Cholesterol Synthesis, Cholesterol Absorption, and Mortality in Hemodialysis Patients

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Summary

Background and objectives Recent clinical trials on cholesterol-lowering in patients with CKD yielded conflicting results, which might have resulted from different treatment strategies. Serum cholesterol levels are determined by endogenous synthesis and intestinal absorption, which are differentially influenced by various classes of cholesterol-lowering agents. Assessing markers of cholesterol metabolism has thus been proposed for guidance of lipid-lowering therapy. This study analyzed surrogate markers of cholesterol absorption and synthesis in hemodialysis (HD) patients.

Design, setting, participants, & measurements In 113 HD patients, lathosterol was measured as a marker of cholesterol synthesis and cholestanol was measured as a marker of cholesterol absorption via gas chromatography. Controls were 229 healthy persons. Overall survival in HD patients was recorded over 3.4-year follow-up.

Results Compared with controls, HD patients had lower lathosterol and higher cholestanol levels (P<0.001 for both). During follow-up, 58 patients died; higher cholestanol (indicating higher cholesterol absorption) predicted poor outcome among HD patients in multivariate Cox regression analysis after adjustment for potential confounders (hazard ratio for cholestanol above median, 2.24 [95% confidence interval (CI), 1.29–3.89]; P=0.004), whereas lower lathosterol (indicating lower cholesterol synthesis) did not (hazard ratio for lathosterol below median, 1.43 [95% CI, 0.81–2.50]; P=0.22).

Conclusions This analysis of markers of cholesterol metabolism characterizes HD patients as cholesterol absorbers. In longitudinal analysis, higher levels of cholesterol were associated with all-cause mortality.

Introduction

Patients undergoing hemodialysis (HD) suffer from a mortality rate that reaches approximately 20% per year (1). Although this enormous death toll is prominently driven by high cardiovascular mortality, traditional pathways of atherogenesis can only partly explain this phenomenon (2).

In the course of progressive loss of renal function, derangements in lipid metabolism occur (3), and recent data revealed surprisingly low LDL cholesterol levels in HD patients (4–6). In two previous large-scale trials—4D (Die Deutsche Diabetes Dialyse Studie) and AURORA (A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis)—statin monotherapy failed to show a survival benefit (4,6) despite leading to an LDL cholesterol reduction of a magnitude shown to significantly reduce cardiovascular morbidity and mortality in the general population (7). In contrast, SHARP (the Study of Heart and Renal Protection) achieved for the first time a significant, albeit modest, reduction of cardiovascular events in patients with CKD by combining inhibition of cholesterol synthesis via statin treatment and inhibition of intestinal cholesterol absorption via ezetimibe (8).

The different results obtained in 4D (6) and AURORA (4) versus SHARP (8) remain elusive but might be partly due to the differing treatment strategies used. Notably, serum cholesterol levels are determined by both endogenous synthesis and intestinal absorption of exogenous cholesterol. Both components of cholesterol metabolism can be assessed by measurement of lathosterol, an established surrogate marker that reflects hepatic synthesis, and cholestanol, an accepted marker of exogenous absorption (9). Because individuals with high cholesterol absorption are less likely to benefit from statin treatment (10), it has been suggested for the general population to stratify patients according to their pattern of cholesterol metabolism as “synthesizer type,” “mixed type,” and “absorber type” in order to guide cholesterol-lowering therapy (11).

However, a comprehensive assessment of cholesterol metabolism has not been performed in HD patients. In our opinion, such an analysis is needed because its implications might be similar to those for the general population.

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Materials and Methods

Study Population
We recruited 159 HD patients in four outpatient dialysis practices throughout the Federal State of Saarland, Germany, in April 2008. Comorbidity and medication were determined by chart review and standardized interviews at baseline. Patients taking ezetimibe or statin medication were excluded from the analysis (n = 46), leaving 113 patients for further analysis. Median vintage of dialysis treatment was 3.4 years (interquartile range, 1.7–5.8 years). Patients with a history of diabetes mellitus, a spontaneous plasma glucose level of ≥200 mg/dl, hypoglycemic treatment, or self-reported diabetes mellitus were categorized as diabetic. Patients were defined as active smokers if they were current smokers or had stopped smoking less than 1 month before entry into the study. Body mass index was calculated as body weight in kg/height in m² (2).

Before an HD session, systolic and diastolic BP were measured and mean BP was calculated as follows:

\[
\text{mean BP} = \frac{\text{diastolic BP} + \left(\text{systolic BP} - \text{diastolic BP}\right)}{3}
\]

Informed consent was obtained from all patients, and the study design was approved by the local ethics committee. All participants were followed from the baseline examination until death or August 31, 2011. Complete follow-up data were available for 112 of 113 patients.

To compare measures of cholesterol metabolism in HD patients with those in individuals with intact renal function, 229 persons from a previously described cohort (12) without manifest cardiovascular disease, known diabetes mellitus, or intake of statins or ezetimibe served as controls.

Biochemical Analysis
Blood samples were taken from all participants before the start of an HD session. To recruit patients from the afternoon HD sessions, no effort was made to collect fasting blood samples. However, we are certain that collection of nonfasting samples does not interfere with our analysis: Markers of cholesterol synthesis and absorption are mostly bound to LDL cholesterol (13), which is not affected by timing of meals.

Plasma concentrations of cholesterol were measured by gas chromatography–flame ionization detection (GC-FID). Fifty micrograms 5α-cholestanol (Serva) (50 μl from a stock solution of 5α-cholestanol in cyclohexane; 1 mg/ml) and 1 μg epicoprostanol (Sigma) (10 μl from a stock solution epicoprostanol in cyclohexane; 100 μg/ml) were added to 100 μl of plasma. After addition of 1 ml NaOH (1 M) in 90% ethanol, alkaline hydrolysis was performed for 120 minutes at 50°C, the solution was neutralized with phosphoric acid (50%, vol/vol), and the sterols were extracted twice with 4 ml of cyclohexane. The organic solvents were evaporated and the residual sterols were derivatized to trimethylsilyl (TMSi) ethers by adding 1 ml TMSi-reagent (pyridine:hexamethydisilazane:trimethylchlorosilane, 9:3:1, by volume; all reagents were from Merck) and incubation for 1 hour at 64°C. The derivatization reagent was evaporated under nitrogen and the silyl sterol ethers from plasma were dissolved in 160 μl n-decane. Eighty microliters of the solution was transferred into microvials for gas chromatography–mass spectrometry–selected ion monitoring (GC-MS-SIM) of cholesterol precursors. The residual 80 μl was diluted with 400 μl n-decane for analysis of cholesterol by GC-FID.

Plasma cholesterol was quantified by GC-FID on a Hewlett-Packard 6890 series II plus gas chromatograph (Agilent Technologies, Böblingen, Germany) using 5α-cholestanol as an internal standard. An aliquot of 1 μl was injected in a splitless mode at 280°C by an automated sampler and injector (Hewlett-Packard 7683). Hydrogen was used as the carrier gas with an inlet pressure of 9.9 psi, resulting in a total gas flow of 1.1 ml/min; the temperature of the FID was kept at 280°C. The sterols were separated on a cross-linked methyl silicone DB-XLB 122-1232 fused silica capillary column (J&W, Folsom, CA) (30 m × 0.25 mm [i.e., 0.25–μm film thickness]) in a Hewlett-Packard 6890 gas chromatograph. The oven temperature was initially kept at

![Figure 1](https://example.com/figure1.png)

Figure 1. | Markers of cholesterol absorption and synthesis in hemodialysis (HD) patients and in controls with intact renal function. (A) Cholestanol (cholesterol absorption marker). (B) Lathosterol (cholesterol synthesis marker). Data are presented as means ± SDs.
150°C for 3 minutes and was then increased at 30°C increments to a final temperature of 290°C. The ratios of the cholesterol areas to the area of internal standard were calculated and multiplied by the added amount of the internal standard (50 μg 5α-cholestane) to reveal absolute cholesterol concentrations.

GC-MS-SIM was performed on a Hewlett-Packard GC-mass selective detector system (5890 series II GC) combined with a 5971 mass selective detector (Agilent Technologies) equipped with a DB-XLB 122-1232 fused silica capillary column (J&W) (30 m × 0.25 mm [i.e., ×0.25-μm film thickness]) in the splitless mode using helium (1 ml/min) as the carrier gas. The temperature program was as follows: 150°C for 1 minute, followed by 20°C increases per minute up to 260°C, and 10°C increases per minute up to 280°C (for 15 minutes). Neutral sterols were monitored as their TMSi derivatives in the selected ion monitoring mode using the following masses: epiconprostane (internal standard) mass-to-charge ratio (m/z) 370 (M+OTMSi), cholesterol at m/z 458 (M+), cholestanol at m/z 306 (M+OTMSi-CH3-C3H9), and lathosterol at m/z 458 (M+). Peak integration was performed manually and sterols were quantified from selected ion monitoring analyses against internal standard (epiconprostane) using standard curves for the listed sterols. Identity of all sterols was proven by comparison with the full-scan mass spectra of authentic compounds (range, m/z 50-500). Additional qualifier (characteristic fragment) ions were used for structural identification. All the preceding determined precursors were sufficiently separated on the column from additional precursors, such as 7-dehydrocholesterol, methyl precursors, or plant sterols, present in plasma samples. The intra- and interday coefficients of variation for all sterols were less than 3%. Accuracy of the method was established by recovery experiments, day-to-day variation (below 3%), limit of detection, and limit of quantification below the present concentrations for each sterol.

In line with common practice (14), serum cholestanol and lathosterol levels are expressed as cholestanol-to-cholesterol ratios and lathosterol-to-cholesterol ratios.

### Statistical Analyses

Categorical variables are presented as percentage of patients and were compared using the Fisher exact test. Continuous data are expressed as means ± SDs and were compared using the Mann-Whitney test (variables that were not normally distributed are expressed as medians [interquartile ranges]). Spearman correlation coefficients were calculated for analyzing the relationship between continuous data. To analyze the association of cholesterol metabolism with total survival, we stratified patients by median levels of lathosterol and cholestanol, respectively, into two groups. Kaplan-Meier curves were drawn, and event-free survival was compared using the log-rank test. Subsequently, Cox proportional hazards models were calculated that include clinical variables that predicted survival in univariate analysis. To illustrate the association of cholesterol metabolism with patient survival after adjustment for potential confounders, Cox regression survival plots were drawn with separate lines for patients stratified by median values of cholestanol and lathosterol, respectively.

Statistical analyses were performed with SPSS 18.0.2. The level of significance was set at P≤0.05.

### Results

Mean age of HD patients was 64.6±15.3 years; controls were slightly but statistically significantly younger (59.7±13.4 years; P=0.002). Gender distribution was even (HD group: 63 men and 50 women; control group: 108 men and 121 women; P=0.17).

We found strikingly higher levels of cholestanol (Figure 1A), indicating high cholesterol absorption, and lower lathosterol levels (Figure 1B), reflecting cholesterol synthesis, in HD patients than in controls.

Baseline cholestanol was associated with low body mass index (r = -0.18; P=0.06) and high C-reactive protein levels (r = 0.31; P=0.001), whereas lathosterol was associated with high body mass index (r = 0.34; P<0.001) and low C-reactive protein levels (r = -0.21; P=0.03). No association was found between either lathosterol or cholestanol and age, mean BP, or serum albumin.

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics</th>
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<td>Characteristic</td>
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<tr>
<td>Age (yr)</td>
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<td>Women</td>
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<tr>
<td>Diabetes mellitus</td>
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<tr>
<td>Prevalent cardiovascular disease</td>
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<tr>
<td>Vintage of dialysis treatment (yr)</td>
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<td>Body mass index (kg/m²)</td>
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<td>Cholesterol level (mg/dl)</td>
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<td>C-reactive protein level (mg/L)</td>
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<td>Albumin level (g/l)</td>
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<td>Sevelamer use</td>
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Data are means ± SDs or numbers (percentages) of patients, as appropriate. Because of skewed distribution, data on dialysis vintage and C-reactive protein level are given as medians and interquartile ranges.
During follow-up, 58 of 113 patients died. Patients who died were older (age, 72.5 ± 11.5 years in those who died and 56.3 ± 14.5 years in those who did not; P < 0.001), had lower serum albumin (37 ± 4 g/L and 40 ± 4 g/L, respectively; P = 0.03), and had lower mean BP (106 ± 18 mmHg and 116 ± 18 mmHg, respectively; P = 0.002). No significant differences existed at baseline between survivors and nonsurvivors regarding gender distribution, prevalent diabetes mellitus, prevalent cardiovascular disease, vintage of dialysis treatment, body mass index, smoking status, cholesterol level, C-reactive protein level, and sevelamer use. Table 1 shows baseline characteristics stratified by median cholestanol level.

In univariate analysis, a trend for higher mortality was found in patients with lathosterol levels below the median (Figure 2A) and in patients with cholestanol levels above the median (Figure 2B).

To assess the association of markers of cholesterol synthesis and absorption with outcome after correction for traditional outcome predictors, we next built Cox regression analyses that adjusted for variables associated with mortality in univariate analysis: serum albumin, age, and mean BP. As depicted in Figure 3, cholestanol levels above the median were an independent predictor of mortality, whereas lathosterol levels below the median did not achieve statistical significance. Higher cholestanol remained a significant predictor of mortality after adjustment for further potential confounders, namely body mass index, C-reactive protein, intake of sevelamer, and prevalent diabetes mellitus (hazard ratio, 1.98 [95% confidence interval, 1.07–3.69]; P = 0.03).

**Discussion**

We found higher levels of cholestanol, a marker of intestinal cholesterol absorption, along with lower levels of lathosterol, a marker of hepatic cholesterol synthesis, in HD patients compared with healthy controls. Therefore, we...
suggest that most HD patients could be considered “absorbers” according to the proposed classification in the general population (11). Moreover, patients with higher serum levels of cholestanol (indicating higher cholesterol absorption) experienced increased mortality in our cohort. These findings are important given that statin monotherapy failed to achieve a benefit in 4D (6) and AURORA (4), whereas addition of ezetimibe to statin treatment reduced cardiovascular events in SHARP (8). Of note, in contrast to SHARP, 4D and AURORA found larger reductions in LDL cholesterol, which should translate into a bigger clinical benefit, bearing recent meta-analysis data in mind (7). Thus, the results from SHARP suggest that in CKD, inhibition of intestinal cholesterol absorption might confer an additional benefit beyond its effect on LDL cholesterol-lowering. LDL cholesterol independent effects of cholesterol absorption inhibitors, such as ezetimibe, and bile acid sequestrants might comprise lower absorption of potentially atherogenic plant sterols (15). Admittedly, these additional effects are not well established.

The question arises: Which mechanisms induced the shift toward cholesterol absorption in HD patients in the present study? It is conceivable that reduced endogenous cholesterol synthesis could lead to a consecutive increase in cholesterol absorption; this would closely resemble the situation seen with statin therapy in the general population, which induces a secondary increase in cholesterol absorption markers (14). Alternatively, the observed shift toward higher cholesterol absorption might be due to reduced hepatic clearance of chylomicrons and VLDL in chronic renal failure (16) and a consecutive increase in chylomicron and VLDL-bound absorption markers. However, both synthesis and absorption markers are largely transported via LDL.

Figure 3. | Cholesterol metabolism and overall survival after adjustment for age, serum albumin, and mean BP. Patients were stratified by markers of cholesterol (A) synthesis (lathosterol) and (B) absorption (cholestanol). Cox regression survival plot.
As opposed to chylomicrons and VLDL, the LDL serum concentration is determined by LDL biosynthesis, excretion via bile acids, and tissue uptake (16).

Taken together, a true increase of cholesterol absorption in HD patients rather than a secondary increase due to reduced hepatic clearance of carrier proteins is more likely to be responsible for our results. However, no experimental data are available to underscore our observation, and clinical information on markers of cholesterol metabolism in HD patients has been restricted so far to a single cross-sectional study comprising 8 dialysis patients and 16 controls (17). A major limitation of our study is its rather small sample size. This limitation partly results from the necessary exclusion of patients receiving lipid-lowering medication because both statin and ezetimibe treatment interferes with plasma sterol levels. In parallel, the exclusion of patients taking lipid-lowering drugs inevitably resulted in underrepresentation of patients with overt hypercholesterolemia. As a further limitation, the use of cholestanol and lathosterol as markers of cholesterol absorption and synthesis is derived from the general population because their use has not formally been validated in dialysis patients.

In summary, we found that compared with healthy controls, HD patients have higher serum levels of cholestanol, a marker of cholesterol absorption. Moreover, HD patients with higher cholestanol levels faced worse clinical outcome. By suggesting that HD patients are “cholesterol absorbers,” our data might provide new information to improve understanding of the differing results of cholesterol-lowering trials in patients with CKD. We hope that our report instigates a replication in a larger cohort because a confirmation of our results would probably have an important effect on clinical decision-making.

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Disclosures
D.L. received lecturing fees from Danone and Unilever. O.W. received lecture fees from Daichii-Sankyo, MSD, Essex, and Raisio Nutrition Ltd. The other authors have no competing interests to declare.

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