were prepared by coupling polyclonal affinity-purified antisera to Affi-Gel 10 as previously described [21, 27, 28]. Aliquots of VLDL + IDL fractions (0.15 - 0.3 mL) were applied to immunosorbers and incubated overnight at room temperature. The elution of retained and unretained fractions was monitored by measuring absorption at 280 nm by a single path monitor UV-1 optical unit (Pharmacia Fine Chemicals, Piscataway, NJ, USA) attached to a recorder. The non-adsorbed proteins and lipoproteins (the unretained fractions) were eluted with the running buffer (0.05 mol/L Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl and 1.5 mg/mL EDTA). After the absorbance at 280 nm returned to the baseline, the apoB-containing lipoproteins bound to the immunosorber (the retained fractions) were desorbed and eluted with 4.5 mol/L sodium thiocyanate at a flow rate of 30 mL/h. The bottom layer of Sephadex G-25 allowed an immediate separation of retained lipoproteins from the desorbent, which resulted in two distinct peaks at 280 nm [27]; the first peak consisted of adsorbed lipoproteins and the second peak contained sodium thiocyanate. The unretained (U) and retained (R) fractions from each immunosorber were placed in dialysis bags (5000 molecular wt cut-off) and concentrated to 5 to 10 mL volumes by reverse dialysis against a saturated aqueous solution of polyvinylpyrrolidone K30 (Acros Organics, Pittsburgh, PA, USA) placed outside dialysis bags. Dialysis bags were rinsed with distilled water and placed into solid sucrose to finish concentrating U- and R-fractions to a volume of 1 to 1.5 mL. After recording final volumes, the apoB contents of U- and R-fractions were quantified by electroimmunoassay [29].

The R-fraction from the anti-apoA-II immunosorber contained Lp-A-II:B:C:D:E particles and the U-fraction the remaining apoB-containing lipoprotein families. If the U-fraction reacted positively with antibodies to apoA-II, it was rechromatographed on the anti-apoA-II immunosorber until free of apoA-II. The R-fraction from the anti-apoC-III immunosorber contained Lp-B:C, Lp-B:C:E, and Lp-A-II:B:C:D:E particles, while the U-fraction contained cholesterol-rich Lp-B and Lp-B:E particles. If this latter fraction still reacted positively with anti-apoC-III serum, it was rechromatographed until free of apoC-III. The R-fraction from the anti-apoE immunosorber consisted of Lp-B:C:E, Lp-A-II:B:C:D:E and a negligible amount of Lp-B:E; the U-fraction contained Lp-B and Lp-B:C particles. If still reacting positively with anti-apoE serum, the U-fraction was rechromatographed until free of apoE-containing lipoproteins. The VLDL + IDL fractions as well as R- and U-fractions from all three immunosorbers were analyzed for apoB contents. The levels of Lp-B:C particles were calculated by subtracting apoB values of apoE-R fraction from those of apoC-III-R fraction. The concentrations of Lp-B:C:E particles were determined by subtracting apoB levels of apoA-II-R faction from corresponding apoB levels of apoE-R fraction. The Lp-A-II:B:C:D:E concentrations were expressed as apoB levels of apoA-II-R fraction. The acceptable recoveries of apoB from R- and U-fractions ranged between 75 and 90%; all values were adjusted to the apoB levels of starting VLDL + IDL and the concentrations of Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E particles were expressed in terms of corresponding apoB values (mg apoB/100 mg TG). The between assay coefficients of variations for anti-apoA-II R-fraction, anti-apoC-III R-fraction and anti-apoE R-fraction were 9 to 10%, 8 to 9% and 6 to 7%, respectively.

Determination of lipids and apolipoproteins

Plasma total cholesterol (TC; CHOP/PAP; Boehringer, Mannheim, Germany) and TG (kits for total TG and free glycerol; Abbott Laboratories, Diagnostic Division, Irving, TX, USA) concentrations were determined enzymatically on an Abbott VP-Super System Analyzer. High-density lipoprotein (HDL) cholesterol was measured by a modified heparin-manganese precipitation procedure of Warnick and Albers [30]. These three assays were standardized with plasma calibrators and control samples supplied by the Centers for Disease Control, Atlanta, Georgia. VLDL-cholesterol levels were assumed to equal one fifth of the plasma TG concentration, and low-density lipoprotein (LDL) cholesterol levels were determined by the method of Friedewald, Levy and Fredrickson [31]. Cholesterol esters (CE), free cho-

Table 1. Concentrations of plasma lipids in uremic patients and normal controls

		Total cholesterol	Triglycerides	VLDL-C	LDL-C	HDL-C
Subjects	*N*	*mmol/L*				
CRF-I	18	6.2 ± 1.6[a]	2.2 ± 1.2[a]	1.0 ± 0.5[a]	4.4 ± 1.3[a]	1.0 ± 0.3
CRF-II	8	6.1 ± 1.2[a]	1.9 ± 0.3[b]	0.9 ± 0.2[b]	4.4 ± 1.2[a]	0.8 ± 0.14[a]
Controls	10	4.9 ± 0.8	1.2 ± 0.3	0.6 ± 0.1	3.4 ± 0.8	1.1 ± 0.2

Data are mean ± SD. Abbreviations are: CRF-I, patients before dialysis; CRF-II, patients on hemodialysis.

CRF-I, CRF-II vs. Controls; [a] $P < 0.02$ and [b] $P < 0.001$

lesterol (FC) and TG in VLDL + IDL were determined by GC according to a procedure by Kuksis et al [26].

The quantitative determination of apolipoproteins was performed by previously described electroimmunoassay for apoA-I [32], apoB [29], apoC-III [33], and apoE [34]. The measurement of apoC-III bound to apoA- (HDL) and apoB- (VLDL + LDL) containing lipoproteins was performed on heparin-Mn^{++} supernates (apoC-III-HS) and heparin-Mn^{++} precipitate (apoC-III-HP), respectively, according to a previously described procedure [35]. The apoC-III-ratio was calculated as apoC-III-HS / apoC-III-HP.

Determination of glomerular filtration rate

Glomerular filtration rate (GFR) was determined as the plasma or renal clearance of ^{51}Cr-EDTA [36]. The renal clearance method was used when the GFR value was below 20 mL/min/1.75 m^2 BSA.

Statistical analysis

Conventional statistical methods were used to describe the salient features of the results. The Student unpaired two-tailed *t* test was used to test the significance of differences between means. A difference between variables with *P* value <0.05 was considered significant. The correlation coefficients were calculated by Spearman's rank correlation method.

RESULTS

Plasma lipid and apolipoprotein profiles

There were no significant differences in the levels of plasma lipids between CRF-I and CRF-II patients (Table 1). On the other hand, both patient groups had significantly higher concentrations of TC, TG, VLDL-C and LDL-C than controls; however, only CRF-II patients had significantly lower levels of HDL-C than control subjects.

Except for significantly lower apoA-I levels ($P < 0.001$), the apolipoprotein profile of CRF-II patients did not differ from that of CRF-I patient group (Table 2). Although both CRF-I and CRF-II patients had significantly higher concentrations of apolipoproteins B, C-III, E and C-III-HP (apoC-III bound to apoB-containing lipoproteins) than controls, the difference in the levels of these apolipoproteins between patients and controls was greater in the CRF-II than CRF-I patient group. ApoA-I levels were significantly lower in CRF-II, but not CRF-I, in comparison with controls. There was no difference in the levels of apoC-III-HS (apoC-III bound to apoA-I-containing lipoproteins) between patient and control subjects. The apoC-III-HS/apoC-III-HP ratio was higher in controls than patient groups, but the significance level was only reached between controls and CRF-II patients.

Kinetic properties of VLDL + IDL

To determine the contribution of compositional abnormalities to the accumulation of TG-rich lipoproteins in patients with renal insufficiency, the lipolytic rate constants of VLDL + IDL fractions were determined in CRF-I and CRF-II patients and compared with corresponding rate constants of asymptomatic control subjects. Results presented in Table 3 show no difference in the mean k_1 values between patients at the earlier stages of renal dysfunction (CRF-I) and patients on hemodialysis (CRF-II). However, the k_1 values of both patient groups were significantly ($P < 0.0005$ for CRF-I and $P < 0.001$ for CRF-II patients) lower than those of control subjects. The time course of TG lipolysis catalyzed in a standardized assay by bovine LPL showed significant differences between patients and controls at all time points (from 10 to 60 min; Fig. 1). These results suggest the existence of considerable abnormalities in the composition and/or concentration of apolipoprotein-defined lipoprotein families in VLDL + IDL of patients with renal insufficiency.

Composition of VLDL + IDL

To identify the possible compositional abnormalities, the VLDL + IDL fractions from patients and controls were characterized by measurements of lipids, apolipoproteins and apoB-containing lipoprotein families.

The determination of neutral lipid composition of VLDL + IDL fractions revealed significant differences in the percentage composition of TG, CE and FC between patients and controls (Table 4). The percentage composition of neutral lipids of VLDL + IDL from CRF-I patients was very similar, if not identical, to that of VLDL + IDL from CRF-II patients. However, when compared to neutral lipids of controls, the relative content of TG was significantly lower and the relative contents of CE and FC were significantly higher in both patient groups.

Concentrations of apolipoproteins B, C-III and E (normalized to 100 mg TG per VLDL + IDL fraction) differed significantly between and, in some cases, within the patient groups and controls (Table 5). The CRF-I patients had significantly lower levels of apoB ($P < 0.01$)

Table 2. Concentrations of plasma apolipoproteins in uremic patients and normal controls

		Apolipoproteins *mg/dL*						
	N	A-I	B	C-III	E	C-III-HS	C-III-HP	C-III ratio
CRF-I	18	116 ± 22	127 ± 36[c]	19.4 ± 8.9[b]	13.0 ± 5.6[a]	5.2 ± 2.0	11.5 ± 7.3[a]	0.80 ± 0.85
CRF-II	8	84 ± 9.4[b]	139 ± 37[c]	22.9 ± 3.9[d]	16.0 ± 6.7[b]	7.3 ± 3.8	13.4 ± 3.3[d]	0.62 ± 0.42[a]
Controls	10	120 ± 16	91 ± 14	11.6 ± 2.8	9.7 ± 4.5	4.5 ± 0.8	4.7 ± 1.5	0.95 ± 0.30

Data are mean ± SD. Abbreviations are in Table 1.
CRF-I, CRF-II vs. Controls: [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, [d] $P < 0.0005$

Table 3. Kinetic rate constants for lipolysis of VLDL + IDL of uremic patients and normal controls

Subjects	*N*	Lipolytic rate constant k_1 min^{-1}	*P* value
CRF-I	18	0.0341 ± 0.0111	<0.0005
CRF-II	8	0.0352 ± 0.0076	<0.001
Controls	10	0.0515 ± 0.0103	

Data are mean ± SD. Abbreviations are in Table 1. *P* values denote the significance of difference when CRF-I and CRF-II are compared to normal controls.

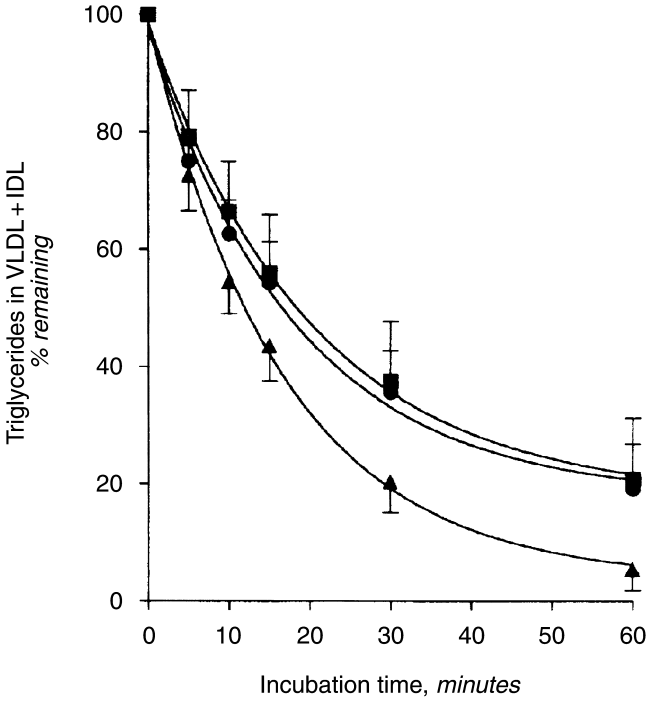

Fig. 1. Time course of the lipolytic degradation of very-low density lipoprotein (VLDL) + intermediate density lipoprotein (IDL) from uremic patients and controls catalyzed by bovine milk lipoprotein lipase. Symbols are: (■) predialytic patients (CRF-I); (●) patients on hemodialysis (CRF-II); (▲) controls. There was no significant difference between CRF-I and CRF-II at all time points, and there were no significant differences between controls and both CRF-I and CRF-II at all time points except at five minutes.

and apoE ($P < 0.05$) than CRF-II patients. However, both patient groups had significantly higher concentrations of apoB and apoC-III, but not apoE, than normal controls. In comparison with predialytic patients, the

Table 4. Neutral lipid composition of VLDL + IDL of uremic patients and normal controls

		Neutral lipids %		
Subjects	*N*	Triglycerides	Cholesterol ester	Free cholesterol
CRF-I	18	60.4 ± 5.5[c]	30.7 ± 5.5[c]	8.9 ± 1.2[c]
CRF-II	8	59.7 ± 6.3[b]	30.6 ± 6.6[a]	9.7 ± 1.9[c]
Controls	10	72.5 ± 9.5	21.0 ± 8.7	6.5 ± 1.2

Data are mean ± SD. Abbreviations are in Table 1.
CRF-1, CRF-II vs. Controls: [a] $P < 0.01$, [b] $P < 0.005$, [c] $P < 0.001$

Table 5. Concentrations of apolipoproteins per 100 mg triglycerides in VLDL + IDL of uremic patients and normal controls

		Apolipoproteins *mg/100 mg TG*				
Subjects	*N*	B	C-III	E	C-III/B	E/B
CRF-I	18	28.2 ± 9.6[c]	6.3 ± 2.3[d]	5.4 ± 3.3	0.22 ± 0.09	0.16 ± 0.08[a]
CRF-II	8	44.0 ± 18.8[d]	5.8 ± 1.9[b]	9.8 ± 9.6	0.17 ± 0.08	0.21 ± 0.14
Controls	10	18.9 ± 5.1	3.8 ± 1.5	4.9 ± 3.2	0.22 ± 0.11	0.26 ± 0.19

Data are mean ± SD. Abbreviations are the same as Table 1.
CRF-I, CRF-II vs. Controls: [a] $P < 0.05$, [b] $P < 0.025$, [c] $P < 0.005$, [d] P 0.0005

Table 6. Concentrations of complex ApoB-containing lipoprotein families per 100 mg triglycerides in VLDL + IDL of uremic patients and normal controls

		Lp-A-II:B:C:D:E	Lp-B:C	Lp-B:C:E
Subjects	*N*	*mg/100 mg TG*		
CRF-I	18	4.6 ± 3.7	9.8 ± 7.8[b]	12.3 ± 7.0
CRF-II	8	5.2 ± 5.5	9.1 ± 5.4[c]	17.9 ± 0.8[d]
CRF-I + II	26	4.7 ± 4.1	9.6 ± 7.1[c]	13.6 ± 6.6[a]
Controls	10	3.6 ± 1.7	2.7 ± 1.5	8.4 ± 4.5

Data are mean ± SD. Abbreviations are in Table 1.
CRF-I, CRF-II, CRF-I + II vs. Controls: [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.005$, [d] $P < 0.001$

VLDL + IDL fractions of patients on hemodialysis were enriched with apoB and apoE, but this increase had no additional effect on the lipolytic rate.

Due to significant increases of apoB and apoC-III concentrations of VLDL + IDL fractions from uremic patients, it was of considerable interest to determine the levels of individual TG-rich lipoprotein families, all of which are characterized by apolipoproteins B and C-III as main protein constituents. As shown in Table 6, the levels of Lp-A-II:B:C:D:E, Lp-B:C, and Lp-B:C:E parti-

Table 7. Correlations between lipolytic rate constant, k_1, and neutral lipids and Lp-A-II:B:C:D:E particles in VLDL + IDL of uremic patients

Patients	*N*	Correlation variables *r*	Coefficients	*P* value
CRF-I + II	26	Free cholesterol *%*	−0.81	<0.001
CRF-I + II	26	Esterified cholesterol *%*	−0.63	<0.001
CRF-I + II	26	Triglycerides *%*	0.72	<0.001
CRF-I + II	26	Lp-A-II:B:C:D:E *%*	−0.47	<0.03
CRF-I + II	26	Lp-A-II:B:C:D:E *mg/100 mg TG*	−0.46	<0.03

Abbreviations are in Table 1.

cles, normalized to 100 mg TG of VLDL + IDL fractions, were determined in both patient groups and compared separately and as a combined patient population with those of normal subjects. Numerically, the levels of Lp-A-II:B:C:D:E particles were higher in all three patient groups than controls. The concentration of Lp-B:C particles was significantly higher in CRF-I ($P < 0.01$) and CRF-II ($P < 0.005$) patients and in the combined patient group ($P < 0.005$) in comparison with control subjects. The Lp-B:C:E particle levels of uremic patients were also higher than those of normal controls, but the statistical significance was only reached in patients on hemodialysis ($P < 0.001$) and in the combined patient population ($P < 0.05$). These results indicate that in both patient groups, the accumulation of TG-rich lipoproteins in VLDL + IDL fraction is due to a 3.5-fold increase in the levels of Lp-B:C particles accompanied by smaller increases in the levels of Lp-A-II:B:C:D:E and Lp-B:C:E particles.

Correlations between lipolytic rate constant and lipoproteins

Since there was no difference in the mean values of the lipolytic rate constant (k_1) between the CRF-I and CRF-II patient groups (Table 3), these two groups were combined to determine any possible correlations between the k_1 values and lipids, apolipoproteins and lipoprotein families of VLDL + IDL fractions. There were no significant correlations between the k_1 values and any of the plasma lipids and apolipoproteins. However, all three neutral lipid constituents of VLDL + IDL correlated significantly with k_1 values ($P < 0.001$); the relative contents of FC ($r = -0.81$) and CE ($r = -0.63$) correlated negatively, while the percent content of TG correlated positively ($r = 0.72$) with the lipolytic rate constant (Table 7). In contrast, none of the measured apolipoproteins of VLDL + IDL correlated significantly with the k_1 values.

Among the three major TG-rich lipoprotein families of VLDL + IDL fraction neither Lp-B:C nor Lp-B:C:E correlated with the k_1 values. However, there was a significant ($P < 0.03$) negative correlation between the lipolytic rate constant and Lp-A-II:B:C:D:E particles expressed both in terms of the percent content ($r = -0.47$) and concentration ($r = -0.46$) of Lp-A-II:B:C:D:E particles.

In contrast to uremic patients, there was no correlation between plasma or VLDL + IDL lipids, apolipoproteins and lipoprotein families and k_1 values in control subjects.

The lipolytic rate constant was not correlated with GFR in CRF-I patients.

DISCUSSION

The results of present study have provided additional evidence that the characteristic accumulation in renal insufficiency of intact and/or partially metabolized TG-rich apoB-containing lipoproteins is related to a defect(s) in the lipolytic degradation of these lipoproteins. The retarded catabolism of TG-rich VLDL and IDL has been generally attributed to the reduced enzymic activity of LPL [7, 8, 10–13]. However, it has been reported more recently that a low LPL activity may not be the only factor responsible for impaired degradation of TG-rich lipoproteins [14–16]. Arnadottir et al have shown that, due to abnormal lipid and apolipoprotein composition, VLDL isolated from patients on hemodialysis is a poor substrate for bovine LPL.

To further explore the possible underlying defect(s) responsible for the slow lipolysis of TG-rich lipoproteins, the present study has been extended to renal patients with a wider spectrum of renal dysfunction by including patients before dialysis as well as patients on hemodialysis. Since the intact and partially delipidized TG-rich lipoproteins accumulate in both VLDL and IDL density regions [6, 37, 38], these two lipoprotein density fractions were combined and characterized by the determination of lipolytic rate constant (k_1) and lipid, apolipoprotein and apoB-containing lipoprotein particle profiles.

In contrast to a previously reported study by Arnadottir et al [15] using a fluorescent phospholipid analog to determine the lipolytic kinetics of VLDL from hemodialysis patients, we have determined the lipolytic rates by measuring the actual TG mass remaining after lipolysis of VLDL + IDL fractions. Whereas the former study [15] showed a difference in the extent of lipolysis, but not in the initial rate of lipolysis between VLDL from patients and controls, the direct measurement of TG in the present study showed that both the initial rate and extent of lipolysis were significantly different between VLDL + IDL substrates from patients and controls. These differences in kinetic findings were due most probably to different methodologies used in these two studies.

The lipolytic rates of VLDL + IDL fractions from patients before dialysis and patients on hemodialysis were significantly lower than those from asymptomatic controls, indicating that TG-rich lipoproteins of very low and intermediate density properties from both patient groups

are a poor substrate for bovine milk LPL, and that this metabolic defect is already present at the earlier stages of renal insufficiency. The possibility that the decreased lipolytic rate was due to increased plasma triglyceride levels was ruled out, because the k_1 values of VLDL + IDL fractions from asymptomatic subjects with mild hypertriglyceridemia were within the normal range (data not shown). Both patient groups had abnormal plasma lipid and apolipoprotein levels in comparison with normal controls. The only significant differences between the CRF-I and CRF-II patients were in the levels of plasma apoA-I ($P < 0.001$) and apoC-III-HS ($P < 0.05$; Table 2). However, since these two patient groups had similar k_1 values, the apoA-I-containing lipoproteins were ruled out as a significant factor affecting the lipolytic rates.

The lipid and apolipoprotein composition of VLDL + IDL fractions characterized by high percent contents of FC and CE, relatively low percentage of TG and increased levels of apolipoproteins B and C-III suggested that the reduced lipolytic rates of TG-rich lipoproteins in both CRF-I and CRF-II patients may be related to increased concentrations of apoC-III- and apoB-containing lipoprotein families of abnormal neutral lipid composition. To test this possibility, it was necessary to determine the potential effect of three major TG-rich lipoprotein families, that is, Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E, on the lipolytic rate constants of VLDL + IDL from renal patients and control subjects. In all patients, there was a significant increase in the levels of Lp-B:C and Lp-B:C:E and a numerical increase in the concentration of Lp-A-II:B:C:D:E particles. However, both the relative and absolute contents of Lp-A-II:B:C:D:E particles showed a statistically significant negative correlation (Table 7) with the lipolytic rate constant of VLDL + IDL fractions, indicating that even a slight increase in the levels of this complex lipoprotein family had a significant effect on the lipolytic degradation of TG-rich lipoproteins. Moreover, the significant inverse correlation between the lipolytic rate and percent contents of FC and CE of VLDL + IDL on one hand and a positive correlation with that of TG (Table 7) on the other showed clearly that neutral lipid composition of substrate is also a significant factor affecting the lipolytic rates: the higher the relative contents of FC and CE and the lower the relative content of TG, the lower the k_1 values and *vice versa*. It has been previously established that Lp-A-II:B:C:D:E particles isolated from VLDL of patients with Tangier disease or type V hyperlipoproteinemia have k_1 values significantly lower than those of Lp-B:C and Lp-B:C:E particles [21]. However, the Lp-A-II:B:C:D:E, Lp-B:C and Lp-B:C:E particles from Tangier or type V hyperlipoproteinemic subjects had normal neutral lipid composition (CE = 14.5%, FC = 6.8%, TG = 78.6%) in comparison with that of VLDL + IDL from renal patients (CE = 37.5%, FC = 8.3%, TG = 54.1%). Furthermore, the mean values of neutral lipid composition of Lp-A-II:B:C:D:E particles (CE = 40.3%, FC = 8.1%, TG = 52.5%, $N = 8$) and Lp-B:C + Lp-B:C:E particles (CE = 35.2%, FC = 7.5%, TG = 57.2%, $N = 8$) from renal patients were found to be significantly ($P < 0.0001$ for CE and TG, but NS for FC) different from those of corresponding Lp-A-II:B:C:D:E particles (CE = 20.7%, FC = 7.2%, TG = 72.0%, $N = 6$) and Lp-B:C + Lp-B:C:E particles (CE = 12.5%, FC = 6.9%, TG = 80.6%, $N = 6$) from control subjects. Taken together, these findings suggest that the levels of Lp-A-II:B:C:D:E particles and the abnormal neutral lipid composition of all TG-rich lipoprotein particles represent the main factors affecting the lipolytic rates of VLDL + IDL fractions in patients with renal insufficiency. This is, to our knowledge, the first indication that a specific apolipoprotein-defined lipoprotein family within a heterogeneous lipoprotein density class is closely associated with a defective metabolic process in renal disease.

Arnadottir et al found a significant inverse correlation between the extent of lipolysis and increased levels of apoC-III bound to apoB-containing lipoproteins [15]. In contrast, results of this study showed no significant correlation between the lipolytic rate constant, k_1, and the increased levels of apoC-III bound to apoB-containing lipoproteins. However, there was a significant inverse correlation between the k_1 values and the levels of Lp-A-II:B:C:D:E particles, as one of the apoC-III-containing TG-rich lipoproteins. These differences were due most probably to differences in kinetic parameters, substrates, and selection of patients. However, the results of both studies suggested a possible association of apoC-III with impaired catabolism of some of the TG-rich lipoproteins.

There are several reports in the literature indicating the occurrence of LPL inhibitors in uremic plasma at density higher than 1.25 g/mL [6, 16]. However, since in this study VLDL + IDL were isolated ultracentrifugally at a density less than 1.019 g/mL, the possible presence of inhibitors can be ruled out.

By measuring oxidative stress in VLDL + IDL of patients and controls using a newly developed procedure [39], it was shown that the lipolytic rate constant, k_1, was not correlated with the oxidative indexes ($r = 0.18$, $P = 0.33$ for oxidative index 1 and $r = 0.13$, $P = 0.48$ for oxidative index 2); this finding ruled out the possible contribution of oxidized lipids to reduced k_1-values. Similarly, the measurement of five molecular species of TG separated by GC on the basis of the fatty acid chain lengths showed no significant difference in the composition of TG molecular species between patients and controls; based on this finding, a possible contribution of fatty acids to reduced k_1 values has been ruled out.

The lack of a significant correlation between k_1 and GFR values suggests that the reduced lipolytic rate of VLDL + IDL fractions may not be associated with the

degree of renal functional impairment. It appears that once the renal dysfunction has resulted in abnormal composition and concentration of TG-rich lipoproteins, occurring already at the early stages of renal insufficiency [40], the reduced lipolytic rate will not be affected by further changes in renal function.

One can only speculate about the sequence of events leading to the abnormal neutral lipid composition of TG-rich lipoprotein families and increased levels of Lp-A-II:B:C:D:E particles as the most probable factors contributing to decreased lipolytic rates of VLDL + IDL fractions in renal insufficiency. This chain of events may be initiated by a reduced plasma lipolytic activity shown to occur already at the early stages of renal insufficiency [10, 41]. Consequently, a partial hydrolysis of TG would result in a changed neutral lipid composition of TG-rich lipoproteins characterized by increased contents of FC and CE and decreased content of TG [14, 15, 38, 42–44]. The increased cholesterol ester transfer from HDL to VLDL and IDL, at least in patients with microalbuminuria or proteinuria [45], and/or the proposed hepatic formation of cholesterol-rich VLDL [43] may represent the other sources of cholesterol-enriched VLDL and IDL in renal insufficiency. The reduced lipolytic activity and decreased substrate affinity for LPL (due to increased cholesterol/triglyceride ratio of substrate) would then result in the accumulation of partially delipidized Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E particles as lipoprotein family constituents of VLDL + IDL fractions. Due to their inherently low affinity for LPL, any increases in the levels of Lp-A-II:B:C:D:E particles would further contribute to the low k_1-values of VLDL + IDL fractions.

Although the reduced lipolytic rate of VLDL + IDL fractions does not seem to be associated with the degree of renal dysfunction, the accumulation of modified Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E particles may play a significant pathophysiologic role in renal disease. We have shown recently in a prospective study [46] that the rate of progression of human chronic renal insufficiency was significantly associated with the sum of triglyceride-rich Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E particles ($Lp\text{-}B_{complex}$) of very low and intermediate densities, but not with cholesterol-rich Lp-B particles, the main lipoprotein species of LDL. This finding has been recently supported by results of the prospective Atherosclerosis Risk in Community Study (ARIC) showing that high TG and low HDL-C, but not LDL-C, predict an increased risk of renal dysfunction [47]. It still remains to be determined, however, whether there are differences among the three major TG-rich lipoprotein families in the degree of kidney damage.

The TG-rich lipoprotein families may not only contribute to the progression of renal dysfunction but also play a role in the development and progression of atherosclerotic lesions. Results of the Monitored Atherosclerosis Regression Study (MARS) have indicated that the progression of coronary artery disease in non-renal subjects is significantly associated with increased levels of directly measured $Lp\text{-}B_c$ and Lp-A-II:B:C:D:E particles [48]. More recently, apoC-III bound to apoB-containing lipoproteins was found to be an independent predictor of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) Trial [49]. In contrast to Lp-B:C:E, the Lp-B:C particles seem to have a very low binding affinity for the LDL receptors [50, 51] and, thus, may have longer residence time in the systemic circulation than apoE-containing Lp-B:C:E or Lp-A-II:B:C:D:E particles. It has already been shown in a preliminary study that Lp-B:C particles are taken up by human THP1 macrophage at a higher rate than other apoB-containing lipoprotein families; macrophages incubated with Lp-B:C were readily transformed into foam cells suggesting a marked atherogenic potential of these lipoprotein particles (abstract; Koren et al, *Atherosclerosis* 109:217, 1994). However, it still remains to be established to what extent individual TG-rich lipoprotein particles also may contribute to the accelerated atherosclerosis in patients with CRF.

In conclusion, results of this study have provided additional evidence that the accumulation in renal insufficiency of intact and/or partially delipidized TG-rich apoB-containing lipoproteins is related, at least in part, to a defect in the lipolytic degradation of these lipoproteins. The lipolytic rate constant, k_1, of combined VLDL and IDL fractions from patients with chronic renal failure before dialysis and patients on hemodialysis was significantly lower than that of asymptomatic control subjects. The levels of all three major apolipoprotein-defined Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E particles occurring in VLDL + IDL fractions were increased in renal patients in comparison with controls and had an abnormal neutral lipid composition characterized by increased relative contents of free and esterified cholesterol and decreased percentage of triglycerides. Correlation analyses have shown that increased levels of Lp-A-II:B:C:D:E particles and the abnormal neutral lipid composition of all TG-rich lipoproteins represent the main factors affecting the in vitro lipolytic rates of VLDL + IDL fractions in patients with renal insufficiency. Whether these factors also affect lipolytic processes in vivo remains to be established in future studies.

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APPENDIX

Abbreviations used in this article are: apo, apolipoprotein; CE, cholesterol esters; CRF, chronic renal failure; FC, free cholesterol; GC, gas chromatography; HDL, high density lipoproteins; HP, heparin-manganese precipitate; HS, heparin-manganese supernate; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; Lp-B:C, lipoprotein B:C; Lp-B:C:E, lipoprotein B:C:E; Lp-A-II:B:C:D:E, lipoprotein A-II:B:C:D:E; LPL, lipoprotein lipase; TC, total cholesterol; TG, triglycerides; VLDL, very low density lipoproteins.

REFERENCES

1. Bagdade JD, Porte D, Bierman EL: Hypertriglyceridemia: a metabolic consequence of chronic renal failure. *N Engl J Med* 279:181–185, 1968
2. Bagdade JD, Albers JJ: Plasma high-density lipoprotein concentration in chronic-hemodialysis and renal transplant patients. *N Engl J Med* 296:1436–1439, 1977
3. Nestel PJ, Fidge NH, Tan MH: Increased lipoprotein remnant formation in chronic renal failure. *N Engl J Med* 307:329–333, 1982
4. Ron D, Oren I, Aviram M, *et al*: Accumulation of lipoprotein remnants in patients with chronic renal failure. *Atherosclerosis* 46: 67–75, 1983
5. Attman PO, Alaupovic P, Gustafson A: Serum apolipoprotein profile of patients with chronic renal failure. *Kidney Int* 32:368–375, 1987
6. Attman P-O, Samuelsson O, Alaupovic P: Lipoprotein metabolism and renal failure. *Am J Kidney Dis* 21:573–592, 1993
7. Applebaum-Bowden D, Goldberg AP, Hazzard WR, *et al*: Post-heparin plasma triglyceride lipase in chronic hemodialysis: Evidence for a role for hepatic lipase in lipoprotein metabolism. *Metabolism* 28:917–924, 1979
8. Savdie E, Gibson JC, Crawford GA, *et al*: Impaired plasma triglyceride clearance as a feature of both uremic and posttransplant triglyceridemia. *Kidney Int* 18:774–782, 1980
9. Chan MK, Varghese Z, Persaud JW, *et al*: Hyperlipidemia in patients on maintenance hemo- and peritoneal dialysis: the relative pathogenetic roles of triglyceride production and triglyceride removal. *Clin Nephrol* 17:183–190, 1982
10. McCosh EJ, Solangi K, Rivers JM, Goodman A: Hypertriglyceridemia in patients with chronic renal insufficiency. *Am J Clin Nutr* 28:1036–1043, 1975
11. Ibels LS, Reardon MF, Nestel PJ: Plasma post-heparin lipolytic activity and triglyceride clearance in uremic and hemodialysis patients and renal allograft recipients. *J Lab Clin Med* 87:648–658, 1976
12. Chan MK, Persaud J, Varghese Z, Moorhead JF: Pathogenetic roles of post-heparin lipases in lipid abnormalities of hemodialysis patients. *Kidney Int* 25:812–818, 1984
13. Mordasini R, Frey F, Flury W, *et al*: Selective deficiency of hepatic triglyceride lipase in uremic patients. *N Engl J Med* 297:1362–1366, 1977
14. Arnadottir M, Thysell H, Dallongeville J, *et al*: Evidence that reduced lipoprotein lipase activity is not a primary pathogenetic factor for hypertriglyceridemia. *Kidney Int* 48:779–784, 1995
15. Arnadottir M, Dallongeville J, Fruchart J-C, Nilsson-Ehle P: Very-low-density lipoprotein of uremic patients is a poor substrate for bovine lipoprotein lipase in vitro. *Metabolism* 45:686–690, 1996
16. Arnadottir M: Pathogenesis of dyslioprotinemia in renal insufficiency: The role of lipoprotein lipase and hepatic lipase. *Scand J Clin Lab Invest* 57:1–11, 1997
17. Ewing AM, Freeman NK, Lindgren FT: The analysis of human serum lipoprotein distribution. *Adv Lipid Res* 3:25–61, 1965
18. Nestel P, Billington T, Tada N, *et al*: Heterogeneity of very-low-density lipoprotein metabolism in hyperlipidemic subjects. *Metabolism* 32:810–817, 1983
19. Demant T, Shepherd J, Packard CJ: Very low density lipoprotein apolipoprotein B metabolism in humans. *Klin Wochenschr* 66:703–712, 1988
20. Alaupovic P: Apolipoprotein composition as the basis for classifying plasma lipoproteins. Characterization of ApoA- and ApoB-containing lipoprotein families. *Prog Lipid Res* 30:105–138, 1991
21. Alaupovic P, Knight-Gibson C, Wang C-S, *et al*: Isolation and characterization of an apoA-II-containing lipoprotein (Lp-A-II:B complex) from plasma very low density lipoproteins of patients with Tangier disease and type V hyperlipoproteinemia. *J Lipid Res* 32:9–19, 1991
22. Lee DM: Apolipoprotein B-48: problems related to quantification. *Methods Enzymol* 263:146–165, 1996
23. Lee DM, Downs D: A quick and large-scale density gradient subfractionation method for low density lipoproteins. *J Lipid Res* 23:14–27, 1982
24. Posner I, Wang C-S, McConathy WJ: The comparative kinetics of soluble and heparin Sepharose-immobilized bovine lipoprotein lipase. *Arch Biochem Biophys* 226:306–316, 1983
25. McConathy WJ, Gesquiere JC, Bass H, *et al*: Inhibition of lipoprotein lipase activity by synthetic peptides of apolipoprotein-C-III. *J Lipid Res* 33:995–1003, 1992
26. Kuksis A, Myher JJ, Marai L, Geher K: Determination of plasma lipid profiles by automated gas chromatography and computerized data analysis. *J Chromatogr Sci* 13:423–430, 1981
27. Dashti N, Alaupovic P, Knight-Gibson C, Koren E: Identification and partial characterization of discrete apolipoprotein B-containing lipoprotein particles produced by human hepatoma cell line HepG2. *Biochemistry* 26:4837–4846, 1987
28. Alaupovic P, Hodis HN, Knight-Gibson C, *et al*: Effects of lovastatin on apoA- and apoB-containing lipoprotein families in a subpopulation of patients participating in the Monitored Atherosclerosis Regression Study (MARS). *Arterioscler Thromb* 14:1906–1914, 1994
29. Curry MD, Gustafson A, Alaupovic P, McConathy WJ: Electroimmunoassay, radioimmunoassay, and radial immunodiffusion assay evaluated for quantification of human apolipoprotein B. *Clin Chem* 24:280–286, 1978
30. Warnick GR, Albers JJ: A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high density lipoprotein cholesterol. *J Lipid Res* 19:65–76, 1978
31. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499–502, 1972
32. Fesmire JD, McConathy WJ, Alaupovic P: Use and significance of reference serum as a secondary standard for electroimmunoassay of apolipoprotein A-I. *Clin Chem* 30:712–716, 1984
33. Curry MD, McConathy WJ, Fesmire JD, Alaupovic P: Quantitative determination of human apolipoprotein C–III by electroimmunoassay. *Biochim Biophys Acta* 617:505–513, 1980
34. Curry MD, McConathy WJ, Alaupovic P, *et al*: Determination of human apolipoprotein E by electroimmunoassay. *Biochim Biophys Acta* 439:413–425, 1976
35. Attman P-O, Alaupovic P: Lipid and apolipoprotein profiles of uremic dyslipoproteinemia—relation to renal function and dialysis. *Nephron* 57:401–410, 1991
36. Jagenburg R, Attman P-O, Aurell M, Bucht H: Determination of glomerular filtration rate in advanced renal insufficiency. *Scand J Urol Nephrol* 12:133–137, 1978
37. Attman P-O, Tavella M, Knight-Gibson C, *et al*: Apolipoprotein B-containing lipoprotein particles in progressive renal insufficiency. *Contrib Nephrol* 98:11–19, 1992
38. Attman P-O, Alaupovic P, Tavella M, Knight-Gibson C: Abnormal lipid and apolipoprotein composition of major lipoprotein

density classes in patients with chronic renal failure. *Nephrol Dial Transplant* 11:63–69, 1996
39. Lee DM: A simple and sensitive method in using the ratios of cholesteryl ester molecular species as indexes of oxidative stress in plasma and lipoprotein fractions. *Atherosclerosis* 146:221–235, 1999
40. Samuelsson O, Attman P-O, Knight-Gibson C, *et al*: Lipoprotein abnormalities without hyperlipidaemia in moderate renal insufficiency. *Nephrol Dial Transplant* 9:1580–1585, 1994
41. Crawford GA, Savdie E, Steward JH: Heparin-released plasma lipases in chronic renal failure and after renal transplantation. *Clin Sci* 57:155–165, 1979
42. Norbeck HE, Carlson LA: Increased frequency of late pre-β lipoproteins (Lpβ) in isolated serum very low density lipoproteins in uraemia. *Eur J Clin Invest* 10:423–426, 1980
43. Norbeck HE, Rössner S: Intravenous fat tolerance test with Intralipid in chronic renal failure. *Acta Med Scand* 211:69–74, 1982
44. Joven J, Villela E, Ahmad S, *et al*: Lipoprotein heterogeneity in end-stage renal disease. *Kidney Int* 43:410–418, 1993
45. Dullaart RPF, Gansevoort RT, Dikkeschei BD, *et al*: Role of elevated lecithin-cholesterol acyltransferase and cholesterol ester transfer protein activities in abnormal lipoproteins from proteinuric patients. *Kidney Int* 44:91–97, 1993
46. Samuelsson O, Attman P-O, Knight-Gibson C, *et al*: Complex apolipoprotein B-containing lipoprotein particles are associated with a higher rate of progression of human chronic renal insufficiency. *J Am Soc Nephrol* 9:1482–1488, 1998
47. Muntner P, Coresh J, Smith JC, *et al*: Plasma lipids and risk of developing renal dysfunction: The Atherosclerosis Risk in Communities Study. *Kidney Int* 58:293–301, 2000
48. Alaupovic P, Mack WJ, Knight-Gibson, Hodis HN: The role of triglyceride-rich lipoprotein families in the progression of atherosclerotic lesions as determined by sequential coronary angiography from a controlled clinical trial. *Arterioscler Thromb Vasc Biol* 17: 715–722, 1997
49. Sacks FM, Alaupovic P, Moye LA, *et al*: VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) Trial. *Circulation* 102:1886–1892, 2000
50. Agnani G, Bard JM, Candelier L, *et al*: Interaction of LpB, LpB:E, LpB:C–III, and LpB:C–III:E lipoproteins with the low density lipoprotein receptor of HeLa cells. *Arterioscler Thromb* 11:1021–1029, 1991
51. Clavey V, Lestavel-Delattre S, Copin C, *et al*: Modulation of lipoprotein B binding to the LDL receptor by exogenous lipids and apolipoproteins CI, CII, CIII, and E. *Arterioscler Thromb Vasc Biol* 15:963–971, 1995