Non-Insulin-Dependent Diabetes Mellitus and Hypertriglyceridemia Impair Lipoprotein Metabolism in Chronic Hemodialysis Patients

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Abstract. Patients with diabetes mellitus undergoing chronic hemodialysis treatment have the worst outcome on dialysis due to an increased rate of cardiovascular complications. Nearly all patients present with dyslipidemia, a prominent vascular risk factor, probably responsible for the high rate of vascular injury. Since both uremia and diabetes predispose to hypertriglyceridemia, the present study was conducted to investigate the influence of diabetes mellitus and/or hypertriglyceridemia on lipoprotein metabolism in hemodialysis patients. LDL was isolated and characterized from hyper- and normotriglyceridemic diabetic and nondiabetic hemodialysis patients (n = 40; 10 in each group); also, LDL-receptor-dependent uptake and intracellular cholesterol metabolism were studied in HepG2 cells. In addition, scavenger-receptor-mediated uptake was examined in mouse peritoneal macrophages. LDL isolated from nondiabetic normotriglyceridemic hemodialysis patients exhibited impaired cellular uptake via the LDL receptor. Additionally, intracellular sterol synthesis was less inhibited and cholesterol esterification was reduced compared with LDL from healthy control subjects. Reduction of catabolic capacities was more marked in hemodialysis patients who were either diabetic or hypertriglyceridemic and even more pronounced in patients presenting with a combination of both diabetes and hypertriglyceridemia. Hypertriglyceridemic and diabetic patients showed reduced lipase activity and increased LDL oxidation. Furthermore, they accumulated a fraction of small, dense LDL, and LDL was predominantly taken up via the scavenger-receptor pathway in peritoneal macrophages. This study elucidates the distinct influence of diabetes and/or hypertriglyceridemia in hemodialysis patients on cellular LDL metabolism via specific and nonspecific metabolic pathways. Furthermore, it underscores the cumulative impact of these pathologic entities on impairment of lipoprotein metabolism and increase of cardiovascular risk.

Patients with renal failure undergoing chronic hemodialysis treatment are known to be at increased atherogenic risk (1). In diabetic patients on hemodialysis treatment, morbidity and mortality are even higher compared with nondiabetic patients. One of every two patients with non-insulin-dependent diabetes mellitus (NIDDM) dies during the first 3 yr of hemodialysis treatment, and in more than 60% the cause of death is of vascular origin (2).

Dyslipidemia is common in uremic and nonuremic patients with diabetes mellitus and is regarded as playing a major role in the progression of atherosclerosis (3,4). One main component of such dyslipidemia is impaired uptake of LDL via LDL-receptor-mediated pathways, which has recently been described for diabetic subjects (5,6), hypertriglyceridemic patients (7,8), and hemodialysis patients (9).

In addition to uremia and dialysis-induced changes of lipoprotein metabolism, alteration of receptor-mediated lipoprotein pathways in diabetic hemodialysis patients could be due to modifications in lipoprotein composition and configuration via glycosylation (10,11). Enrichment of small, dense and triglyceride-rich LDL in diabetes mellitus has been described before (12). This alteration in distribution of LDL subfractions may additionally diminish receptor-specific uptake because small and triglyceride-rich LDL are known to exhibit impaired affinity to the LDL-receptor.

Because hydrolysis of triglyceride-rich lipoproteins is dependent on lipoprotein lipase and hepatic lipase activity, changes in enzyme activity may considerably influence lipoprotein metabolism, preferably affecting triglyceride-rich lipoproteins. Besides uremic factors leading to impaired lipase activity in patients with renal failure, chronic heparin treatment in hemodialysis patients might deplete endothelial lipase stores, resulting in hypertriglyceridemia based on an increase in half-life of triglyceride-rich lipoproteins.

Circulating lipoproteins are exposed to oxidative stress,
which increases parallel to their half-life. The degree of alteration is determined by the exposure time to oxidative agents and the susceptibility of the lipoproteins to oxidation (13).

Since oxidative stress and glycosylation can lead to alteration in conformation of lipoproteins (14), oxidized and/or glycated LDL exhibit impaired binding to the LDL receptor. As a result, it remains longer in circulation, which closes a circulus vitiosus that can only be interrupted by uptake of modified lipoproteins via the nonsaturable scavenger-receptor pathway (12). Hence, in hemodialysis patients with diabetes mellitus and/or hypertriglyceridemia, a number of conditions are present that may account for altered cellular LDL uptake and intracellular cholesterol metabolism.

To differentiate the impact of these conditions, we examined the cellular LDL uptake in patients on hemodialysis treatment suffering from additional diabetes and/or hypertriglyceridemia, performed detailed subfractional characterization, and measured the susceptibility to oxidation and the oxidative state of LDL. Furthermore, uptake of LDL in mouse peritoneal macrophages and total and hepatic lipase levels were determined, and data were related to the alterations in receptor-specific uptake.

The present study, therefore, was designed to elucidate metabolism of lipoproteins under the condition of hemodialysis treatment, diabetes mellitus, hypertriglyceridemia, and their combinations and, furthermore, to examine the influence and interaction of these common conditions and their potential impact on the pathogenesis of atherosclerosis.

Materials and Methods

Patients

After an overnight fast, blood was drawn from 40 patients undergoing chronic hemodialysis treatment (>6 mo on treatment), and serum was isolated for the studies performed in vitro. Standard hemodialysis treatment was performed three times weekly in all patients using high-flux polysulfone membranes (F60) and receiving 600 to 1000 IU/h unfractionated heparin. Patients were selected from a larger group of hemodialysis patients according to their metabolic condition.

Twenty patients (17 men, three women) with NIDDM for more than 5 yr were selected. Among the 20 patients with NIDDM, 10 exhibited hypertriglyceridemia (serum triglycerides > 2.28 mmol/L). Their mean age was 67 ± 10 yr and their body mass index was 23.9 ± 3.2 kg/m² for hypertriglyceridemic (body weight, 70.6 ± 9.3 kg) and 23.5 ± 2.9 kg/m² for normotriganlgemic (body weight, 69.7 ± 8.7 kg) subjects. Their mean blood glucose concentration was 9.8 ± 2.2 mmol/L and HbA1c was 7.4 ± 1.6%. Six patients were taking insulin, whereas 14 were on oral antidiabetic drugs (glibenclamide). Except for one patient who had cystic kidney disease, the underlying cause for chronic renal failure was diabetic nephropathy in all patients. Body mass index, body weight, and other markers of metabolic control such as blood glucose and HbA1c did not differ significantly between hypertriglyceridemic and normotriganlgemic diabetic subjects.

For comparison, an analogous group of nondiabetic hemodialysis patients (n = 20) was selected, consisting of 12 men and eight women. Their mean age was 68 ± 12 yr and their body mass index was 24.1 ± 3.6 kg/m² for hypertriglyceridemic (body weight, 68.3 ± 11.2 kg) and 22.4 ± 3.2 kg/m² for normotriganlgemic (body weight, 63.0 ± 12.7 kg) subjects. Fifteen patients were suffering from various types of chronic glomerulonephritis, three had polycystic kidney disease, one suffered from chronic pyelonephritis, and one from vascular nephropathy. Among the 20 patients without diabetes, 10 exhibited hypertriglyceridemia (serum triglycerides > 2.28 mmol/L).

Patients with primary disorders of lipid metabolism were excluded. No patient was on immunosuppressive or lipid-lowering drugs. Informed consent was obtained from all patients. Sixteen normolipidemic and normoglycemic healthy age-matched subjects (12 men, four women) acted as control subjects.

Cell Cultures

HepG2 cells and mouse peritoneal macrophages (P388D) were obtained from American Type Culture Collection (Rockville, MD). HepG2 cells were grown in minimum essential medium supplemented with 5% fetal calf serum, 2.5 mmol L-glutamine, 1 mmol pyruvate, and 1% nonessential amino acids, pH 7.4, and incubated in 95% air/5% CO2 at 37°C. Macrophages were grown in RPMI 1640 supplemented with 10% fetal calf serum, 2.5 mmol L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mmol pyruvate, and 0.5 mL of insulin-transferrin-sodium-selenite.

Lipase Activity

Total post-heparin lipase activity was determined by the method of Krauss et al. (15), using triglyceride hydrolysis as an indicator of total lipase activity. Levels of hepatic lipase were measured by inhibition of lipoprotein lipase with protamine sulfate and 1 M sodium chloride. Lipoprotein lipase levels were obtained by subtraction of hepatic lipase values from total lipase activity. The coefficient of variation for lipase activity measurements was 12.4%.

Lipid and Apolipoprotein Analysis

Cholesterol, triglycerides, and phospholipids were determined enzymatically on an automated analyzer (Wako R-30, Wako, Neuss, Germany) within coefficients of variation of 1.3% for cholesterol, 2.1% for triglycerides, and 1.8% for phospholipids. Lipoprotein concentrations were determined using a combined ultracentrifugation and precipitation procedure as described in the “Manual of Laboratory Operations” of the Lipid Research Clinics (16) with slight modifications (17). Briefly, VLDL were prepared from 500 μL of serum by ultracentrifugation for 18 h at 1.063 kg/L, 30,000 min⁻¹. VLDL cholesterol was calculated from the difference of total cholesterol and cholesterol in the 1.063 kg/L infranatant. HDL cholesterol and LDL cholesterol were determined by precipitation of LDL in the 1.063 kg/L bottom fraction. Apolipoprotein B (apoB) was determined by kinetic nephelometry (Array Protein System, Beckman, Brea, CA). The protein content of cells and lipoproteins was measured according to the Lowry method. The degree of glycation of apoB was determined with the “Glycacor” enzyme immunoassay from Exocell (Philadelphia, PA). The concentration of glycated apoB was expressed as percentage of LDL apoB.

Isolation of LDL

LDL (d = 1.019 to 1.060 kg/L) were isolated from fasting citrated (3.8 g/L) plasma by sequential ultracentrifugation (19), dialyzed against 5 mol/L Tris-HCl, pH 7.4, 154 mmol/L NaCl, 250 mmol/L ethylenediaminetetra-acetic acid · Na2, and sterilized by passage through a 0.45-μm filter (Millipore, Molsheim, France). Purity of lipoproteins was tested by agarose gel electrophoresis (REP-HDLplus cholesterol electrophoresis, Helena Diagnostika, Hartheim, Germany).
Subfractionation of LDL

Total LDL \((d = 1.019 \text{ to } 1.060 \text{ kg/L})\) were separated into six classes by equilibrium density gradient centrifugation as described (18). Density ranges of the subfractions as determined by precision refractometry (19) of blank gradients were: LDL-1 \(<1.031 \text{ kg/L} \); LDL-2 1.031 to 1.034 kg/L; LDL-3 1.034 to 1.037 kg/L; LDL-4 1.037 to 1.040 kg/L; LDL-5 1.040 to 1.044 kg/L; LDL-6 1.044 to 1.060 kg/L. All centrifugation steps were performed at 18°C using partially filled polycarbonate tubes (6 ml) in a 50 Ti rotor (Beckman, Palo Alto, CA). LDL subfractions were not used for uptake experiments but were quantified by the determination of apoB in each fraction.

Radioiodination of Lipoproteins

Radioiodination of LDL (Amersham-Buchler, Braunschweig, Germany) was performed by the method of McFarlane (20) as modified by Bilheimer \textit{et al.} (21). \(^{125}\text{I} \)-LDL were sterilized and used within 2 wk.

Preparation of Lipoprotein-Deficient Serum

Human lipoprotein-deficient serum (LDS) was prepared by ultra-centrifugation (150,000 \(\times\) g for 48 h at 10°C), adjusting the plasma to \(d = 1.250 \text{ g/ml} \) by addition of solid KBr. The resulting LDS was dialyzed against 154 mmol/L NaCl, 250 mmol/L ethylenediaminetetra-acetic acid, and 5 mmol/L Hepes, pH 7.4, heat-inactivated at 54°C for 1 h, and sterilized by passage through a 0.45- \(\mu\)m filter. The protein content was adjusted to 40 g/L.

Oxidation of Lipoproteins

Oxidation of lipoproteins was determined by detection of malondialdehyde by thiobarbituric acid using the TBARS assay for thiobarbituric acid-reactive substances as described (22). Briefly, LDL suspension samples were boiled at 100°C after addition of 0.2% thiobarbituric acid and butanol. After centrifugation at 3000 U/min, supernatant containing red chromogen was measured photometrically at 532 nm against pure butanol.

Susceptibility of Lipoproteins to Oxidation

Conjugated dienes and susceptibility to oxidation were determined with the method published by Esterbauer \textit{et al.} (23). LDL were oxidized in the presence of 1 mM CuSO\(_4\) solution. This copper-induced oxidation altered light absorption at 233 nm, which was measured by spectrophotometry every 10 min for 3 h. Measurements revealed typical plots including lag phase, acceleration phase, and deceleration phase. Conjugated dienes were calculated based on the absorption at the beginning of measurements and the specific absorption factor for conjugated dienes \(e_{234} = 29,500 \text{ L} \times \text{Mol}^{-1} \times \text{cm}^{-1}\).

Lag time was determined by measuring the time between the lag phase and the acceleration phase within every absorption plot. Lipoproteins exhibiting a short lag time were more susceptible to oxidation than those with a prolonged lag time. In conclusion, short lag time can be regarded as a marker for reduced resistance against oxidation.

Binding, Internalization, and Degradation of Lipoproteins

To elucidate the impact of lipoprotein alterations in patients on hemodialysis for their metabolic pathway, kinetics of receptor-mediated intracellular lipoprotein uptake as described by Goldstein \textit{et al.} (24) were conducted.

To upregulate LDL receptors, HepG2 cells were preincubated with medium containing 10% (vol/vol) LDS. After 48 h, LDS-containing medium was replaced by \(^{125}\text{I} \)-labeled LDL with or without a 25-fold excess of unlabeled lipoproteins. The cultures were incubated for 5 h at 37°C. The medium was removed and the cells were placed on ice. The culture dishes were then washed 5 times with cold buffer containing 154 mmol/L NaCl, 50 mmol/L Tris, pH 7.4, and 2 mg/ml bovine serum albumin (fatty acid free). Binding, internalization, and degradation were measured by standard methods (24,25). Heparin-releasable radioactivity represented the binding at 37°C. After releasing the cell surface-bound radioactivity with heparin, monolayers were dissolved in 1 ml of 0.1N NaOH and radioactivity and protein were determined. Radioactivity in the pellet was considered a measure of internalization. Non-iodide TCA-soluble radioactivity in the culture medium served as a measure of lipoprotein degradation. Nonspecific binding, internalization, and degradation were estimated in the presence of a 25-fold excess of unlabeled ligand. The intra-assay coefficients of variation for degradation, internalization, and binding were 3.6, 10.6, and 12.8%, respectively. For increased reliability, all experiments were performed in triplicate. In each experiment, LDL from healthy control subjects were run in parallel with LDL from patients. Cell density and temperature conditions were kept constant in all experiments throughout the study.

Cellular Sterol Synthesis and Cholesterol Esterification

Cell cultures were incubated in Dulbecco’s modified Eagle’s medium containing 10% (vol/vol) LDS for 48 h and thereafter with varying concentrations of lipoproteins for 12 h. Cellular sterol synthesis was estimated from the rate of incorporation of \(^{14}\text{C} \) (Du Pont, Dreieich, Germany) into sterols. Cellular cholesterol esterification was measured as described (25). For increased reliability, all experiments were performed in duplicate.

Statistical Analyses

Results are reported as means \(\pm\) SEM. Comparison of means was performed using paired or unpaired \(t\) test as appropriate. Scatchard regression analysis was performed with the results of LDL uptake experiments, and the coefficient of regression \((r)\) was found to be greater than 0.95 in all experiments.

Results

Serum Lipid Concentration and Lipid Distribution among the Various Lipoprotein Classes

The various groups of hemodialysis patients were selected according to their serum triglyceride levels and the presence of diabetes. Therefore, serum triglycerides were higher in the diabetic hypertriglyceridemic group compared with diabetic normotriglyceridemic patients (5.42 \(\pm\) 3.24 mmol/L \textit{versus} 1.43 \(\pm\) 0.42 mmol/L, respectively), whereas they were 3.39 \(\pm\) 0.77 mmol/L in hypertriglyceridemic, nondiabetic subjects (HTG) and 1.31 \(\pm\) 0.36 mmol/L in nondiabetic, normotriglyceridemic subjects (NTG). Consequently, serum cholesterol differed between control subjects (4.19 \(\pm\) 0.75 mmol/L) and nondiabetic and diabetic hypertriglyceridemic patients (5.34 \(\pm\) 0.98 mmol/L and 6.37 \(\pm\) 1.68 mmol/L, respectively). While VLDL cholesterol levels were elevated in hypertriglyceridemic patients, LDL cholesterol levels did not differ significantly. HDL cholesterol was reduced in hypertriglyceridemic nondiabetic patients and to an even greater extent in hypertriglyceridemic diabetic subjects compared with healthy control sub-
jects. Compared with healthy control subjects, total serum triglycerides were elevated in all patients, thus representing an elevation of both VLDL and LDL triglycerides (Table 1).

ApoB Concentrations
ApoB serum concentrations were significantly elevated in hypertriglyceridemic subjects, with hypertriglyceridemic diabetic patients expressing the highest serum concentrations (121 ± 6 32 mg/dl versus 74 ± 15 mg/dl for control subjects), suggesting an accumulation of VLDL and HDL particles (Table 1). The concentration of glycated apoB, determined by enzyme immunoassay, was significantly elevated in diabetic patients.

Composition and Subfractionation of LDL
The composition of LDL in hemodialysis patients compared with healthy control subjects was altered significantly. LDL of patients in all groups exhibited a higher content of triglycerides at the expense of esterified cholesterol, whereas free cholesterol, phospholipids, and protein did not change significantly (Table 2).

Subfractionation of LDL revealed that the increase in LDL triglycerides in the hemodialysis patients was due to elevation of the concentrations of LDL-1 (1.019 to 1.031 kg/L) and LDL-6 (1.044 to 1.063 kg/L) (Figure 1). The largest enrichment in LDL-6 was found in diabetic hypertriglyceridemic patients (84.4 ± 6 32 mg/dl versus 29.0 ± 3.1 mg/dl for healthy control subjects).

Oxidation and Susceptibility to Oxidation
To determine lipid peroxides in LDL, thiobarbituric acid-reactive substances (TBARS) were measured (Table 3). Although TBARS were elevated in all groups of hemodialysis patients compared with control subjects, only the increase of TBARS in diabetic hypertriglyceridemic LDL was significant (0.158 ± 0.126 μmol/ml versus 0.027 ± 0.016 μmol/ml for control subjects).

Conjugated dienes and susceptibility of LDL to oxidation were determined by spectrophotometry after copper-induced oxidation. Measurements revealed typical plots including lag phase, acceleration phase, and deceleration phase. Diene formation was elevated in all groups, but elevation was significant only in diabetic LDL (2.61 ± 0.5 μmol for diabetic normotriglyceridemic LDL and 2.51 ± 0.39 μmol for diabetic hypertriglyceridemic LDL versus 2.08 ± 0.28 μmol for healthy subjects).

Lag time was revealed by measuring the time between the lag phase and the acceleration phase within every absorption plot. Lipoproteins exhibiting a short lag time were more susceptible to oxidation than those with a prolonged lag time. However, there were no significant differences in lag time among the various hemodialysis groups or in comparison to the healthy control subjects.

Lipase Activity
To evaluate the influence of lipase activity on lipoprotein metabolism, activities of total lipase, hepatic lipase, and lipoprotein lipase were determined. Total lipase, hepatic lipase,

<table>
<thead>
<tr>
<th>Table 1. Lipid distribution among the various lipoprotein density classes and serum concentration of native and glycosylated apoB.</th>
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</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>NTG</td>
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<tr>
<td>HTG</td>
</tr>
<tr>
<td>NIDDM</td>
</tr>
<tr>
<td>Control subjects</td>
</tr>
</tbody>
</table>

* Results are given as mean ± SEM. NTG: serum triglycerides < 2.28 mmol/L (n = 10); HTG: serum triglycerides > 2.28 mmol/L (n = 10); apoB: apolipoprotein B; NIDDM: non-insulin-dependent diabetes mellitus; apoB glyc: glycosylated apoB.

b P < 0.05 versus control subjects.
and serum lipase were markedly reduced in all groups compared with healthy control subjects, but did not vary significantly among the different groups of hemodialysis patients (Figure 2).

**Cellular Uptake of LDL Isolated from Hemodialysis Patients**

To examine the receptor-mediated cellular uptake of lipoproteins from patients undergoing hemodialysis treatment, increasing concentrations of $^{125}$I-labeled LDL (5 to 100 µg protein per ml) were incubated with HepG2 cells. LDL receptors were upregulated by incubating the cells with a medium containing 10% (vol/vol) LDS for 48 h. Binding, internalization, and degradation of LDL of healthy control subjects and hemodialysis patients in HepG2 was dependent on concentration and was saturable. However, uptake and degradation of LDL from hemodialysis patients were markedly lower compared with control LDL, especially when dialysis patients were both diabetic and hypertriglyceridemic (Figure 3). Scatchard plot analysis revealed lower maximum capacities ($V_{max}$) for binding, internalization, and degradation in every group of hemodialysis patients. In patients with either hypertriglyceridemia or diabetes, only the maximum capacity was slightly reduced, whereas the decrease in $V_{max}$ was pronounced in hemodialysis patients who were both diabetic and hypertriglyceridemic (Table 4). In contrast, $K_D$ values of LDL from hemodialysis patients did not differ among the groups or compared with healthy control subjects.

**Effect of LDL Isolated from Hemodialysis Patients on Cellular Sterol Synthesis and Cholesterol Esterification**

**Incorporation of [14C] Acetate into Sterols.** To determine the influence of LDL from hemodialysis patients on intracellular cholesterol metabolism, HepG2 cells were incubated with LDL from patients and healthy control subjects. After 48 h of incubation in medium containing 10% LDS, the cells were incubated for 16 h with LDL at a final concentration of 20 µg of protein per ml medium (Figure 4). LDL from hemodialysis patients was less effective than control LDL in inhibiting sterol synthesis from acetic acid, even though the differences were not always significant. The diminished capability to inhibit sterol synthesis was most obvious in hypertriglyceridemic LDL from both nondiabetic and diabetic subjects.

**[3H]-Oleate Incorporation into Cholesteryl Esters.** Receptor-mediated endocytosis of lipoproteins results in a stimulation of acyl cholesterol acyl transferase activity. Therefore, incorporation of oleate into cholesteryl esters was used as a measure of sufficient cellular uptake of lipoproteins. LDL from all groups of hemodialysis patients was less effective in stimulating cholesteryl esterification than control LDL (Figure 5).

### Table 2. Lipid and protein composition of LDL in hemodialysis patients

<table>
<thead>
<tr>
<th>Group</th>
<th>FC</th>
<th>CE</th>
<th>TG</th>
<th>PL</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemodialysis NTG</td>
<td>11 ± 1</td>
<td>25 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28 ± 2</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>HTG</td>
<td>9 ± 1</td>
<td>25 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Hemodialysis NIDDM NTG</td>
<td>10 ± 2</td>
<td>23 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>HTG</td>
<td>8 ± 2</td>
<td>23 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27 ± 2</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Control subjects</td>
<td>10 ± 1</td>
<td>30 ± 2</td>
<td>6 ± 1</td>
<td>29 ± 1</td>
<td>25 ± 1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results are given as mean ± SEM, in %. NTG, serum triglycerides <2.28 mmol/L ($n = 10$); HTG, serum triglycerides ≥2.28 mmol/L ($n = 10$); FC, free cholesterol; CE, esterified cholesterol; TG, triglycerides; PL, phospholipids.

<sup>b</sup> $P < 0.05$ versus control subjects.
Although cholesterol esterification was only moderately reduced by LDL from hypertriglyceridemic or diabetic hemodialysis patients, the most striking decrease in cholesterol esterification was achieved by LDL from patients with both diabetes and hypertriglyceridemia.

**Cellular Uptake of LDL by Peritoneal Macrophages (P388D)**

In contrast to the LDL receptor-mediated uptake in HepG2 cells, peritoneal macrophages, which are known to possess scavenger receptors, exhibited enhanced uptake of modified LDL particles. The higher the modification of lipoproteins the more enhanced was the uptake of LDL. Therefore, the highest uptake was observed for LDL isolated from diabetic hypertriglyceridemic patients reflecting the combination of both modification of lipid composition and protein component (Figure 6).

**Discussion**

In end-stage renal disease, dyslipidemia resulting from both diabetes mellitus and hemodialysis treatment is evident in a considerable number of patients (3,4). Although the impact of each single factor for the progression of atherosclerosis is difficult to distinguish, the sum of alterations results in impaired lipoprotein metabolism and, therefore, bears enhanced atherogenic risk (1).

In the present study, LDL isolated from nondiabetic nor-

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**Table 3. TBARS, diene formation, and lag time**

<table>
<thead>
<tr>
<th>Group</th>
<th>TBARS (μmol/mg LDL-Protein)</th>
<th>Diene Formation (μmol/mg LDL-Protein)</th>
<th>Lag Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemodialysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTG</td>
<td>0.65 ± 0.59</td>
<td>23.5 ± 1.5</td>
<td>113 ± 20</td>
</tr>
<tr>
<td>HTG</td>
<td>0.58 ± 0.28</td>
<td>24.6 ± 3.9</td>
<td>91 ± 19</td>
</tr>
<tr>
<td>Hemodialysis NIDDM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTG</td>
<td>0.82 ± 0.80</td>
<td>26.1 ± 5.0b</td>
<td>90 ± 10</td>
</tr>
<tr>
<td>HTG</td>
<td>1.58 ± 1.26b</td>
<td>25.1 ± 3.9b</td>
<td>126 ± 23</td>
</tr>
<tr>
<td>Control subjects</td>
<td>0.27 ± 0.16</td>
<td>20.8 ± 2.8</td>
<td>103 ± 16</td>
</tr>
</tbody>
</table>

*Results are given as mean ± SEM. NTG, serum triglycerides <2.28 mmol/L (n = 10); HTG, serum triglycerides >2.28 mmol/L (n = 10); TBARS, thiobarbituric acid-reactive substances.  
bP < 0.05 versus control subjects.*
motriglyceridemic hemodialysis patients exhibited impaired receptor-specific uptake, reduced inhibition of intracellular sterol synthesis, and reduced cholesterol esterification compared with LDL from healthy control subjects. Reduction of catabolic capacities was found to be more distinct in patients who were either diabetic or hypertriglyceridemic, and it was most pronounced in patients presenting with a combination of both diabetes and hypertriglyceridemia.

Therefore, the data suggest that a number of conditions are present which may account for diminished cellular LDL uptake and impairment of intracellular cholesterol metabolism in hemodialysis patients. The present study attempted to dissect various factors such as size, configuration, and composition of LDL particles, which all may contribute to an impairment of LDL receptor-mediated catabolism.

Prominent compositional and configurational changes of LDL particles occur in hypertriglyceridemia (26), which severely influence their receptor-mediated catabolism (27). Independent of the etiology of hypertriglyceridemia, triglyceride-rich LDL exhibit changes in conformation of apoB, which impairs binding to LDL receptors (28). Triglyceride-rich LDL are known to be smaller and exhibit higher density than control LDL (29). Furthermore, configurational changes in apoB independent of their triglyceride content have been described for small, dense LDL (30). Consequently, small, dense LDL exhibit reduced receptor-specific uptake (31) and therefore bear increased atherogenicity (32). In the present study, subfractionation of LDL from hemodialysis patients revealed accumula-

### Table 4. Maximum capacity ($V_{max}$) and affinity ($K_D$) of LDL in HepG2 cells obtained by Scatchard plot analysis

<table>
<thead>
<tr>
<th>Group</th>
<th>Binding</th>
<th>Internalization</th>
<th>Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemodialysis</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NTG</td>
<td>482</td>
<td>2388</td>
<td>2426</td>
</tr>
<tr>
<td>(32)</td>
<td>(35)</td>
<td>(21)</td>
<td></td>
</tr>
<tr>
<td>HTG</td>
<td>402&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2120&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2135&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(35)</td>
<td>(43)</td>
<td>(21)</td>
<td></td>
</tr>
<tr>
<td>Hemodialysis NIDDM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTG</td>
<td>490</td>
<td>2358</td>
<td>2133&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(36)</td>
<td>(38)</td>
<td>(28)</td>
<td></td>
</tr>
<tr>
<td>HTG</td>
<td>444&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1856&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1804&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(36)</td>
<td>(34)</td>
<td>(20)</td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td>562</td>
<td>2635</td>
<td>2560</td>
</tr>
<tr>
<td>(35)</td>
<td>(38)</td>
<td>(20)</td>
<td></td>
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<sup>a</sup> Results are given as $V_{max}$ ($K_D$). $V_{max}$ is given in ng LDL-protein/mg cell protein and $K_D$ is given in μg LDL-protein/ml medium; NTG, serum triglycerides <2.28 mmol/L (n = 10); HTG, serum triglycerides >2.28 mmol/L (n = 10).<sup>b</sup> $P < 0.05$ versus control subjects.

Figure 4. Inhibition of intracellular cholesterol synthesis in HepG2 cells by LDL of control subjects (●), hemodialysis patients with serum triglycerides <2.28 mmol/L (NTG, □), hemodialysis patients with serum triglycerides >2.28 mmol/L (HTG, △), NIDDM hemodialysis patients with triglycerides <2.28 mmol/L (DM-NTG, ○), and NIDDM hemodialysis patients with triglycerides >2.28 mmol/L (DM-HTG, ●). Incorporation of $^{14}$C acetate into sterols is expressed in percentage of acetate found in nonsaponifiable lipids. Data are given as mean ± SEM of 10 subjects in every group. All measurements were carried out in duplicate. *<sup>P < 0.05</sup> versus control subjects.

Figure 5. Influence of LDL from healthy control subjects (●), non-diabetic hemodialysis patients without (■) or with (▲) hypertriglyceridemia, and NIDDM hemodialysis patients without (▼) or with (●) hypertriglyceridemia on formation of cholesteryl esters in HepG2 cells. Data are given as mean ± SEM of 10 subjects in every group. All measurements were carried out in triplicate. For symbols without error bars, the SEM was less than symbol size.
Reduced receptor-mediated LDL uptake leads to intravascular LDL accumulation and, as a result, prolongs circulation time due to an increased half-life (12). This also may promote the process of modification by glycosylation and oxidation. Since glycosylation is closely related to the oxidative state (37), susceptibility to oxidation and oxidation products (TBARS) were measured. LDL from hemodialysis patients was found to be more susceptible to oxidation than control LDL. This may be due to elevated products of lipid peroxidation in hemodialysis patients (38) and to the lack of antioxidant agents such as vitamins C and E (39). Furthermore, triglyceride-rich LDL are known to be more susceptible to oxidation (40).

Although the distinct influence of diabetes and hypertriglyceridemia per se on impairment of lipoprotein metabolism is obvious, the number of patients included in the present study did not allow us to definitively quantify the impact of each single factor. With respect to the present findings, some cautious conclusions may be drawn: lipoproteins from hemodialysis patients exhibit several structural and compositional alterations that impair their receptor-specific uptake. The reduction in receptor-specific metabolism is greater when diabetes mellitus or hypertriglyceridemia occur additionally, and is most evident if both diabetes mellitus and hypertriglyceridemia are superimposed. Consequently, lipoprotein clearance is impaired, prolonged circulation is likely to occur, and exposure to oxidative stress is increased. Accumulated and altered LDL is predominantly taken up by scavenger receptors of macrophages favoring foam cell formation and the development of atherosclerotic plaques (41). Therefore, diabetes and hypertriglyceridemia appear to promote atherosclerosis and enhance cardiovascular risk via the influence on cellular LDL metabolism in hemodialysis patients. In the general population, several epidemiologic studies have identified hypertriglyceridemia in the presence or absence of diabetes mellitus as an independent risk factor for enhanced cardiovascular morbidity and mortality (42– 44). In view of their cumulative effect on atherosclerosis and mortality, treatment of diabetes and hypertriglyceridemia becomes unavoidable. Large-scale interventional cardiovascular end point studies are required to prove whether advances in quality of life and long-term survival can be made in diabetic patients on hemodialysis.

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