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A broad-based metabolic approach to study VLDL apoB100 metabolism in patients with ESRD and patients treated with peritoneal dialysis

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A broad-based metabolic approach to study VLDL apoB100 metabolism in patients with ESRD and patients treated with peritoneal dialysis.

Background. Dyslipidemia is often observed in patients with end-stage renal disease (ESRD) and is associated with cardiovascular diseases. Peritoneal dialysis treatment may further deteriorate the lipoprotein abnormalities, suggesting that peritoneal dialysis alters lipid metabolism.

Methods. To study the mechanisms involved in these abnormalities in peritoneal dialysis, we measured insulin sensitivity, free fatty acids release, de novo lipogenesis (DNL), very low-density lipoprotein (VLDL) apoB100 kinetics and cholesterol synthesis in vivo in ESRD ($N = 6$), peritoneal dialysis patients ($N = 5$), and controls ($N = 7$) using stable isotopes.

Results. Insulin sensitivity, as assessed by an euglycemic hyperinsulinemic clamp, tended to be lower in ESRD and peritoneal dialysis compared to controls [$P = 0.08$ by analysis of variance (ANOVA)]. Free fatty acid release during the euglycemic hyperinsulinemic clamp tended to be higher in ESRD and peritoneal dialysis compared to controls ($P = 0.08$ by ANOVA), while DNL and fractional cholesterol synthesis were normal. VLDL-1 apoB100 ($P < 0.05$) and VLDL-2 apoB100 pool sizes ($P < 0.05$) were significantly higher in peritoneal dialysis patients compared to controls. The increased VLDL-1 apoB100 pool size was explained by increased VLDL-1 apoB100 synthesis ($P < 0.05$) in combination with reduced VLDL-1 apoB100 catabolism ($P < 0.01$), while the increased VLDL-2 apoB100 pool was explained by reduced catabolism ($P < 0.01$).

Conclusion. Both VLDL-1 apoB100 and VLDL-2 apoB100 pool sizes are increased in peritoneal dialysis patients, due to disturbances both in synthesis and catabolism. VLDL-1 apoB100 production is, at least partially, explained by increased free fatty acid availability secondary to peripheral insulin resistance, thus identifying insulin resistance as potential therapeutic target in peritoneal dialysis patients.

Key words: ESRD, peritoneal dialysis, stable isotopes, dyslipidemia, VLDL, insulin, apoB100, triglycerides, cholesterol, free fatty acids, de novo lipogenesis, lipoproteins.

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In renal failure, multiple risk factors are present that contribute to cardiovascular diseases [1, 2]. One of these risk factors, dyslipidemia, is already observed early in the course of renal disease [3] and has been forwarded as an independent risk factor for development of cardiovascular disease in end-stage renal disease (ESRD) and renal replacement therapy [4, 5]. Hemodialysis as well as peritoneal dialysis can both provide adequate relief of uremic symptoms, but the dialysis techniques appear to have differential effects on uremic dyslipidemia. Plasma triglyceride levels, plasma cholesterol and apoB100 levels are higher in peritoneal dialysis compared to hemodialysis, indicating that differences in lipoprotein metabolism are partly attributable to peritoneal dialysis treatment rather than uremia per se [6, 7]. The increase in apoB100 in peritoneal dialysis patients is most markedly in the very low-density lipoprotein (VLDL) fraction an to a minor extent in intermediate-density lipoprotein (IDL) and low-density lipoprotein (LDL) [8], while long-term treatment with peritoneal dialysis may further deteriorate some of these lipoprotein abnormalities [9, 10].

Several groups have studied the metabolism of dyslipidemia in ESRD before and after dialysis treatment and there has been debate about the mechanisms responsible for the dyslipidemia. Some groups reported impaired removal of triglyceride-rich lipoproteins [11–13], while

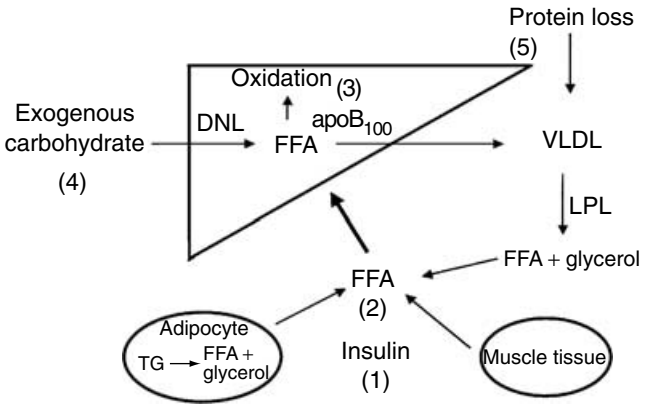

Fig. 1. Hypothetical model for hypertriglyceridemia in end-stage renal disease (ESRD). One of the key players is insulin. Reduced insulin sensitivity present in peripheral tissues in ESRD (1), contributes to increased free fatty acid (FFA) release (2). These free fatty acids are subsequently oxidized or esterified, where after, if not stored, free fatty acids are directed to very low-density lipoprotein (VLDL) apolipoprotein B (apoB100) synthesis (3). As peritoneal dialysis treatment is initiated, aggravation of hypertriglyceridemia occurs, that could be the result of stimulation of de novo lipogenesis (DNL) due to glucose loading (4) or substantial protein loss (5). High plasma concentrations of triglyceride (TG)-rich lipoproteins leads to increased release of free fatty acids and generation of remnants as a result of lipolysis by lipoprotein lipase (LPL). These free fatty acids are subjected to the liver, thereby setting up a vicious cycle and further driving VLDL production.

other studies suggest overproduction of lipoproteins, in particular VLDL [13, 14] as well.

In Figure 1, we propose a model for dyslipidemia focused on hypertriglyceridemia in ESRD that takes into account different metabolic pathways as proposed recently in more detail [15]. One of the key players in this model is insulin resistance [Fig. 1 (1)] [16]. Reduced insulin sensitivity is present in renal failure [17, 18]. As a consequence of peripheral insulin resistance, increased free fatty acids [Fig. 1 (2)] are transported to the liver, where they are subjected to oxidation or re-esterification. Subsequently, re-esterified fatty acids are either stored as cytosolic triglycerides or secreted in VLDL apolipoprotein B100 (apoB100) containing particles [Fig. 1 (3)] [19]. As VLDL apoB100 is a substrate driven process, such increased free fatty acid delivery to the liver may result in enhanced VLDL release. Dialysis treatment may aggravate these processes. In particular, in peritoneal dialysis in which substrate availability for VLDL secretion may also be enhanced due to de novo lipogenesis (DNL) associated with the repetitive glucose loading by the dialysis fluid on a background of insulin resistance [Fig. 1 (4)] [9, 20]. In addition, peritoneal dialysis is associated with substantial protein loss in the dialysate [21–23], which may in an analogous way as reported in the nephrotic syndrome further aggravate lipoprotein abnormalities [Fig. 1 (5)] [24]. High plasma concentrations of triglyceride-rich lipoproteins (VLDL and chylomicrons) [25] subsequently lead to an increase in the release of free fatty acids and generation of remnants thereby initiating a vicious cycle and further driving VLDL production.

In this report, we tested this model in patients with ESRD and patients on peritoneal dialysis treatment, using endogenous labeling with [1-^{13}C]-valine for apoB100, [1-^{13}C]-acetate for DNL and cholesterol synthesis and [2,2-$^{2}H_2$]-palmitate to determine free fatty acid release.

METHODS

Subjects

Six patients with ESRD (three males and three females), five peritoneal dialysis patients (two males and three females), and seven controls (five males and two females) participated in the study. Two patients in the ESRD group had reflux nephropathy, one patient had interstitial nephritis, one patient had IgA nephropathy, one patient had chronic pyelonephritis and the cause of renal disease in one patient was unknown. The patients in the peritoneal dialysis group had glomerulonephritis, interstitial nephritis, reflux nephropathy, and in two patients the cause of renal failure was unknown. Patients on peritoneal dialysis treatment included those on continuous ambulatory peritoneal dialysis (CAPD) or CAPD in combination with automated peritoneal dialysis (APD). Patients underwent dialysis for at least 9 months. Two patients from the ESRD group (patients 1 and 2) dropped out (one kidney transplantation and one death, respectively). The other patients had not started peritoneal dialysis yet. Patients with type 2 diabetes, familial hypercholesterolemia (FH), familial combined hyperlipidemia (FCH) and/or underlying cardiovascular disease and patients with volume overload were excluded from the study. The clinical data of the patients and controls are shown in Table 1. Patients and controls were matched for age, weight, body mass index (BMI), and body surface area (BSA). The calculated creatinine clearance, using the Cockcroft-Gault formula, was significantly decreased in ESRD compared to controls (9 ± 1 mL/min/1.73 m^2 versus 92 ± 10 mL/min/1.73 m^2, $P < 0.001$). Mean Kt/V value in the peritoneal dialysis group, where V was estimated by the formula of Watson and Watson, was 2.01 ± 0.16 (range 1.70 to 2.55). Lipid-lowering medication was stopped at least 4 weeks before participation in the study and specific medication other than vitamins, erythropoietin, iron supplementation, sodium bicarbonate, and phosphate binder supplementation are presented in Table 1. Patients 4 and 9 smoked cigarettes. Before isotope infusion, the patients recorded their food intake for 3 days and collected 24-hour urine for 3 consecutive days. Additionally, the peritoneal dialysis group also collected 24-hour dialysis fluid for 3 days.

Control subjects also recorded their food intake for 3 days and collected one 24-hour urine sample. All patients and volunteers agreed to participate after signing an

Table 1. Clinical data of the patients and control subjects

	Renal disease	Age *years*	Gender	Weight *kg*	BMI kg/m^2	BSA m^2	Creatinine clearance *mL/min/ 1.73* m^2	Kt/V	Medication
ESRD patients ($N = 6$)									
1	Chronic pyelonephritis	59	M	82.6	27.9	2.0	8	—	1
2	Reflux nephropathy	49	M	72.6	23.7	1.9	7	—	1
3	Interstitial nephritis	51	M	85.7	25.9	2.1	11	—	—
4	Reflux nephropathy	29	F	71.1	26.1	1.8	14	—	1,2
5	IgA nephropathy	19	F	83.0	31.2	1.9	9	—	1
6	Unknown	56	F	74.4	25.7	1.9	9	—	—
Mean ± SEM		44 ± 7^{NS}	3 M/3 F	78.3 ± 2.5^{NS}	26.8 ± 0.9^{NS}	1.9 ± 0.0^{NS}	9 ± 1^{a}	—	
Peritoneal dialysis patients ($N = 5$)									
7	Glomerulonephritis	49	M	87.1	26.0	2.1	—	1.70	2
8	Unknown	54	M	78.3	26.2	1.9	—	2.55	1
9	Interstitial nephritis	54	F	69.3	24.0	1.8	—	1.90	1
10	Unknown	45	F	58.8	20.8	1.7	—	1.70	1
11	Reflux nephropathy	53	F	68.1	28.0	1.7	—	2.19	2
Mean ± SEM		51 ± 2^{NS}	2 M/3 F	72.3 ± 4.8^{NS}	25.0 ± 1.2^{NS}	1.8 ± 0.0^{NS}	—	2.01 ± 0.16	
Control subjects ($N = 7$)									
Mean ± SEM		48 ± 3	5M/2F	77.4 ± 5.1	24.3 ± 0.9	1.9 ± 0.1	92 ± 10	—	—

Abbreviations are: ESRD, end-stage renal disease; BMI, body mass index; BSA, body surface area; 1, angiotensin-converting enzyme (ACE) inhibitor; 2, β-blocker; NS, not significant.

[a] $P < 0.001$, compared to controls.

informed consent form, in accordance with the Helsinki Declaration of Human Rights. The Institutional Ethical Committee for studies in humans approved this study.

Study design

The subjects were studied on two occasions. In the first study, apoB100 kinetics, DNL and whole-body cholesterol synthesis were studied. In the second study, which was performed within 2 to 3 weeks after the first infusion, insulin sensitivity and free fatty acid release into blood were studied. Infusion protocols on day 1 and day 2 are depicted in Figure 2, respectively.

On day 1, the subjects came to the research unit in the morning after 10 hours of fasting. Two intravenous cannulas were placed (brachial vein) for blood sampling and infusion of the labeled valine. No food was given during the tracer infusion and the subjects were only allowed to drink water. If peritoneal dialysis patients were studied, the last bag of peritoneal dialysate had been drained for at least 6 hours prior to the start of tracer infusion. During the infusion period, no dialysis was performed.

At baseline (t = 0), ESRD patients received a priming dose of 11.5 μmol/kg L-[1-^{13}C]-valine (isotope mole fraction > 0.99) (Mass Trace, Woburn, MA, USA) intravenously in 2 minutes, followed by a continuous infusion of 11.5 μmol/kg/hour L-[1-^{13}C]-valine for 10 hours. The priming dose was 8 μmol/kg for peritoneal dialysis patients and 15 μmol/kg for control subjects, followed by a continuous infusion of 8 μmol/kg/hour and 15 μmol/kg/hour for 10 hours, respectively. During the valine infusion, the subjects also received every 30 minutes an oral dose of

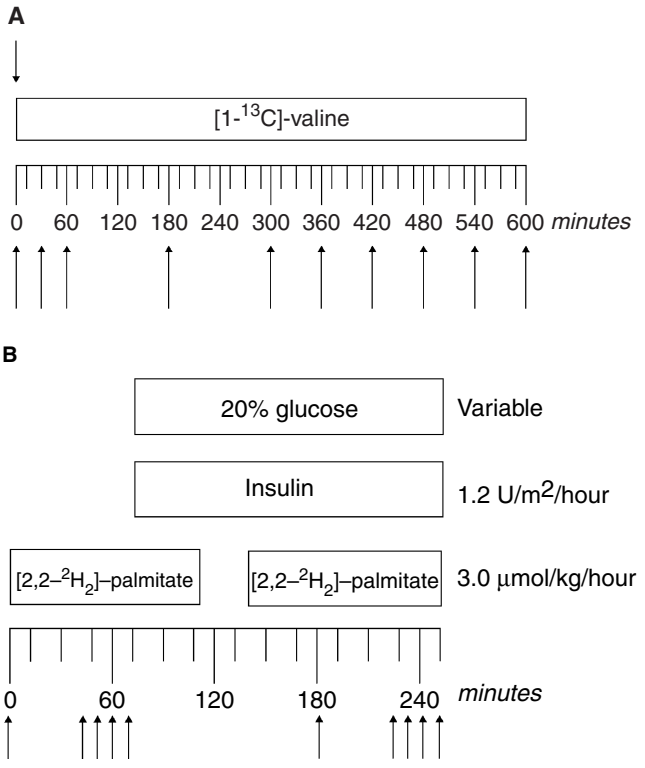

Fig. 2. Infusion scheme. (*A*) Day 1. Subjects received a priming dose of [1-^{13}C]-valine directly followed by a continuous infusion of the same amount of tracer for 600 minutes. In addition, subjects received every half hour 500 mg [1-^{13}C]-acetate, which was administered orally. The arrows indicate the time-points for collection of blood. (*B*) Day 2. Subjects received a continuous infusion of [2,2-^{2}H$_2$]-palmitate 70 minutes before starting the clamp and during the last 70 minutes of the clamp. Euglycemic hyperinsulinemic clamp was performed at an insulin infusion rate of 1.2 U/m^2/hour. The arrows indicate the time-points for collection of blood for determining of free fatty acid release. Additionally blood samples were taken in order to measure insulin sensitivity.

500 mg sodium-[1-^{13}C]-acetate (isotope mole fraction > 0.99) (Mass Trace), which was dissolved in water. Blood samples were taken in heparin-containing and ethylenediaminetetraacetic acid (EDTA)-containing tubes from the contralateral arm at t = 0, 30, 60, 180, 300, 360, 420, 480, 540, and 600 minutes. Samples were kept on ice until plasma was separated by centrifugation (15 minutes, 3000 rpm, 4°C) and stored at −80°C.

Before the second study, the subjects were fasting again for 10 hours and the conditions in relation to food, time of dialysis and collection of urine and dialysis fluid were similar as described above. At baseline (t = 0), the subjects received a continuous intravenous infusion of 3.0 μmol/kg/hour potassium-[2,2-$^{2}H_2$]-palmitate (isotope mole fraction > 0.98) (ARC, Amsterdam, The Netherlands), which was bound to 20% albumin according to the protocol described in [26]. After 70 minutes, the [2,2-$^{2}H_2$]-palmitate infusion was stopped, whereafter insulin sensitivity of the subjects was assessed for 3 hours by a euglycemic hyperinsulinemic clamp. Human insulin (Actrapid Human) (Novo Nordisk, Gentofte, Denmark) was infused intravenously at a rate of 1.2 U/m^2/hour for 3 hours (plateau value ~40 mU/L). Plasma glucose concentrations were measured every 5 minutes using arterialized venous blood with a YSI 200 Stat Plus Glucose analyzer (Salm en Kipp, Breukelen, The Netherlands) and the 20% glucose solution was infused at a variable rate to maintain euglycemia at 5.0 mmol/L. During the last 70 minutes of the clamp, [2,2-$^{2}H_2$]-palmitate was infused again. Before and during the clamp, blood samples were taken in EDTA and heparin containing tubes from the contralateral at t = 0, 40, 50, 60, 70, 180, 220, 230, 240, and 250 minutes. Samples were kept on ice until plasma was separated by centrifugation (15 minutes, 3000 rpm, 4°C) and stored at −80°C.

Preinfusion measurements

Plasma urea, plasma creatinine, plasma glucose, as well as urea and creatinine in urine and dialysis fluid were measured with standard laboratory methods on a Vitros 950AT (Johnson & Johnson, Ortho Clinical Diagnostics, Rochester, NY, USA). Total plasma triglycerides and cholesterol were analyzed by standard enzymatic assays on a Cobas Mira S (ABX, Montpellier, France), while free fatty acids were analyzed by a standard enzymatic assay on a Cobas Fara (ABX). Total plasma apoB and apoB in the different lipoprotein fractions were measured using a nephelometric assay (Dade Behring, Marburg, Germany). Insulin was measured with a competitive radioimmunoassay using a polyclonal antiinsulin-antibody (Caris 46), ^{125}I-insulin (IM166) (Amersham, Roosendaal, The Netherlands) as a tracer and Humuline (YV2632 AMV Lilly, Indianapolis, USA) as a standard. ApoE genotyping was done according to the method described by Slooter et al [27].

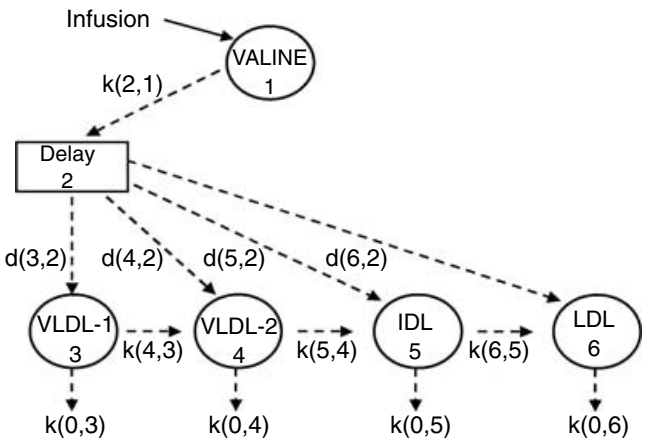

Fig. 3. Multicompartmental model for apolipoprotein B (apoB100) metabolism. Compartment 1 represents plasma valine into which the valine tracer was injected. Compartment 2 represents a delay compartment and valine in incorporated in very low-density lipoprotein (VLDL-1) apoB100, VLDL-2 apoB100, intermediate-density lipoprotein (IDL) apoB100, and low-density lipoprotein (LDL) apoB100 via compartments 3, 4, 5, and 6. The k-values represent rate constants.

ApoB100 metabolism

The isolation of free amino acids from plasma was performed as described in detail elsewhere [28].

The apoB100 containing lipoproteins (VLDL-1, VLDL-2, IDL, and LDL) were isolated from 2 mL EDTA plasma using a discontinuous salt gradient by cumulative ultracentrifugation as described in [29]. ApoB concentrations were determined using a nephelometric assay (Dade Behring). The detection limit of the apoB assay was 12 mg/L. In patients who had detectable lipoprotein(a) [Lp(a)] concentration in the IDL and LDL fraction, the samples were pretreated with lysine-sepharose 4B to remove the Lp(a) as described in [30]. ApoB100 from the isolated lipoproteins was then precipitated using isopropanol and isolated as described before [31]. The preparation of N(O,S) methoxycarbonylmethylester derivatives of apoB100, plasma valine, and mass spectrometric measurements were described in detail elsewhere [31, 32]. ApoB100 production was measured as the rate of incorporation of ^{13}C-enriched valine into circulating VLDL-1, VLDL-2, IDL, and LDL apoB100. The best fit to the actual data points was determined using the SAAM II software (Simulation Analysis and Modelling, version 1.1.1) (SAAM Institute, Seattle, WA, USA). The compartment model is depicted in Figure 3. Compartment 1 represents plasma valine into which the valine tracer was injected. Compartment 2 represents delay compartment and valine is incorporated into VLDL-1 apoB100, VLDL-2 apoB100, IDL apoB100, and LDL apoB100 via

compartments 3, 4, 5, and 6. The absolute synthesis rate (ASR), which is the amount of protein synthesized per day, was calculated as the product of the fractional synthesis rate (FSR) and the plasma pool (plasma volume × plasma concentration). Plasma volumes in all subjects were calculated from BSA using the formulas described by Hurley [33].

DNL

After isolation of the different lipoprotein fractions, the VLDL-1 and VLDL-2 fraction were used to determine the contribution of new-formed ^{13}C-palmitate from [1-^{13}C]-acetate to total palmitate according to the protocol described in [34].

In short, in glass tubes, 2 mL methanol/hexane (4:1, vol/vol) was added to 100 μL VLDL-1 and 100 μL VLDL-2 fraction. After vortexing, 200 μL acetylchlorid was added very slowly and incubated for 1 hour at 100°C, where after cooling to room temperature, 5 mL 6% potassium carbonate was added. After centrifugation, the hexane fraction containing the palmitate was transferred to a vial and aspirated under N_2. The residue was dissolved in 50 μL hexane.

For selected ion monitory (SIM) gas chromatography-mass spectrometry (GC-MS) analysis, 1 μL was delivered by automatic injection to a HP-5890 gas chromatograph split injection port (1:20) leading to a 0.2 mm × 25 m Chrompack CP-sil 19CB (WCOT Fused Silica) capillary column. The injection port contained a glass wool liner. The carrier gas was helium at a linear rate of 1 mL/min. The injection temperature was 240°C and the detection temperature was 280°C. A HP-5989B mass spectrometer was used as detector. Measurements were done in the electron impact mode (EI) at 70 eV. The ion source temperature was 250°C and the quadropole temperature was 150°C. SIM measurements were done for quantification of the mass fragments M + 0 (m/z 270), M + 1 (m/z 271), M + 2 (m/z 272). The peak area for M + 0, M + 1, and M + 2 were integrated, after which the ratios of M + 0/(M + 0...M + 2), M + 1/(M + 0...M + 2), and M + 2/(M + 0...M + 2) were calculated. Based upon these ratios the FSR of DNL was determined using mass isotopomer distribution analysis (MIDA) as described in [35].

Fractional cholesterol synthesis

For cholesterol determination, 50 μL plasma was deproteinized with 2 mL ethanol/aceton (1:1, vol/vol) and centrifuged for 10 minutes at 4000 rpm. The upper layer was dried under nitrogen and derivatized with 50 μL Bis (trimethylsilyl) trifluoroacetamide (BSTFA)/pyridine (5:1, vol/vol) for 60 minutes at 60°C.

For SIM-GC-MS analysis, 2 μL of the derivative mixture was delivered by automatic injection to a HP-5890 gas chromatograph as described above. Mass spectrometric data were collected in the selected ion mode at M + 0 (m/z 368), M + 1 (m/z 369), M + 2 (m/z 370), and M + 3 (m/z 371). FSR of cholesterol was calculated using MIDA as described in [36].

Palmitate release

The extraction of plasma lipids and the procedure for rapid isolation of plasma long chain free fatty acids, including palmitate, was done according to the method described in [37, 38]. After isolation of the long chain free fatty acids, the fraction was dried under N_2 at room temperature, derivatized and the methylesters were chromatographed as described in the section DNL. SIM measurements were done for quantification of the mass fragments M + 0 (m/z 270) and M + 2 (m/z 272). A palmitate calibration curve with increased concentrations of [2,2-$^2H_2$]-palmitate (0% to 5%) was used to calculate the enrichment of the plasma samples.

The palmitate concentration in the infusate was determined by gas chromatography measurements. Five hundred microliters infusate was added to 50 μL internal standard (3-phenyl-buteric acid). After adding HCl to a pH of 1 to 2, the sample was extracted twice with ethylacetate. The ethylacetate fraction was dried and derivatized with 50 μL BSTFA/pyridine (5:1, vol/vol) for 60 minutes at 60°C. One μL was injected on a Varian 3400 gas chromatograph.

Free fatty acid kinetics were described by a single pool model [39]. The rate of appearance (Ra) of palmitate is considered to refer to the total rate of appearance of the tracee and was calculated using the equation of Steele [40] for steady-state conditions by dividing palmitate infusion rate by palmitate enrichment at plateau. During the last 30 minutes of the clamp, where a steady state was achieved, Ra palmitate was calculated again.

Euglycemic hyperinsulinemic clamp

The glucose infusion rate (GIR) required to maintain euglycemia (in mmol/min) during the steady state in the last 30 minutes of the clamp was calculated. Fat free mass was calculated according to Deurenberg et al [41]. The GIR was expressed per kg fat free mass or per m^2 (M value). Insulin sensitivity was calculated as M, expressed either per fat free mass or m^2, divided by the average plasma insulin concentration, during the last 30 minutes of the clamp.

Statistical analysis

Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA) assessed the significance of differences and multiple comparisons were done using Bonferroni *t* test. If the tests for normality or equal variance test failed, a Kruskal-Wallis one-way ANOVA on

RANKS was performed and multiple comparisons were done using Dunn's test. Correlations were performed by linear regression analysis.

RESULTS

Baseline data

Plasma concentrations of apoB, free fatty acids, triglycerides, cholesterol, apoB pool size, and apoE genotyping are shown in Table 2. Total plasma apoB and total apoB pool size tended to be greater in dialysis patients compared to control subjects. Since apoE2 genotype per se is associated with increased triglyceride concentrations, apoE genotyping of all subjects was determined. No subjects had apoE2 genotype, while patients 5 and 8 and 2 controls had apoE3/E4 genotype, whereas the other subjects had apoE3/E3 genotype.

Total plasma triglyceride concentrations tended to be higher in both patients groups, but were only significant higher in peritoneal dialysis patients compared to control subjects ($P < 0.05$). Plasma cholesterol was not different between the three groups.

apoB100 kinetics

VLDL-1 apoB100 kinetic is shown in Table 3. VLDL-1 apoB100 pool size was significantly higher in the peritoneal dialysis group compared to controls ($P < 0.01$). The ASR of VLDL-1 apoB100 was higher in the peritoneal dialysis group compared to controls ($P < 0.05$). Transfer to VLDL-2 apoB100 ($P < 0.05$) and total VLDL-1 apoB100 FCR ($P < 0.01$) were significantly lower in the peritoneal dialysis subjects. VLDL-1 apoB100 direct catabolism was similar for both patients groups and controls.

VLDL-2 apoB100 kinetics are shown in Table 4. VLDL-2 apoB100 pool size was significantly higher in the peritoneal dialysis patients compared to controls ($P < 0.01$). Transfer to IDL apoB100 was significantly lower in the peritoneal dialysis group ($P < 0.01$). Total VLDL-2 apoB100 fractional catabolic rate was significant lower for ESRD ($P < 0.05$) and peritoneal dialysis patients ($P < 0.01$) compared to control subjects. VLDL-2 apoB100 ASR tended to be lower in both patient groups compared to controls, while no significant difference was found for VLDL-2 apoB100 direct catabolism.

IDL apoB100 kinetics in all patients and control subjects are shown in Table 5. IDL apoB100 tended to be higher in both patient groups compared to control subjects, but were not significantly different. No significant differences were found for IDL apoB100 synthesis rate, transfer to LDL apoB100, direct IDL apoB100 catabolism, or total IDL apoB100 catabolism in patients compared to control subjects.

Table 2. Biochemical parameters in plasma from patients and control subjects

	ApoB *g/L*	ApoB pool size *mg*	Triglycerides *mmol/L*	Cholesterol *mmol/L*	Free fatty acids *mmol/L*	Insulin *mU/L*	Glucose *mmol/L*	M value *µmol glucose/min/kg fat free mass*	Insulin sensitivity *µmol glucose/min/kg fat free mass/mU insulin/L*	ApoE genotyping
ESRD patients ($N = 6$)										
1	0.64	1974	1.1	2.8	0.38	16	5.6	17	0.54	E3/E3
2	1.76	5144	4.7	8.2	1.92	30	6.8	15	0.32	E3/E3
3	1.58	5124	2.3	5.7	0.53	18	5.3	24	0.52	E3/E3
4	0.67	1705	1.5	2.4	0.31	26	6.1	51	0.85	E3/E3
5	1.17	3198	2.1	4.3	0.93	26	4.3	—	—	E3/E4
6	1.12	2971	1.4	4.6	1.01	7	5.3	43	0.94	E3/E3
Mean ± SEM	1.15 ± 0.18^{NS}	3353 ± 609^{NS}	2.3 ± 0.5^{NS}	4.7 ± 0.8^{NS}	0.85 ± 0.24^{NS}	21 ± 4^{b}	5.6 ± 0.2^{NS}	30 ± 7^{NS}	0.63 ± 0.12^{NS}	
Peritoneal dialysis patients ($N = 5$)										
7	1.10	3611	2.9	3.7	0.55	5	5.6	21	0.63	E3/E4
8	1.48	4472	3.5	5.4	3.86	12	5.7	40	0.83	E3/E3
9	1.05	2679	1.8	4.9	0.52	15	5.2	39	0.90	E3/E3
10	1.65	3784	9.1	6.5	2.13	27	5.3	27	0.61	E3/E3
11	1.14	2766	5.5	4.4	0.34	13	6.4	30	0.43	E3/E3
Mean ± SEM	1.34 ± 0.11^{NS}	3462 ± 335^{NS}	4.6 ± 1.3^{a}	5.0 ± 1.3^{NS}	1.48 ± 0.68^{NS}	14 ± 3^{NS}	5.6 ± 0.2^{NS}	31 ± 4^{NS}	0.68 ± 2.1^{NS}	
Control Subjects (N = 7)										
Mean ± SEM	0.89 ± 0.08	2636 ± 266	1.1 ± 0.2	3.8 ± 0.3	0.78 ± 0.31	6 ± 1	5.7 ± 0.3	41 ± 8	1.14 ± 0.26	5 E3/E3, 2 E3/E4

Abbreviations are: ESRD, end-stage renal disease; NS, not significant. Due to technical problems, no clamp study could be performed in patient 5.
$^{a}P < 0.05$; $^{b}P < 0.01$, compared to controls.

Table 3. Very low-density lipoprotein (VLDL-1) apolipoprotein B (apoB100) (Sf 60 to 400) metabolism in patients and control subjects

	VLDL-1 apoB100 *mg*	ASR VLDL-1 ApoB100 *mg/day*	ASR VLDL-1 apoB100 *mg/kg/day*	Transfer to VLDL-2 *pools/day*	Direct catabolism *pools/day*	Total VLDL-1 FCR *pools/day*
ESRD patients ($N = 6$)						
1	105	613	7	2.7	3.1	5.8
2	555	946	13	1.7	0.0	1.7
3	297	652	8	2.2	0.0	2.2
4	128	710	10	5.6	0.0	5.6
5	152	993	12	5.0	1.5	6.5
6	43	915	12	21.3	0.0	21.3
Mean ± SEM	213 ± 76^{NS}	805 ± 68^{NS}	10 ± 1^{NS}	6.4 ± 3.0^{NS}	0.8 ± 0.5^{NS}	7.2 ± 2.9^{NS}
Peritoneal dialysis patients ($N = 5$)						
7	345	1156	13	1.7	1.6	3.3
8	427	1464	19	1.5	1.9	3.4
9	223	879	13	3.9	0.0	3.9
10	686	601	10	0.9	0.0	0.9
11	362	621	9	1.7	0.0	1.7
Mean ± SEM	408 ± 77^{b}	944 ± 165^{a}	13 ± 2^{a}	2.0 ± 0.5^{a}	0.7 ± 0.4^{NS}	2.7 ± 0.6^{b}
Control subjects (N = 7)						
Mean ± SEM	68 ± 12	507 ± 58	7 ± 1	7.4 ± 2.1	1.4 ± 1.1	8.8 ± 1.9

Abbreviations are: ESRD, end-stage renal disease; NS, not significant; Sf, svedberg flotation; ASR, absolute synthesis rate; FCR, fractional catabolic rate.
[a] $P < 0.05$, compared to controls; [b] $P < 0.01$, compared to controls.

Table 4. Very low-density lipoprotein (VLDL-2) apolipoprotein B (apoB100) (Sf 20 to 60) metabolism in patients and control subjects

	VLDL-2 apoB100 *mg*	ASR VLDL-2 apoB100 *mg/day*	ASR VLDL-2 apoB100 *mg/kg/day*	Transfer to IDL *pools/day*	Direct catabolism *pools/day*	Total VLDL-2 FCR *pools/day*
ESRD patients ($N = 6$)						
1	64	331	4	6.5	3.1	9.6
2	538	213	3	2.2	0.0	2.2
3	463	449	5	2.4	0.0	2.4
4	153	376	5	7.1	0.0	7.1
5	231	412	5	3.5	1.5	5.0
6	89	66	1	11.0	0.0	11.0
Mean ± SEM	256 ± 81^{NS}	307 ± 59^{NS}	4 ± 1^{NS}	5.5 ± 1.4^{NS}	0.8 ± 0.5^{NS}	6.2 ± 1.3^{a}
Peritoneal dialysis patients ($N = 5$)						
7	290	369	4	1.7	1.6	3.3
8	230	299	4	2.2	1.9	4.1
9	256	142	2	4.0	0.0	4.0
10	458	79	1	1.5	0.0	1.5
11	291	247	4	3.0	0.0	3.0
Mean ± SEM	292 ± 35^{c}	227 ± 52^{NS}	3 ± 1^{NS}	2.5 ± 0.5^{a}	0.7 ± 0.4^{NS}	3.2 ± 0.5^{b}
Control subjects ($N = 7$)						
Mean ± SEM	89 ± 10	419 ± 66	5 ± 1	8.5 ± 1.6	1.4 ± 1.1	9.2 ± 1.2

Abbreviations are: ESRD, end-stage renal disease; ASR, absolute synthesis rate; Sf, svedberg flotation; IDL, intermediate-density lipoprotein; NS, not significant; FCR, fractional catabolic rate.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$, compared to controls.

LDL apoB100 kinetics in all patients and control subjects are shown in Table 5. LDL apoB100 pool size tended to be lower in peritoneal dialysis patients. LDL apoB100 synthesis rate, expressed in mg/day tended to be lower in ESRD, but was significantly lower in peritoneal dialysis patients compared to control subjects (30 ± 9 mg/day versus 145 ± 31 mg/day, $P < 0.05$). No significant difference was found for total LDL apoB100 fractional catabolic rate in patients and control subjects. ApoB100 recovery in all subjects was constant and was $85.2\% \pm 1.0\%$ (range 83% to 91%).

Insulin sensitivity

Plasma insulin concentrations at the start of the clamp were higher in ESRD and peritoneal dialysis patients compared to controls, while plasma insulin concentrations were only significant higher in the ESRD group than in controls ($P < 0.01$). During the last 30 minutes of the clamp, an insulin plateau value of ~40 mU/L was achieved in the three groups. Fasting glucose levels were not significantly different between patients and controls at the beginning as well as at the end of the clamp. The M value (Table 2), which is an estimate of whole body

Table 5. Intermediate-density lipoprotein (IDL) apolipoprotein B (apoB100) (Sf 12–20) and low-density lipoprotein (LDL) apoB100 (Sf 0 to 12) metabolism in patients and control subjects

	IDL apoB100 *mg*	ASR IDL apoB100 *mg/day*	ASR IDL apoB100 *mg/kg/day*	Transfer to LDL *pools/day*	Direct catabolism *pools/day*	Total LDL FCR *pools/day*	LDL apoB100 *mg*	ASR LDL apoB100 mg/day	Total IDL FCR *pools/day*
ESRD patients ($N = 6$)									
1	146	183	2	3.3	0.8	4.1	611	136	1.0
2	522	31	0	2.3	0.0	2.3	1367	3	0.9
3	592	35	0	1.9	0.1	2.0	1733	7	0.6
4	157	12	0	3.5	3.5	7.0	731	27	0.8
5	440	31	0	1.4	0.6	2.0	1221	66	0.5
6	223	156	2	5.1	0.0	5.1	1351	129	0.9
Mean ± SEM	347 ± 80^{NS}	74 ± 30^{NS}	1 ± 0^{NS}	2.9 ± 0.5^{NS}	0.8 ± 0.6^{NS}	3.7 ± 0.8^{NS}	1169 ± 173^{NS}	61 ± 24	0.8 ± 0.1^{NS}
Peritonal dialysis patients ($N = 5$)									
7	268	61	1	2.1	0.0	2.1	845	22	0.7
8	302	67	1	1.9	0.0	1.9	1008	63	0.6
9	348	44	1	3.1	0.0	3.1	842	31	1.3
10	364	13	0	1.2	0.7	1.9	680	9	0.6
11	267	48	1	3.4	0.0	3.4	491	27	1.9
Mean ± SEM	310 ± 20^{NS}	46 ± 10^{NS}	1 ± 0^{NS}	2.3 ± 0.4^{NS}	0.1 ± 0.1^{NS}	2.4 ± 0.3^{NS}	773 ± 88^{NS}	30 ± 9^{a}	1.0 ± 0.3^{NS}
Control subjects ($N = 7$)									
Mean ± SEM	194 ± 12	61 ± 29	1 ± 0	3.8 ± 0.5	0.4 ± 0.2	4.2 ± 0.6	1239 ± 165	145 ± 31	0.7 ± 0.1

Abbreviations are: ASR, absolute synthesis rate; NS, not significant; FCR, fractional catabolic rate.
[a] $P < 0.05$, compared to controls.

Table 6. De novo lipogenesis (DNL), total cholesterol synthesis, and palmitate fluxes in patients and control subjects

	DNL VLDL-1 %	DNL VLDL-2 %	FSR cholesterol %/*day*	Ra palmitate, basal *μmol/kg/min*	Ra palmitate, insulin *μmol/kg/min*	% reduction
ESRD patients ($N = 6$)						
1	1.6	1.1	2.0	2.3	1.4	41
2	2.2	0.9	0.7	2.2	1.7	20
3	1.9	7.0	0.6	3.1	1.2	58
4	3.9	3.1	1.3	3.0	1.7	42
5	0.2	2.9	1.3	-	-	-
6	1.9	4.8	1.2	3.4	2.1	37
Mean ± SEM	2.0 ± 0.5^{NS}	3.3 ± 0.9^{NS}	1.2 ± 0.2^{NS}	2.8 ± 0.2^{NS}	1.6 ± 0.2^{NS}	39 ± 6^{NS}
Peritoneal dialysis patients ($N = 5$)						
7	2.2	1.3	1.7	2.2	1.3	43
8	1.5	1.1	2.4	2.6	2.2	14
9	3.3	0.2	1.5	3.9	2.4	38
10	3.1	2.9	0.9	3.4	1.3	61
11	0.8	0.2	1.0	1.6	1.0	36
Mean ± SEM	2.2 ± 0.5^{NS}	3.0 ± 0.9^{NS}	1.5 ± 0.3^{NS}	2.7 ± 0.4^{NS}	1.6 ± 0.3^{NS}	38 ± 8^{NS}
Control subjects ($N = 7$)						
Mean ± SEM	1.5 ± 0.6	1.6 ± 0.9	1.5 ± 0.2	2.7 ± 0.4	1.0 ± 0.2	59 ± 7

Abbreviations are: ESRD, end-stage renal disease; VLDL, very low-density lipoprotein; Ra, rate of appearance; NS, not significant; FSR, fractional synthesis rate.

insulin sensitivity, was not different between the groups. Insulin sensitivity (Table 2) tended to be lower in both patient groups compared to controls ($P = 0.08$ by ANOVA). Similar conclusions were drawn, when the data were expressed per BSA (data not shown).

DNL and fractional cholesterol synthesis

DNL, the hepatic production of fatty acids using glucose as substrate, is measured as ^{13}C-palmitate enrichment in VLDL-1 and VLDL-2. Data are shown in Table 6. No significant difference was found for DNL measured in VLDL-1 and VLDL-2 fraction in patients and controls.

No significant difference was found for fractional cholesterol synthesis in patients and control subjects (Table 6).

Lipolysis

Mean plasma palmitate enrichment of all subjects measured at plateau during basal conditions was 1.73% ±

0.17% (range 0.67% to 2.91%) and 3.18% ± 0.28% (range 1.06% to 5.88%) at the end of the insulin infusion. The rate of appearance of palmitate is shown in Table 6. Under basal conditions Ra palmitate was not different among the three groups. During the euglycemic hyperinsulinemic clamp, Ra palmitate tended to be higher in ESRD and peritoneal dialysis subjects compared to controls ($P = 0.08$ by ANOVA). Palmitate release was 20% higher in ESRD and peritoneal dialysis patients compared to controls.

Correlations

Considering the data in controls and patients together, several parameters were tested. VLDL-1 apoB100 pool size and VLDL-2 apoB100 pool size as well as IDL apoB100 pool size correlated only with its corresponding total FCR (VLDL-1, $r^2 = 0.381$, $P < 0.01$; VLDL-2, $r^2 = 0.755$, $P < 0.0001$; IDL, $r^2 = 0.47$, $P < 0.01$) and not with its synthesis rate. This observation is confirmed, when the patient group ($N = 11$) was tested separately (VLDL-1, $r^2 = 0.376$, $P = 0.02$; VLDL-2, $r^2 = 0.746$, $P < 0.01$; IDL, $r^2 = 0.492$, $P < 0.05$). VLDL-1 apoB100 ASR correlated with Ra palmitate during insulin infusion ($r^2 = 0.404$, $P = 0.01$).

No association was observed for VLDL-1 apoB100 FCR, VLDL-2 apoB100 FCR, transfer to VLDL-2 and transfer to IDL with M value or insulin sensitivity index. Furthermore, no association was observed for VLDL-1 apoB100 secretion either expressed as mg/day or mg/kg/day with insulin sensitivity index, or plasma insulin in patients and controls.

DISCUSSION

There is still debate about the mechanisms explaining altered VLDL apoB100 kinetics in chronic renal failure, since an impaired triglyceride removal or apoB100 clearance alone [11, 12] or reduced clearance in combination with increased triglyceride synthesis rate or VLDL apoB100 overproduction [13, 14] have been reported. Our data support the concept that both overproduction of VLDL-1 apoB100 as well as decreased clearance of VLDL-1 apoB100 and VLDL-2 apoB100 is involved. Previous studies have measured only total VLDL metabolism. However, evidence is present that VLDL-1 apoB100 and VLDL-2 apoB100 are independently regulated [42], which may not be reflected by total VLDL kinetics. In the present study, the combination of VLDL-1 apoB100 and VLDL-2 apoB100 secretion rates was not different between patients and controls ($P = 0.17$, by ANOVA), explaining the discrepancy in literature concerning VLDL apoB100 overproduction.

Increased VLDL production can be secondary to the observed insulin resistance since we showed that insulin sensitivity tended to be lower in patients with ESRD and peritoneal dialysis patients compared to controls ($P = 0.08$ by ANOVA) (Table 2) as also reported by other groups [17, 18]. As peritoneal dialysis is initiated, insulin sensitivity remains similar, since no differences in mean M value or insulin sensitivity index between the ESRD and peritoneal dialysis group is observed. A reduction in peripheral insulin sensitivity has been postulated to lead to increased free fatty acid availability from muscle and adipose tissue that could, by increased substrate delivery to the liver, stimulate VLDL-1 apoB100 release [43, 44]. In agreement with this hypothesis, we found impaired suppression of free fatty acids by insulin in ESRD and peritoneal dialysis patients (Table 6). In addition, VLDL-1 apoB100 production was strongly associated with free fatty acid release (Ra palmitate) during insulin infusion ($r^2 = 0.404$, $P = 0.01$). While VLDL-1 apoB100 release tended to be higher in ESRD patients, it clearly reached significance in the patients that were on peritoneal dialysis.

As free fatty acid fluxes were similar for peritoneal dialysis and ESRD patients while VLDL apoB100 release was higher in peritoneal dialysis, the contribution of different sources of substrate for VLDL apoB100 release in peritoneal dialysis patients such as DNL, or altered free fatty acid oxidation should be taken into account [19]. We measured the contribution of DNL to VLDL-triglyceride availability. From our data it is clear, that DNL probably does not contribute to increased VLDL-triglyceride availability, since no significant differences were found for patients and control subjects (Table 6). DNL is below 5% in both patients groups, data that are in agreement to what is reported for healthy controls [35]. This suggests no direct effect of glucose absorption via the peritoneum on VLDL-triglyceride availability in peritoneal dialysis patients, although an indirect effect cannot be excluded. In peritoneal dialysis, free fatty acid oxidation has been reported to be normal or reduced depending on the amount of glucose absorbed via the peritoneum [45, 46]. Taken together, increased free fatty acids per se or increased free fatty acid flux in combination with altered free fatty acid oxidation contributes to increased VLDL-1 apoB100 release in peritoneal dialysis.

In addition to increased VLDL-1 apoB100 secretion, defects in VLDL-1 apoB100 and VLDL-2 apoB100 catabolism are observed in peritoneal dialysis compared to ESRD. Correlation studies showed that VLDL-1 apoB100 and VLDL-2 apoB100 pool sizes were associated with its corresponding total FCR, but not with its ASR. VLDL-2 apoB100 ASR tended to be lower in peritoneal dialysis patients, despite an increased VLDL-2 apoB100 pool size. This suggests that catabolism is more predominant for VLDL-1 apoB100 and VLDL-2

apoB100 pool size than synthesis. One can speculate about the disturbances for catabolism like lipoprotein lipase (LPL) or apoprotein loss.

LPL is an insulin-sensitive enzyme, which is a mediator for hydrolysis of triglycerides. Therefore, it is tempting to speculate that LPL expression and activity could be disturbed in chronic renal failure. Unfortunately, no clear consensus can be drawn for LPL activity, since LPL activity is reported to be normal or reduced in chronic renal failure [6, 47]. In the subjects studied, no association was observed for VLDL-1 apoB100 FCR, VLDL-2 apoB100 FCR, transfer to VLDL-2 and IDL with indexes of insulin sensitivity (M value and insulin sensitivity index). In addition, hemodialysis patients have a lipid phenotype that is much milder compared to peritoneal dialysis, while LPL is similar [6], suggesting that LPL per se is putatively not a limiting factor for VLDL catabolism. In the nephrotic syndrome, a decreased clearance of VLDL apoB100 has also been reported [24], while Deighan et al [48] showed that VLDL-1 particles deficient in apoCII, apoCIII, and apoE are circulating in this condition. Such an observation was also found more recently by Moberly et al [49] for peritoneal dialysis subjects. Since in vitro evidence suggests that apoCII, apoCIII, and apoE are important for binding and clearance of lipoprotein particles, it is tempting to speculate that (apo)protein loss in dialysis fluid may have contributed to defective clearance of VLDL particles in peritoneal dialysis.

Alternatively, protein loss could also be a stimulus for increased (lipo)protein synthesis. It has been suggested that urinary albumin loss in nephrotic syndrome results in enhanced hepatic (lipo)protein synthesis [24, 28]. In ESRD and peritoneal dialysis, protein loss in urine and dialysis fluid is considerable [22, 50, 51], ranging from 5 to 15 g/day. In order to cope this loss, the human body has to adapt to this response by alterations in synthesis or catabolism of the lost protein. Recently, we have shown that albumin and fibrinogen are coordinately upregulated in ESRD and peritoneal dialysis [51]. Therefore, it is tempting to speculate that secretion rates of other liver derived proteins like VLDL-1 apoB100 might be close related to albumin ASR. However, no close correlation was observed between albumin or fibrinogen synthesis rate and VLDL-1 apoB100 synthesis rate, nor total protein loss and VLDL-1 apoB100 synthesis rate in patients with chronic renal failure and control subjects, suggesting that the occurence of a coordinated upregulation is not likely present for VLDL-1 apoB100.

In this study, no significant differences were found for IDL apoB100 kinetics in ESRD and peritoneal dialysis patients, although IDL apoB100 pool size tended to be higher for both patient groups. In other reports, where more subjects were included, IDL apoB100 pool size was significantly higher in ESRD and peritoneal dialysis patients compared to control subjects [6, 49].

No significant difference was observed for LDL apoB100 pool size between patients and controls, which is in accordance with most other studies [6, 52], although an increased LDL apoB100 pool size also has been reported [49]. LDL apoB100 synthesis rate was significantly lower in peritoneal dialysis patients as also observed by Hörkkö et al [52].

The current data are primarily focused om VLDL. Overproduction of VLDL particles in combination with reduced clearance of VLDL particles will inevitably result in the formation of small dense LDL, the atherogenic particle that may contribute to progression of atherosclerosis. Despite disturbances in cholesterol composition among the different lipoprotein fractions in peritoneal dialysis [7, 8, 49], the FSR of cholesterol for both patient groups and controls was within the normal range (Table 6). Care is needed in interpretation of these data, however, as total FSR of cholesterol reflects cholesterol synthesis in all tissues and not only cholesterol circulating in plasma.

Our study does have some limitations. It is important to note that apoB100 metabolism was studied during fasting condition. In the postprandial state, increased concentrations of triglyceride-rich particles (chylomicrons) lead to an increase in the release of free fatty acids and generation of remnants as a result of lipolysis by LPL. Extrapolation of the reported data to the daily situation suggests that VLDL-1 apoB100 synthesis might even be higher. In addition, during both studies, peritoneal dialysis subjects performed no dialysis. We choose this approach, since glucose absorption might affect apoB100 synthesis, via the action of insulin [42].

CONCLUSION

VLDL-1 apoB100 pool in peritoneal dialysis patients is enlarged due increased VLDL-1 apoB100 release in combination with reduced VLDL-1 apoB100 catabolism, while VLDL-2 apoB100 pool results from a decreased catabolism. Such mechanisms are already present in the predialysis state and are aggravated significantly as peritoneal dialysis treatment is initiated. Our data suggest that insulin resistance and associated enhanced free fatty acid delivery to the liver may contribute to the lipid abnormalities in ESRD and peritoneal dialysis. In addition, there appear to be specific peritoneal dialysis–related abnormalities in VLDL catabolism as well. As the resulting prolonged circulation of VLDL-1 particles results in the formation of small dense LDL, the most atherogenic particle, these abnormalities constitute an important therapeutic target in these patients. In particular, the recent introduction of new thiazolidinediones (TZDs), which improve insulin sensitivity may be of interest in this respect.

NOTE ADDED IN PROOF

See additional comments, p. 1116.

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REFERENCES

1. Baigent C, Burbury K, Wheeler D: Premature cardiovascular disease in chronic renal failure. *Lancet* 356:147–152, 2000
2. Levin A, Foley RN: Cardiovascular disease in chronic renal insufficiency. *Am J Kidney Dis* 36:S24–S30, 2000
3. Ozsoy RC, Kastelein JJ, Arisz L, Koopman MG: Atorvastatin and the dyslipidemia of early renal failure. *Atherosclerosis* 166:187–194, 2003
4. Jungers P, Massy ZA, Khoa TN, *et al*: Incidence and risk factors of atherosclerotic cardiovascular accidents in predialysis chronic renal failure patients: A prospective study. *Nephrol Dial Transplant* 12:2597–2602, 1997
5. Webb AT, Reaveley DA, Donnell M, *et al*: Lipids and lipoprotein(a) as risk factors for vascular disease in patients on renal replacement therapy. *Nephrol Dial Transplant* 10:354–357, 1995
6. Deighan CJ, Caslake MJ, McConnell M, *et al*: Atherogenic lipoprotein phenotype in end-stage renal failure: Origin and extent of small dense low-density lipoprotein formation. *Am J Kidney Dis* 35:852–862, 2000
7. Attman PO, Samuelsson OG, Moberly J, *et al*: Apolipoprotein B-containing lipoproteins in renal failure: The relation to mode of dialysis. *Kidney Int* 55:1536–1542, 1999
8. Attman PO, Samuelsson O, Johansson AC, *et al*: Dialysis modalities and dyslipidemia. *Kidney Int* 63 (Suppl 84):S110–S112, 2003
9. Faller B, Lameire N: Evolution of clinical parameters and peritoneal function in a cohort of CAPD patients followed over 7 years. *Nephrol Dial Transplant* 9:280–286, 1994
10. Little J, Phillips L, Russell L, *et al*: Longitudinal lipid profiles on CAPD: Their relationship to weight gain, comorbidity, and dialysis factors. *J Am Soc Nephrol* 9:1931–1939, 1998
11. 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
12. Attman PO, Gustafson A: Lipid and carbohydrate metabolism in uraemia. *Eur J Clin Invest* 9:285–291, 1979
13. Chan PC, Persaud J, Varghese Z, *et al*: Apolipoprotein B turnover in dialysis patients: Its relationship to pathogenesis of hyperlipidemia. *Clin Nephrol* 31:88–95, 1989
14. Verschoor L, Lammers R, Birkenhager JC: Triglyceride turnover in severe chronic non-nephrotic renal failure. *Metabolism* 27:879–883, 1978
15. Prinsen BH, de Sain-van der Velden MG, De Koning EJ, *et al*: Hypertriglyceridemia in patients with chronic renal failure: Possible mechanisms. *Kidney Int* 63(Suppl 84):S121–S124, 2003
16. Cheng SC, Chu TS, Huang KY, *et al*: Association of hypertriglyceridemia and insulin resistance in uremic patients undergoing CAPD. *Perit Dial Int* 21:282–289, 2001
17. DeFronzo RA, Alvestrand A, Smith D, *et al*: Insulin resistance in uremia. *J Clin Invest* 67:563–568, 1981
18. Eidemak I, Feldt-Rasmussen B, Kanstrup IL, *et al*: Insulin resistance and hyperinsulinaemia in mild to moderate progressive chronic renal failure and its association with aerobic work capacity. *Diabetologia* 38:565–572, 1995
19. Lewis GF: Fatty acid regulation of very low density lipoprotein production. *Curr Opin Lipidol* 8:146–153, 1997
20. Lindholm B, Norbeck HE: Serum lipids and lipoproteins during continuous ambulatory peritoneal dialysis. *Acta Med Scand* 220:143–151, 1986
21. Blumenkrantz MJ, Gahl GM, Kopple JD, *et al*: Protein losses during peritoneal dialysis. *Kidney Int* 19:593–602, 1981
22. Kagan A, Bar-Khayim Y, Schafer Z, Fainaru M: Kinetics of peritoneal protein loss during CAPD: I. Different characteristics for low and high molecular weight proteins. *Kidney Int* 37:971–979, 1990
23. Saku K, Sasaki J, Naito S, Arakawa K: Lipoprotein and apolipoprotein losses during continuous ambulatory peritoneal dialysis. *Nephron* 51:220–224, 1989
24. de Sain-van der Velden MG, Kaysen GA, Barrett HA, *et al*: Increased VLDL in nephrotic patients results from a decreased catabolism while increased LDL results from increased synthesis. *Kidney Int* 53:994–1001, 1998
25. Weintraub M, Burstein A, Rassin T, *et al*: Severe defect in clearing postprandial chylomicron remnants in dialysis patients. *Kidney Int* 42:1247–1252, 1992
26. Bisschop PH, Ackermans MT, Endert E, *et al*: The effect of carbohydrate and fat variation in euenergetic diets on postabsorptive free fatty acid release. *Br J Nutr* 87:555–559, 2002
27. Slooter AJ, van Gool WA, van Duijn CM: The role of apolipoproteins E genotyping in the differential diagnosis of dementia. *Ned Tijdschr Geneeskd* 140:2073–2076, 1996
28. de Sain-van der Velden MG, Kaysen GA, de Meer K, *et al*: Proportionate increase of fibrinogen and albumin synthesis in nephrotic patients: Measurements with stable isotopes. *Kidney Int* 53:181–188, 1998
29. Prinsen BH, Romijn JA, Bisschop PH, *et al*: Endogenous cholesterol synthesis is associated with VLDL-2 apoB-100 production in healthy humans. *J Lipid Res* 44:1341–1348, 2003
30. de Sain-van der Velden MG, Reijngoud DJ, Kaysen GA, *et al*: Evidence for increased synthesis of lipoprotein[a] in the nephrotic syndrome. *J Am Soc Nephrol* 9:1474–1481, 1998
31. de Sain-van der Velden MG, Rabelink TJ, Gadellaa MM, *et al*: In vivo determination of very-low-density lipoprotein-apolipoprotein B100 secretion rates in humans with a low dose of L-[1-^{13}C] valine and isotope ratio mass spectrometry. *Anal Biochem* 265:308–312, 1998
32. Prinsen BH, de Sain-van der Velden MG, Kaysen GA, *et al*: Transferrin synthesis is increased in nephrotic patients insufficiently to replace urinary losses. *J Am Soc Nephrol* 12:1017–1025, 2001
33. Hurley PJ: Red cell and plasma volumes in normal adults. *J Nucl Med* 16:46–52, 1975
34. Christie WW: *Lipid Analysis* (2nd ed), New York, Pergammon Press, 1982, pp 52–53
35. Hellerstein MK, Christiansen M, Kaempfer S, *et al*: Measurement of de novo hepatic lipogenesis in humans using stable isotopes. *J Clin Invest* 87:1841–1852, 1991
36. Neese RA, Faix D, Kletke C, *et al*: Measurement of endogenous synthesis of plasma cholesterol in rats and humans using MIDA. *Am J Physiol* 264:E136–E147, 1993
37. Ingalls ST, Kriaris MS, Xu Y, *et al*: Method for isolation of non-esterified fatty acids and several other classes of plasma lipids by column chromatography on silica gel. *J Chromatogr* 619:9–19, 1993
38. Ingalls ST, Xu Y, Hoppel CL: Determination of plasma nonesterified fatty acids and triglyceride fatty acids by gas chromatography of their methyl esters after isolation by column chromatography on silica gel. *J Chromatogr B Biomed Appl* 666:1–12, 1995
39. Miles JM, Ellman MG, McClean KL, Jensen MD: Validation of a new method for determination of free fatty acid turnover. *Am J Physiol* 252:E431–E438, 1987
40. Steele R: Influences of glucose loading and of injected insulin or hepatic glucose output. *Ann NY Acad Sci* 82:420–430, 1959

41. Deurenberg P, Weststrate JA, Seidell JC: Body mass index as a measure of body fatness: Age- and sex-specific prediction formulas. *Br J Nutr* 65:105–114, 1991
42. Malmstrom R, Packard CJ, Watson TD, *et al*: Metabolic basis of hypotriglyceridemic effects of insulin in normal men. *Arterioscler Thromb Vasc Biol* 17:1454–1464, 1997
43. Lewis GF, Steiner G: Acute effects of insulin in the control of VLDL production in humans. Implications for the insulin-resistant state. *Diabetes Care* 19:390–393, 1996
44. Cummings MH, Watts GF, Pal C, *et al*: Increased hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 in obesity: A stable isotope study. *Clin Sci(Lond)* 88:225–233, 1995
45. Delarue J, Maingourd C, Lamisse F, *et al*: Glucose oxidation after a peritoneal and an oral glucose load in dialyzed patients. *Kidney Int* 45:1147–1152, 1994
46. Harty J, Conway L, Keegan M, *et al*: Energy metabolism during CAPD: A controlled study. *Adv Perit Dial* 11:229–233, 1995
47. Chan MK, Persaud J, Varghese Z, Moorhead JF: Pathogenic roles of post-heparin lipases in lipid abnormalities in hemodialysis patients. *Kidney Int* 25:812–818, 1984
48. Deighan CJ, Caslake MJ, McConnell M, *et al*: Patients with nephrotic-range proteinuria have apolipoprotein C and E deficient VLDL-1. *Kidney Int* 58:1238–1246, 2000
49. Moberly JB, Attman PO, Samuelsson O, *et al*: Alterations in lipoprotein composition in peritoneal dialysis patients. *Perit Dial Int* 22:220–228, 2002
50. Kaysen GA, Schoenfeld PY: Albumin homeostasis in patients undergoing continuous ambulatory peritoneal dialysis. *Kidney Int* 25:107–114, 1984
51. Prinsen BH, Rabelink TJ, Beutler JJ, *et al*: Increased albumin and fibrinogen synthesis rate in patients with chronic renal failure. *Kidney Int* 64:1495–1504, 2003
52. Hörkkö S, Huttunen K, Kesaniemi YA: Decreased clearance of low-density lipoprotein in uremic patients under dialysis treatment. *Kidney Int* 47:1732–1740, 1995