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High Cholesterol Absorption Is Associated with Increased Cardiovascular Risk in Hemodialysis Patients: Insights from the AURORA Study

Silbernagel et al.: Cholesterol Absorption and Statins in Hemodialysis

Günther Silbernagel¹, Kévin Duarte², Samir Sadiku³, Günter Fauler³, Winfried März^{3,4,5},

Roland E Schmieder⁶, Alan G Jardine⁷, Ziad A Massy⁸, Nicolas Girerd⁹, Bengt Fellström¹⁰,

Patrick Rossignol², Hubert Scharnagl^{3*}, Faiez Zannad²

¹ Division of Angiology, Department of Internal Medicine, Medical University of Graz;
Auenbruggerplatz 15, 8036 Graz, Austria

² Université de Lorraine, Inserm, Centre d'Investigations Cliniques- 1433, and Inserm U1116;
CHRU Nancy; F-CRIN INI-CRCT, Nancy, France.

³ Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of
Graz; Auenbruggerplatz 15, 8036 Graz, Austria

⁴ Department of Internal Medicine 5 (Nephrology, Hypertensiology, Endocrinology,
Diabetology, Rheumatology), Mannheim Medical Faculty, University of Heidelberg,
Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany

⁵ Synlab Academy, Synlab Holding Germany GmbH, P5, 7, 68167, Mannheim, Germany

⁶ Department of Nephrology and Hypertension, University Hospital Erlangen, Erlangen,
Germany

⁷ Renal Research Group, British Heart Foundation Cardiovascular Research Centre, Institute
of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK.

⁸ Division of Nephrology, Ambroise Paré University Hospital, APHP, Boulogne
Billancourt/Paris, and INSERM U-1018, Centre de recherche en épidémiologie et santé des
populations (CESP), Equipe 5, Paris-Saclay University (PSU) and University of Paris Ouest-
Versailles-Saint-Quentin-en-Yvelines (UVSQ), FCRIN INI-CRCT, Villejuif, France

⁹ Centre d'Investigation Clinique Plurithématique Pierre Drouin & Département de
Cardiologie, Institut Lorrain du Coeur et des Vaisseaux, CHU de Nancy, Nancy, France

¹⁰ Department of Medical Sciences, Uppsala University, Uppsala University Hospital,
Uppsala, Sweden.

* Address for correspondence:

Hubert Scharnagl, PhD

Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of
Graz; Auenbruggerplatz 15, 8036 Graz, Austria

Email: hubert.scharnagl@medunigraz.at

Tel: +43-316-385-86030

Fax: +43-316-385-13430

Abstract

Background: Statin treatment did not reduce the risk of cardiovascular events in hemodialysis patients in the 4D and AURORA trials. Post-hoc-analyses in the 4D study suggested that high cholesterol absorption was associated with increased cardiovascular risk and that atorvastatin would reduce cardiovascular risk in hemodialysis patients with low cholesterol absorption but not in those with high cholesterol absorption.

Methods: AURORA is a randomized, double-blind, placebo-controlled, multicenter trial in hemodialysis patients. The participants were randomly assigned to receive either rosuvastatin, 10 mg daily, or matching placebo. There was a follow-up for cardiovascular death with a median duration of 3.9 years. The cholestanol and lathosterol to cholesterol ratios were used to estimate cholesterol absorption and synthesis, respectively.

Results: Measurement of non-cholesterol sterols was available in 2,332 participants of the 2,733 patients included in the primary analysis of the AURORA study. A total of 598 participants died from cardiovascular diseases. The 3rd vs. the 1st tertile of the cholestanol to cholesterol ratio was significantly associated with increased risk of cardiovascular death (Hazard ratio [95% confidence interval] = 1.36 [1.11-1.65]) in univariate (p = 0.002) and multivariate models (p = 0.034). In contrast, the 3rd vs. the 1st tertile of the lathosterol to cholesterol ratio was significantly associated with decreased risk of cardiovascular death (Hazard ratio [95% confidence interval] = 0.81 [0.67-0.99]) in univariate (p = 0.041) and multivariate (p = 0.019) models. There was no significant interaction between the cholestanol and lathosterol to cholesterol tertiles and treatment group in predicting cardiovascular death.

Conclusions: The present data from the AURORA Study confirm that high cholesterol absorption is associated with increased cardiovascular risk in hemodialysis patients. Assessment of the individual cholesterol absorption rate to guide initiation of statin treatment is not supported by the findings in the AURORA Study.

1 Introduction

2 Epidemiologic and genetic have provided compelling evidence that elevated low density
3 lipoprotein (LDL) cholesterol is one of the major cardiovascular risk factors. There is also broad
4 evidence that cardiovascular risk can be reduced with LDL cholesterol lowering (1). Therefore,
5 the therapeutic targets for LDL cholesterol have again been reduced to even lower levels in the
6 latest European Atherosclerosis Society/European Society of Cardiology (2) and American
7 Heart Association guidelines (3). In patients with hemodialysis, despite having a 5- to 10-fold
8 increased risk for developing cardiovascular disease, the situation appears less conclusive (4,
9 5). The 2 large multi-center, randomized, placebo-controlled, prospective statin trials in
10 hemodialysis patients did not meet the primary endpoint. The 1st trial, which addressed this
11 issue, was the “German Diabetes Dialysis Study” (4D Study). In this trial with 1,255
12 participants, 20 mg of atorvastatin vs. placebo did not reduce the risk of death from cardiac
13 causes, nonfatal myocardial infarction, and stroke during a follow-up period of 4 years (6). The
14 2nd study was the “A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular
15 Hemodialysis: An Assessment of Survival and Cardiovascular Events” (AURORA) Study. In
16 this trial with 2,776 participants, 10 mg of rosuvastatin vs. placebo did not reduce the risk of
17 cardiovascular events during a follow-up period of 3.8 years (7). Of relevance, hemodialysis
18 patients are characterized by high cholesterol absorption (8). In addition, high cholesterol
19 absorption was found to be a cardiovascular risk factor (9) and to be associated with reduced
20 efficacy of simvastatin to prevent cardiovascular events in the 4S Study (Scandinavian
21 Simvastatin Survival Study) (10). Post-hoc analyses in the 4D study were also supportive of
22 this theory as the subgroup with high cholestanol to cholesterol ratio (indicative of high
23 cholesterol absorbers) had increased risk of reaching the primary endpoint and did not profit
24 from statin treatment whereas the subgroup with low cholestanol to cholesterol ratio did (11).
25 To further explore the role of cholesterol metabolism in hemodialysis patients we aimed to
26 measure baseline non-cholesterol sterols in the AURORA Study (7). Our objective was to

1 confirm the previous 4D results that 1) hemodialysis patients with high cholesterol absorption
2 have increased cardiovascular risk and that 2) rosuvastatin treatment may be ineffective in
3 reducing cardiovascular risk in hemodialysis patients with high absorption but still be effective
4 in those with relatively low absorption and thus higher synthesis.

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1 **Methods**

2 **Study design**

3 The study design of the AURORA study has been previously described in detail (7). AURORA
4 is a randomized, double-blind, placebo-controlled, multicenter trial. The study was conducted
5 in accordance with the ethical principles of the Declaration of Helsinki, the Good Clinical
6 Practice guidelines of the International Conference of Harmonisation, and all participants gave
7 written, informed consent. The study was approved by ethics committees at all participating
8 centers. The executive steering committee designed the trial and supervised its conduct in
9 collaboration with the sponsor, AstraZeneca.

10
11 **Patients**

12 A total of 2,776 men and women aged 50 to 80 years who had end-stage renal disease and had
13 been treated with regular hemodialysis or hemofiltration for at least 3 months were recruited
14 from 280 centers in 25 countries. The major exclusion criteria were statin therapy within the
15 previous 6 months, expected kidney transplantation within 1 year, and serious hematologic,
16 neoplastic, gastrointestinal, infectious, or metabolic disease (excluding diabetes) that was
17 predicted to limit life expectancy to less than 1 year. Moreover, subjects with a history of a
18 malignant condition, active liver disease (indicated by an alanine aminotransferase level that
19 was more than three times the upper limit of the normal range), uncontrolled hypothyroidism,
20 and an unexplained elevation in the creatine kinase level to more than three times the upper
21 limit of the normal range were excluded. Eligible patients were randomly assigned (in blocks
22 of four in a 1:1 ratio) to receive either rosuvastatin, 10 mg daily, or matching placebo. There
23 were follow-up visits 3 months after randomization and then every 6 months. Assessments
24 included laboratory evaluations once yearly and, at each visit, pill counts and structured
25 interviews to assess potential adverse events. Blood samples were analyzed at a central
26 laboratory for levels of lipids, high-sensitivity C-reactive protein, troponin, brain natriuretic

1 peptide, and galectin 3. A closeout visit was scheduled at the end of the study. Measurement of
2 non-cholesterol sterols was available in 2,332 participants of the 2,733 patients included in the
3 primary analysis of the AURORA study. We excluded 2 patients because their identifications
4 didn't match with any of the patients included in the AURORA trial.

6 **Endpoints**

7 The primary end point of the present analyses was the time to cardiovascular death. Major
8 cardiovascular events, which included nonfatal myocardial infarction, nonfatal stroke, or death
9 from cardiovascular causes, were included as a secondary endpoint. All myocardial infarctions,
10 strokes or deaths were reviewed and adjudicated by a clinical end-point committee whose
11 members were unaware of the randomized treatment assignments, in order to ensure
12 consistency of the event diagnosis. The median follow-up duration was 3.9 years (inter-quartile
13 range: 2.1 - 4.5).

15 **Non-cholesterol sterol measurement**

16 Measurement of cholestanol and lathosterol was performed using a well-established gas
17 chromatography and mass spectroscopy method (single ion monitoring mode). The significant
18 ions were m/z 445.4 for cholestanol and m/z 458.5 for lathosterol (12).

20 **Statistical analysis**

21 The power calculation of the AURORA study has been described in detail (7). The present
22 analysis were non-prespecified post-hoc analyses performed by an independent statistician,
23 Kévin Duarte. The analysis plan for the present investigations was orientated on a previous
24 report in the 4D study for comparability reasons (11).

25 The baseline characteristics are presented according to randomization group. Categorical data
26 are reported as counts (percentages) and continuous data as means \pm standard deviation or

1 medians with inter-quartile range if distribution was skewed. We calculated ratios of
2 cholestanol and lathosterol to cholesterol. Then tertiles of the cholestanol and lathosterol to
3 cholesterol ratios were formed. Comparison of cardiovascular risk factors according to tertiles
4 of sterol variables were carried out using ANalysis Of VAriance test or non-parametric Kruskal-
5 Wallis test for continuous variables, and Fisher's exact test for categorical variables.
6 We estimated post-hoc power by calculating the cumulative probability of finding significance
7 using non-parametric bootstrapping. We performed Cox model for each bootstrap sample and
8 we recorded whether an effect was significant or not, after which we calculated the percentage
9 of significant effects across all bootstrapped samples (13).
10 To assess the association between sterol variables and outcomes, time-to-event analyses were
11 performed using Cox regression model. Hazard Ratios (HR) are presented with their 95%
12 confidence intervals (CI 95%). We assessed interactions between sterol variables and the study
13 treatment in Cox models. We also performed Cox analyses stratified by sex and analyses
14 including an interaction term between sterol variables and sex.
15 The prognostic value of sterol variables on top of clinical variables was evaluated by the
16 increase Harrell's c-index (14, 15), continuous Net Reclassification Improvement (cNRI) (16),
17 and Integrated Discrimination Improvement (IDI) (16) indexes. cNRI and IDI were calculated
18 at 3.5 years. This timepoint was slightly earlier than the median of follow-up time (3.9 years).
19 All analyses were performed using R software (the R foundation for Statistical Computing).
20 The two-tailed significance level was set at $p < 0.05$. All academic authors received the analyses
21 results and confirm completeness of the reported analyses.

1 **Results**

2 **Baseline characteristics according to randomization groups**

3 Patients in the rosuvastatin and placebo groups had similar age, sex and racial distribution, body
4 mass index, blood pressure, and dialysis modalities. There were also no differences in
5 haemoglobin, albumin, calcium, phosphate, and C-reactive protein. The proportion of current
6 smokers was slightly higher in the placebo group. No significant differences were registered
7 for history of diabetes, history of atherosclerotic vascular disease and current medication. The
8 concentrations of standard lipid, cholestanol, lathosterol, and the cholestanol and lathosterol to
9 cholesterol ratios did not differ between the 2 groups either (*table 1*). The mean values for the
10 cholestanol and lathosterol to cholesterol ratios were 1.39 and 0.82 $\mu\text{mol}/\text{mmol}$, respectively.

11
12 **Baseline characteristics according to tertiles of non-cholesterol sterol to cholesterol ratios**

13 Tertiles of the cholestanol to cholesterol ratio

14 Age, sex distribution, proportion of current smokers, history of diabetes or hypertension did not
15 differ among the tertiles of the cholestanol to cholesterol ratio. Whereas troponin and galectin
16 3 did not differ among the tertiles of the cholestanol to cholesterol ratio, brain natriuretic peptide
17 was positively related to the tertiles of the cholestanol to cholesterol ratio. In contrast, the body
18 mass index, total cholesterol, LDL cholesterol, non-HDL cholesterol, triglycerides, and
19 apolipoprotein B were inversely related to the tertiles of the cholestanol to cholesterol ratio
20 (*table 2*).

21
22 Tertiles of the lathosterol to cholesterol ratio

23 Age, sex distribution, proportion of current smokers, and history of diabetes or hypertension
24 did not differ among the tertiles of the lathosterol to cholesterol ratio. The proportion of males
25 and the body mass index were positively related to tertiles of the lathosterol to cholesterol ratio.
26 Whereas troponin and galectin 3 did not differ among the tertiles of the lathosterol to cholesterol
27 ratio, brain natriuretic peptide was inversely related to the tertiles of the lathosterol to

1 cholesterol ratio. Total cholesterol, LDL cholesterol, non-HDL cholesterol, and apolipoprotein
2 B were inversely related to the lathosterol to cholesterol ratio. In contrast, triglycerides were
3 positively related to the lathosterol to cholesterol ratio (*table 3*).

5 **Association of cholesterol variables with outcome**

6 We found a post-hoc power of 42.8 % to find a significant effect on cardiovascular mortality
7 for the tertiles of lathosterol to cholesterol ratio and 84.5 % for the tertiles of cholestanol to
8 cholesterol ratio.

9 The 3rd vs. the 1st tertile of the cholestanol to cholesterol ratio was significantly associated with
10 increased risk of cardiovascular death in univariate (*central figure*) and multivariate models. In
11 contrast, the 3rd vs. the 1st tertile of the lathosterol to cholesterol ratio was significantly
12 associated decreased risk of cardiovascular death (*table 4*). Consistent results were obtained for
13 major adverse cardiovascular events (*supplementary table 1*).

14 There were 502 patients with cardiovascular death at 3.5 years, 1442 without cardiovascular
15 death at 3.5 years (including 92 with cardiovascular death after 3.5 years), and 401 patients
16 were excluded because they were lost to follow-up at 3.5 years. The cNRI (17.2 [5.1 to 26.6] p
17 = 0.006) and the IDI at 3.5 years (0.4 [0.1 to 1.1]; p = 0.004) were significant for the cholestanol
18 to cholesterol ratio using model 2 with borderline significance using model 3 (16.7 [-1.1 to
19 25.1], p = 0.058; and 0.2 [0.0 to 0.8], p = 0.064; respectively). The c-index was not significantly
20 improved after inclusion of the cholestanol to cholesterol ratio. The cNRI at 3.5 years (17.3 [-
21 0.4 to 22.5], p = 0.056) was at borderline significance and the IDI at 3.5 years (0.2 [0.0 to 0.8];
22 p = 0.028) was significant for the lathosterol to cholesterol ratio using model 2. Both, the cNRI
23 (10.8 [0.2 to 21.6], 0.046) and the IDI (0.3 [0.0 to 0.9], p = 0.026) were significant for the
24 lathosterol to cholesterol ratio using model 3. The c-index did not significantly increase for the
25 lathosterol to cholesterol ratios using both models of adjustment.

1 **Interaction with treatment effect**

2 There was no significant interaction between the cholestanol and lathosterol to cholesterol
3 tertiles and treatment group in predicting cardiovascular death (*table 4*). There was also no
4 significant interaction between the cholestanol and lathosterol to cholesterol tertiles and
5 treatment group in predicting major adverse cardiovascular events (*supplementary table 1*).

7 **Analyses stratified by sex**

8 The associations of the cholestanol and lathosterol to cholesterol ratios with cardiovascular
9 death were similar in females and males (*supplementary table 2*), with no significant
10 interactions (all $p > 0.4$). The associations of the cholestanol and lathosterol to cholesterol ratios
11 with major adverse cardiovascular events were also similar in females and males
12 (*supplementary table 3*), with no significant interactions (all $p > 0.2$).

1 **Discussion**

2 The present findings in the AURORA Study confirm that hemodialysis patients with high
3 cholesterol absorption and low cholesterol synthesis have increased cardiovascular risk, with
4 no differences between in females and males. Hence, the results are supportive of the suggestion
5 to use ezetimibe in hemodialysis patients. In accordance, the “Study of Heart and Renal
6 Protection” (SHARP Study) with 9,270 participants showed, that 20 mg of simvastatin plus 10
7 mg of ezetimibe reduced the risk of the first major atherosclerotic event during a median follow-
8 up of 4.9 years in patients with a wide range of advanced chronic kidney disease. The results
9 did not reach statistical significance for the subgroup of patients with hemodialysis but there
10 was no interaction between hemodialysis treatment and lipid lowering therapy with regard to
11 the primary endpoint (17). Of relevance, ezetimibe has also been demonstrated to reduce
12 cardiovascular events in the “The Improved Reduction of Outcomes: Vytorin Efficacy
13 International Trial” (IMPROVE-IT Study) (18) and has been found particularly beneficial in
14 the elderly (19). The reason for the association of lowest lathosterol to cholesterol tertile with
15 the highest risk of cardiovascular death may be explained by relatively low cholesterol synthesis
16 rates in patients with high cholesterol absorption (9). However, residual confounding by frailty
17 in those with low cholesterol synthesis cannot be completely ruled out (20).

18 According to the 4D Study, triglycerides, VLDL cholesterol, and apolipoprotein C-III were not
19 associated with outcomes (21). Hence, it appears unclear, whether increasing lipoprotein lipase
20 activity for example by blocking angiopoietin-like proteins 3 or 4 (22) will be instrumental to
21 further reduce cardiovascular risk in hemodialysis patients. Rather, future efforts to reduce
22 cardiovascular risk in hemodialysis patients may address hepatic lipase activity. Importantly,
23 low hepatic lipase activity is a feature of uraemia (23) and has been suggested to be a causal
24 cardiovascular risk factor (24).

25 The positive association of brain natriuretic peptide with the cholestanol to cholesterol tertiles
26 and the inverse relationship with the lathosterol to cholesterol tertiles may be a reflection of

1 ischaemic cardiomyopathy secondary to detrimental effects of high cholesterol absorption (9,
2 25). Another possibility may be that the association of high cholesterol absorption and low
3 cholesterol synthesis with increased brain natriuretic peptide is a feature of cardiac cachexia
4 (26).

5 In contrast to the 4D Study (11), the present data did not support that the use of the cholestanol
6 to cholesterol ratio may help to decide on treatment initiation with statins in hemodialysis
7 patients. The difference between the AURORA and 4D results may be due to differences in
8 patient characteristics such as the considerably lower body mass index and the lower proportion
9 of patients with diabetes in the AURORA study. Importantly, type 2 diabetes and the metabolic
10 syndrome are both associated with increased cholesterol synthesis and low cholesterol
11 absorption (25). Paradoxically, IMPROVE-IT trial patients with diabetes experienced a greater
12 reduction of LDL cholesterol and cardiovascular events compared to those without diabetes by
13 treatment with ezetimibe (27). This may partly be due to premedication with statins causing a
14 compensatory increase of cholesterol absorption (28). In addition, the results may due to higher
15 baseline cardiovascular risk in patients with diabetes compared to those without (27). The
16 difference in the results between the 4D and AURORA results with regard to the predictive
17 value of cholesterol absorption on the effectiveness of statin treatment to reduce cardiovascular
18 risk may also be due to power issues. Last but not least, the differences in outcomes between
19 the 4D and AURORA studies may be due to unequal effects of rosuvastatin and atorvastatin on
20 the microinflammatory state in patients with hemodialysis (29).

21 Based on the present observations it appears reasonable to focus on LDL cholesterol as a
22 biochemical parameter to decide on initiation of treatment with statins in hemodialysis patients.
23 Of relevance, post-hoc analyses in the 4D Study showed that atorvastatin treatment was
24 effective in reducing the risk of reaching the primary endpoint in hemodialysis patients with
25 high LDL cholesterol but not in those with low LDL cholesterol (30). Although this interaction
26 was not significant in the AURORA trial, the results appeared to indicate a similar trend

1 (*supplementary table 4*). In addition, baseline cardiovascular risk, general physical condition
2 (e.g. potential frailty), and age should be considered. Recently, a scoring system was introduced
3 to predict the effectiveness of statin treatment to reduce cardiovascular events in hemodialysis
4 patients. This scoring system, which also included the cholestanol to cholesterol ratio, was
5 based on results from the 4D study (31). The current findings indicate that it would be useful
6 to generate an improved algorithm that is based on data from both, the 4D and the AURORA
7 studies.

8 The AURORA Study is the largest randomized, placebo-controlled, multi-center statin trial in
9 hemodialysis patients. Hence, the present report on the role of cholesterol absorption and
10 synthesis in this patient group is also the largest so far. The AURORA Study is also the only
11 cohort in which the hypothesis generated based on the results from the 4D Study could be tested.
12 Moreover, there was a very precise clinical and biochemical characterization of the study
13 participants and a comprehensive, adjudicated, clinical follow-up. Another strong aspect of the
14 present investigation is the measurement of non-cholesterol sterols using an established method
15 by a single technician who was blinded to all clinical and biochemical data of the study
16 participants.

17 It may be a limitation of the present investigation that it was a post-hoc analysis. In addition,
18 the dietary habits of the participants, which may affect cholesterol absorption and associated
19 risk, were not evaluated systematically (32). Furthermore, measurement of non-cholesterol
20 sterols, unlike isotope methods (33), represents an indirect method to evaluate individual
21 cholesterol metabolism. Finally, the findings from the AURORA Study are restricted to patients
22 with 50 to 80 years of age and patients, who expected kidney transplantation within 1 year, had
23 also been excluded according to the AURORA study protocol.

24 In conclusion, the present data add to the evidence that hemodialysis patients with high
25 cholesterol absorption have increased cardiovascular risk. Assessment of the individual

- 1 cholesterol absorption rate to guide initiation of statin treatment is not supported by the findings
- 2 from the AURORA Study.

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1 **Authorship**

2 GS and HS drafted the manuscript. KD performed the statistical analysis. SS and GS performed
3 measurement on non-cholesterol sterols. WM, NG, and PR critically revised the manuscript.
4 RES, AGJ, ZAM, BF, and FZ designed the study. All gave final approval and agree to be
5 accountable for all aspects of work ensuring integrity and accuracy.

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Table 1

Title: Baseline characteristics of the cohort according to randomization group

Characteristic	Overall (n=2375)	Placebo (n=1164)	Rosuvastatin (n=1181)
Age (years)	64.3 ± 8.7	64.4 ± 8.8	64.1 ± 8.6
Female	889 (37.9 %)	429 (36.9 %)	460 (39.0 %)
Race			
Caucasian	1988 (84.8 %)	990 (85.1 %)	998 (84.5 %)
Black	78 (3.3 %)	40 (3.4 %)	38 (3.2 %)
Asian	128 (5.5 %)	62 (5.3 %)	66 (5.6 %)
Hispanic	93 (4.0 %)	46 (4.0 %)	47 (4.0 %)
Other race	58 (2.5 %)	26 (2.2 %)	32 (2.7 %)
Body mass index (kg/m ²)	25.3 ± 4.9	25.3 ± 5.2	25.2 ± 4.7
Systolic Blood Pressure (mmHg)	137.3 ± 24.4	136.9 ± 24.1	137.6 ± 24.7
Diastolic Blood Pressure (mmHg)	75.9 ± 12.7	75.6 ± 12.6	76.2 ± 12.7
Current smoker	362 (15.4 %)	199 (17.1 %)	163 (13.8 %)
Total cholesterol (mmol/L)	4.52 ± 1.09	4.49 ± 1.09	4.55 ± 1.10
LDL cholesterol (mmol/L)	2.57 ± 0.89	2.55 ± 0.88	2.58 ± 0.90
HDL cholesterol (mmol/L)	1.17 ± 0.40	1.16 ± 0.42	1.17 ± 0.38
Non-HDL cholesterol (mmol/L)	3.35 ± 1.08	3.33 ± 1.07	3.38 ± 1.10
Triglycerides (mmol/L)	1.72 ± 1.06	1.72 ± 1.07	1.73 ± 1.05
Apolipoprotein B (mg/dL)	81.4 ± 24.2	81.3 ± 24.4	81.4 ± 23.9
Lathosterol (µmol/L)	2.29 (1.26 - 4.35)	2.31 (1.28 - 4.43)	2.26 (1.25 - 4.24)
Cholestanol (µmol/L)	4.42 (2.55 - 7.41)	4.56 (2.59 - 7.58)	4.33 (2.52 - 7.34)
Ratio Lathosterol/Cholesterol (µmol/mmol)	0.52 (0.29 - 0.99)	0.54 (0.29 - 1.02)	0.51 (0.28 - 0.95)
Ratio Cholestanol/Cholesterol (µmol/mmol)	1.01 (0.59 - 1.72)	1.03 (0.59 - 1.75)	0.99 (0.59 - 1.69)
High sensitivity C-reactive protein (mg/dL)	0.51 (0.21 - 1.43)	0.54 (0.22 - 1.50)	0.48 (0.20 - 1.37)
Hemoglobin (g/dL)	11.7 ± 1.6	11.7 ± 1.6	11.7 ± 1.6
Albumin (g/L)	39.6 ± 3.5	39.6 ± 3.5	39.7 ± 3.5

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Characteristic	Overall (n=2375)	Placebo (n=1164)	Rosuvastatin (n=1181)
Calcium (mmol/L)	2.34 ± 0.22	2.34 ± 0.21	2.35 ± 0.22
Phosphate (mmol/L)	1.78 ± 0.55	1.78 ± 0.53	1.79 ± 0.57
Duration of treatment with hemodialysis (years)	3.5 ± 3.9	3.6 ± 3.9	3.4 ± 3.9
Duration of dialysis sessions (hr/week)	11.8 ± 1.8	11.9 ± 1.7	11.8 ± 1.8
Cause of end-stage renal disease			
Nephrosclerosis	468 (20.0 %)	235 (20.2 %)	233 (19.7 %)
Glomerulonephritis or vasculitis	423 (18.0 %)	211 (18.1 %)	212 (18.0 %)
Diabetes	432 (18.4 %)	202 (17.4 %)	230 (19.5 %)
Tubulointerstitial disease	362 (15.4 %)	174 (14.9 %)	188 (15.9 %)
Hereditary	301 (12.8 %)	158 (13.6 %)	143 (12.1 %)
Other	359 (15.3 %)	184 (15.8 %)	175 (14.8 %)
Clinical history			
Diabetes	604 (25.8 %)	284 (24.4 %)	320 (27.1 %)
Cardiovascular disease	924 (39.4 %)	463 (39.8 %)	461 (39.0 %)
Myocardial infarction	239 (10.2 %)	118 (10.1 %)	121 (10.2 %)
Coronary revascularization	139 (5.9 %)	70 (6.0 %)	69 (5.8 %)
Peripheral vascular disease	346 (14.8 %)	173 (14.9 %)	173 (14.6 %)
Drug therapy			
Angiotensin-converting-enzyme inhibitor or angiotensin-receptor blocker	853 (36.4 %)	428 (36.8 %)	425 (36.0 %)
Calcium-channel blocker	828 (35.3 %)	420 (36.1 %)	408 (34.5 %)
Beta-blocker	867 (37.0 %)	411 (35.3 %)	456 (38.6 %)
Diuretic	696 (29.7 %)	338 (29.0 %)	358 (30.3 %)
Platelet inhibitor	992 (42.3 %)	484 (41.6 %)	508 (43.0 %)
Vitamin D	1088 (46.4 %)	544 (46.7 %)	544 (46.1 %)
Calcium substitution	1753 (74.8 %)	873 (75.0 %)	880 (74.5 %)
Sevelamer	678 (28.9 %)	323 (27.7 %)	355 (30.1 %)
Erythropoietin	2070 (88.3 %)	1031 (88.6 %)	1039 (88.0 %)

Data are shown as mean ± standard deviation, median (inter-quartile range) or frequency (percentage). HDL high density lipoprotein; LDL low density lipoprotein.

Table 2**Title:** Risk factors of cardiovascular disease according to tertiles of the cholestanol/cholesterol ratio

	First tertile (n=778) ≤ 0.71 μmol/mmol	Second tertile (n=777)]0.71 ; 1.39] μmol/mmol	Third tertile (n=777) > 1.39 μmol/mmol	p-value^a
Cholestanol/Cholesterol (μmol/mmol)	0.71	1.01	1.39	-
Age (years)	64.1 ± 8.6	64.2 ± 8.9	64.5 ± 8.6	0.60
Male	459 (59.0 %)	500 (64.4 %)	490 (63.1 %)	0.076
Body mass index (kg/m ²)	25.5 ± 4.9	25.5 ± 5.0	24.8 ± 4.8	0.004
Current smoker	116 (14.9 %)	127 (16.3 %)	117 (15.1 %)	0.69
Systemic hypertension	382 (49.1 %)	387 (49.9 %)	420 (54.1 %)	0.11
Diabetes	195 (25.1 %)	196 (25.2 %)	208 (26.8 %)	0.70
Total cholesterol (mmol/L)	4.74 ± 1.14	4.55 ± 1.04	4.27 ± 1.04	<0.0001
LDL cholesterol (mmol/L)	2.70 ± 0.94	2.60 ± 0.85	2.41 ± 0.85	<0.0001
HDL cholesterol (mmol/L)	1.16 ± 0.41	1.17 ± 0.39	1.17 ± 0.41	0.86
Non-HDL cholesterol (mmol/L)	3.58 ± 1.15	3.38 ± 1.04	3.10 ± 1.01	<0.0001
Triglycerides (mmol/L)	1.90 ± 1.11	1.73 ± 1.15	1.54 ± 0.86	<0.0001
Apolipoprotein B (mg/dL)	85.6 ± 25.2	82.1 ± 23.3	76.3 ± 23.2	<0.0001
Fasting glucose (mmol/L)	5.65 ± 2.02	5.58 ± 1.97	5.46 ± 2.00	0.19
High sensitivity C-reactive protein (mg/dL)	0.48 (0.21 - 1.35)	0.46 (0.19 - 1.40)	0.57 (0.25 - 1.62)	0.002
Troponin I (pg/mL)	17.6 (10.8 - 33.2)	16.8 (9.7 - 30.9)	17.9 (9.9 - 34.2)	0.21
Galectin 3 (ng/mL)	68.9 ± 25.0	68.6 ± 23.5	70.1 ± 25.4	0.48
Brain natriuretic peptide (pg/mL)	101 (34 - 283)	111 (41 - 266)	142 (44 - 461)	<0.0001

Data are shown as mean ± standard deviation, median (inter-quartile range) or frequency (percentage).

^a p-values for means data were calculated with the use of ANalysis Of VAriance test, for medians with the use of Kruskal-Wallis test, and for percentages with the use of Fisher's exact test. HDL high density lipoprotein; LDL low density lipoprotein.

Table 3**Title:** Risk factors of cardiovascular disease according to tertiles of the lathosterol/cholesterol ratio

	First tertile (n=778) ≤ 0.36 μmol/mmol	Second tertile (n=777)]0.36 ; 0.77] μmol/mmol	Third tertile (n=777) > 0.77 μmol/mmol	p-value ^a
Age (years)	64.1 ± 8.8	64.7 ± 8.5	64.0 ± 8.8	0.20
Male	427 (54.9 %)	506 (65.1 %)	516 (66.4 %)	<0.0001
Body mass index (kg/m ²)	24.1 ± 4.3	25.4 ± 4.8	26.3 ± 5.3	<0.0001
Current smoker	127 (16.3 %)	118 (15.2 %)	115 (14.8 %)	0.69
Systemic hypertension	408 (52.4 %)	379 (48.8 %)	402 (51.8 %)	0.32
Diabetes	184 (23.7 %)	210 (27.0 %)	205 (26.4 %)	0.27
Total cholesterol (mmol/L)	4.68 ± 1.11	4.50 ± 1.04	4.38 ± 1.11	<0.0001
LDL cholesterol (mmol/L)	2.78 ± 0.91	2.56 ± 0.83	2.37 ± 0.88	<0.0001
HDL cholesterol (mmol/L)	1.17 ± 0.39	1.19 ± 0.42	1.13 ± 0.39	0.019
Non-HDL cholesterol (mmol/L)	3.50 ± 1.09	3.31 ± 1.02	3.25 ± 1.12	<0.0001
Triglycerides (mmol/L)	1.60 ± 0.83	1.65 ± 0.92	1.93 ± 1.33	<0.0001
Apolipoprotein B (mg/dL)	83.5 ± 24.1	81.3 ± 23.7	79.3 ± 24.6	0.003
Fasting glucose (mmol/L)	5.51 ± 1.93	5.67 ± 2.10	5.51 ± 1.95	0.21
High sensitivity C-reactive protein (mg/dL)	0.48 (0.20 - 1.56)	0.53 (0.22 - 1.43)	0.51 (0.21 - 1.31)	0.75
Troponin I (pg/mL)	18.9 (10.9 - 34.9)	17.3 (10.1 - 33.5)	16.4 (9.6 - 29.1)	0.019
Galectin 3 (ng/mL)	71.2 ± 25.6	68.4 ± 24.0	68.0 ± 24.2	0.020
Brain natriuretic peptide (pg/mL)	136 (46 - 370)	108 (39 - 320)	104 (30 - 242)	<0.0001

Data are shown as mean ± standard deviation, median (inter-quartile range) or frequency (percentage).

^a p-values for means data were calculated with the use of ANalysis Of VAriance test, for medians with the use of Kruskal-Wallis test, and for percentages with the use of Fisher's exact test. HDL high density lipoprotein; LDL low density lipoprotein.

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Table 4

Title: Association of the cholestanol and lathosterol to cholesterol ratios with cardiovascular death and interactions with treatment

		Model 1 ^a		Model 2 ^b		Model 3 ^c		
	N _{CV death} /N (%)	HR (CI 95 %)	p-value	HR (CI 95 %)	p-value	HR (CI 95 %)	p-value	p-value for interaction ^a
Lathosterol/Cholesterol			0.12		0.085		0.064	0.075
First tertile (ref.)	213/778 (27.4%)	1.00	-	1.00	-	1.00	-	
Second tertile	200/777 (25.7%)	0.92 (0.76 - 1.11)	0.38	0.89 (0.73 - 1.08)	0.24	0.88 (0.72 - 1.07)	0.20	
Third tertile	182/777 (23.4%)	0.81 (0.67 - 0.99)	0.041	0.80 (0.65 - 0.97)	0.027	0.78 (0.64 - 0.96)	0.019	
Cholestanol/Cholesterol			0.005		0.008		0.083	0.45
First tertile (ref.)	183/778 (23.5%)	1.00	-	1.00	-	1.00	-	
Second tertile	189/777 (24.3%)	1.06 (0.87 - 1.30)	0.55	1.05 (0.86 - 1.29)	0.64	1.06 (0.86 - 1.30)	0.60	
Third tertile	223/777 (28.7%)	1.36 (1.11 - 1.65)	0.002	1.33 (1.09 - 1.62)	0.004	1.24 (1.02 - 1.52)	0.034	

^a unadjusted.

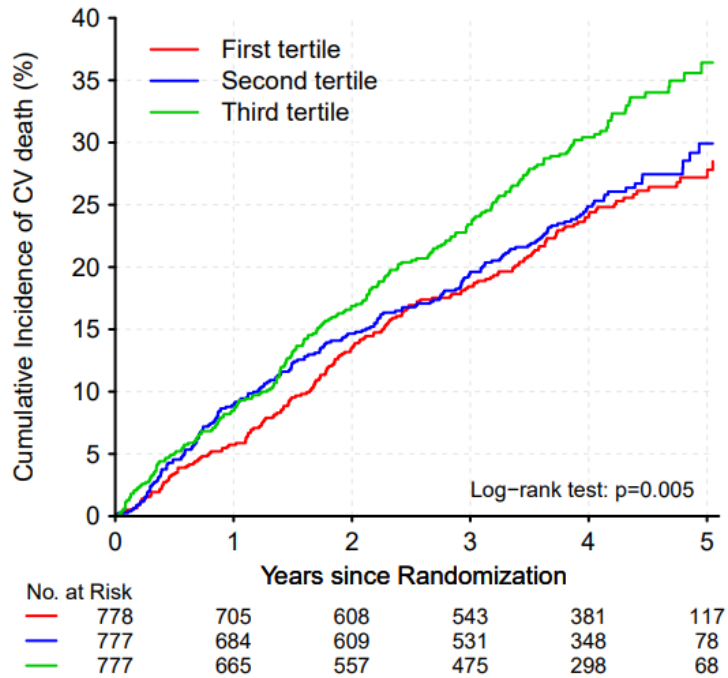
^b adjusted for age and sex.

^c adjusted for age, sex, body mass index, systemic hypertension, diabetes, smoking, log high sensitivity C-reactive protein, and treatment group (rosuvastatin vs placebo).

N_{CV death} number of cardiovascular deaths; N total number; HR hazard ratio; CI confidence interval; ref. reference

Central figure

Title: High cholesterol absorption as reflected by the cholestanol to cholesterol ratio is associated with increased cardiovascular risk in hemodialysis patients



CV cardiovascular

High Cholesterol Absorption Is Associated with Increased Cardiovascular Risk in Hemodialysis Patients: Insights from the AURORA Study

Silbernagel et al.: Cholesterol Absorption and Statins in Hemodialysis

Günther Silbernagel¹, Kévin Duarte², Samir Sadiku³, Günter Fauler³, Winfried März^{3,4,5},

Roland E Schmieder⁶, Alan G Jardine⁷, Ziad A Massy⁸, Nicolas Girerd⁹, Bengt Fellström¹⁰,

Patrick Rossignol², Hubert Scharnagl^{3*}, Faiez Zannad²

¹ Division of Angiology, Department of Internal Medicine, Medical University of Graz;
Auenbruggerplatz 15, 8036 Graz, Austria

² Université de Lorraine, Inserm, Centre d'Investigations Cliniques- 1433, and Inserm U1116;
CHRU Nancy; F-CRIN INI-CRCT, Nancy, France.

³ Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of
Graz; Auenbruggerplatz 15, 8036 Graz, Austria

⁴ Department of Internal Medicine 5 (Nephrology, Hypertensiology, Endocrinology,
Diabetology, Rheumatology), Mannheim Medical Faculty, University of Heidelberg,
Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany

⁵ Synlab Academy, Synlab Holding Germany GmbH, P5, 7, 68167, Mannheim, Germany

⁶ Department of Nephrology and Hypertension, University Hospital Erlangen, Erlangen,
Germany

⁷ Renal Research Group, British Heart Foundation Cardiovascular Research Centre, Institute
of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK.

⁸ Division of Nephrology, Ambroise Paré University Hospital, APHP, Boulogne
Billancourt/Paris, and INSERM U-1018, Centre de recherche en épidémiologie et santé des
populations (CESP), Equipe 5, Paris-Saclay University (PSU) and University of Paris Ouest-
Versailles-Saint-Quentin-en-Yvelines (UVSQ), FCRIN INI-CRCT, Villejuif, France

⁹ Centre d'Investigation Clinique Plurithématique Pierre Drouin & Département de
Cardiologie, Institut Lorrain du Coeur et des Vaisseaux, CHU de Nancy, Nancy, France

¹⁰ Department of Medical Sciences, Uppsala University, Uppsala University Hospital,
Uppsala, Sweden.

* Address for correspondence:

Hubert Scharnagl, PhD

Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of
Graz; Auenbruggerplatz 15, 8036 Graz, Austria

Email: hubert.scharnagl@medunigraz.at

Tel: +43-316-385-86030

Fax: +43-316-385-13430

Abstract

Background: Statin treatment did not reduce the risk of cardiovascular events in hemodialysis patients in the 4D and AURORA trials. Post-hoc-analyses in the 4D study suggested that high cholesterol absorption was associated with increased cardiovascular risk and that atorvastatin would reduce cardiovascular risk in hemodialysis patients with low cholesterol absorption but not in those with high cholesterol absorption.

Methods: AURORA is a randomized, double-blind, placebo-controlled, multicenter trial in hemodialysis patients. The participants were randomly assigned to receive either rosuvastatin, 10 mg daily, or matching placebo. There was a follow-up for cardiovascular death with a median duration of 3.9 years. The cholestanol and lathosterol to cholesterol ratios were used to estimate cholesterol absorption and synthesis, respectively.

Results: Measurement of non-cholesterol sterols was available in 2,332 participants of the 2,733 patients included in the primary analysis of the AURORA study. A total of 598 participants died from cardiovascular diseases. The 3rd vs. the 1st tertile of the cholestanol to cholesterol ratio was significantly associated with increased risk of cardiovascular death (Hazard ratio [95% confidence interval] = 1.36 [1.11-1.65]) in univariate (p = 0.002) and multivariate models (p = 0.034). In contrast, the 3rd vs. the 1st tertile of the lathosterol to cholesterol ratio was significantly associated with decreased risk of cardiovascular death (Hazard ratio [95% confidence interval] = 0.81 [0.67-0.99]) in univariate (p = 0.041) and multivariate (p = 0.019) models. There was no significant interaction between the cholestanol and lathosterol to cholesterol tertiles and treatment group in predicting cardiovascular death.

Conclusions: The present data from the AURORA Study confirm that high cholesterol absorption is associated with increased cardiovascular risk in hemodialysis patients. Assessment of the individual cholesterol absorption rate to guide initiation of statin treatment is not supported by the findings in the AURORA Study.

1 **Introduction**

2 Epidemiologic and genetic have provided compelling evidence that elevated low density
3 lipoprotein (LDL) cholesterol is one of the major cardiovascular risk factors. There is also broad
4 evidence that cardiovascular risk can be reduced with LDL cholesterol lowering (1). Therefore,
5 the therapeutic targets for LDL cholesterol have again been reduced to even lower levels in the
6 latest European Atherosclerosis Society/European Society of Cardiology (2) and American
7 Heart Association guidelines (3). In patients with hemodialysis, despite having a 5- to 10-fold
8 increased risk for developing cardiovascular disease, the situation appears less conclusive (4,
9 5). The 2 large multi-center, randomized, placebo-controlled, prospective statin trials in
10 hemodialysis patients did not meet the primary endpoint. The 1st trial, which addressed this
11 issue, was the “German Diabetes Dialysis Study” (4D Study). In this trial with 1,255
12 participants, 20 mg of atorvastatin vs. placebo did not reduce the risk of death from cardiac
13 causes, nonfatal myocardial infarction, and stroke during a follow-up period of 4 years (6). The
14 2nd study was the “A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular
15 Hemodialysis: An Assessment of Survival and Cardiovascular Events” (AURORA) Study. In
16 this trial with 2,776 participants, 10 mg of rosuvastatin vs. placebo did not reduce the risk of
17 cardiovascular events during a follow-up period of 3.8 years (7). Of relevance, hemodialysis
18 patients are characterized by high cholesterol absorption (8). In addition, high cholesterol
19 absorption was found to be a cardiovascular risk factor (9) and to be associated with reduced
20 efficacy of simvastatin to prevent cardiovascular events in the 4S Study (Scandinavian
21 Simvastatin Survival Study) (10). Post-hoc analyses in the 4D study were also supportive of
22 this theory as the subgroup with high cholestanol to cholesterol ratio (indicative of high
23 cholesterol absorbers) had increased risk of reaching the primary endpoint and did not profit
24 from statin treatment whereas the subgroup with low cholestanol to cholesterol ratio did (11).
25 To further explore the role of cholesterol metabolism in hemodialysis patients we aimed to
26 measure baseline non-cholesterol sterols in the AURORA Study (7). Our objective was to

1 confirm the previous 4D results that 1) hemodialysis patients with high cholesterol absorption
2 have increased cardiovascular risk and that 2) rosuvastatin treatment may be ineffective in
3 reducing cardiovascular risk in hemodialysis patients with high absorption but still be effective
4 in those with relatively low absorption and thus higher synthesis.

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1 **Methods**

2 **Study design**

3 The study design of the AURORA study has been previously described in detail (7). AURORA
4 is a randomized, double-blind, placebo-controlled, multicenter trial. The study was conducted
5 in accordance with the ethical principles of the Declaration of Helsinki, the Good Clinical
6 Practice guidelines of the International Conference of Harmonisation, and all participants gave
7 written, informed consent. The study was approved by ethics committees at all participating
8 centers. The executive steering committee designed the trial and supervised its conduct in
9 collaboration with the sponsor, AstraZeneca.

10
11 **Patients**

12 A total of 2,776 men and women aged 50 to 80 years who had end-stage renal disease and had
13 been treated with regular hemodialysis or hemofiltration for at least 3 months were recruited
14 from 280 centers in 25 countries. The major exclusion criteria were statin therapy within the
15 previous 6 months, expected kidney transplantation within 1 year, and serious hematologic,
16 neoplastic, gastrointestinal, infectious, or metabolic disease (excluding diabetes) that was
17 predicted to limit life expectancy to less than 1 year. Moreover, subjects with a history of a
18 malignant condition, active liver disease (indicated by an alanine aminotransferase level that
19 was more than three times the upper limit of the normal range), uncontrolled hypothyroidism,
20 and an unexplained elevation in the creatine kinase level to more than three times the upper
21 limit of the normal range were excluded. Eligible patients were randomly assigned (in blocks
22 of four in a 1:1 ratio) to receive either rosuvastatin, 10 mg daily, or matching placebo. There
23 were follow-up visits 3 months after randomization and then every 6 months. Assessments
24 included laboratory evaluations once yearly and, at each visit, pill counts and structured
25 interviews to assess potential adverse events. Blood samples were analyzed at a central
26 laboratory for levels of lipids, high-sensitivity C-reactive protein, troponin, brain natriuretic

1 peptide, and galectin 3. A closeout visit was scheduled at the end of the study. Measurement of
2 non-cholesterol sterols was available in 2,332 participants of the 2,733 patients included in the
3 primary analysis of the AURORA study. We excluded 2 patients because their identifications
4 didn't match with any of the patients included in the AURORA trial.

6 **Endpoints**

7 The primary end point of the present analyses was the time to cardiovascular death. Major
8 cardiovascular events, which included nonfatal myocardial infarction, nonfatal stroke, or death
9 from cardiovascular causes, were included as a secondary endpoint. All myocardial infarctions,
10 strokes or deaths were reviewed and adjudicated by a clinical end-point committee whose
11 members were unaware of the randomized treatment assignments, in order to ensure
12 consistency of the event diagnosis. The median follow-up duration was 3.9 years (inter-quartile
13 range: 2.1 - 4.5).

15 **Non-cholesterol sterol measurement**

16 Measurement of cholestanol and lathosterol was performed using a well-established gas
17 chromatography and mass spectroscopy method (single ion monitoring mode). The significant
18 ions were m/z 445.4 for cholestanol and m/z 458.5 for lathosterol (12).

20 **Statistical analysis**

21 The power calculation of the AURORA study has been described in detail (7). The present
22 analysis were non-prespecified post-hoc analyses performed by an independent statistician,
23 Kévin Duarte. The analysis plan for the present investigations was orientated on a previous
24 report in the 4D study for comparability reasons (11).

25 The baseline characteristics are presented according to randomization group. Categorical data
26 are reported as counts (percentages) and continuous data as means \pm standard deviation or

1 medians with inter-quartile range if distribution was skewed. We calculated ratios of
2 cholestanol and lathosterol to cholesterol. Then tertiles of the cholestanol and lathosterol to
3 cholesterol ratios were formed. Comparison of cardiovascular risk factors according to tertiles
4 of sterol variables were carried out using ANalysis Of VAriance test or non-parametric Kruskal-
5 Wallis test for continuous variables, and Fisher's exact test for categorical variables.
6 We estimated post-hoc power by calculating the cumulative probability of finding significance
7 using non-parametric bootstrapping. We performed Cox model for each bootstrap sample and
8 we recorded whether an effect was significant or not, after which we calculated the percentage
9 of significant effects across all bootstrapped samples (13).
10 To assess the association between sterol variables and outcomes, time-to-event analyses were
11 performed using Cox regression model. Hazard Ratios (HR) are presented with their 95%
12 confidence intervals (CI 95%). We assessed interactions between sterol variables and the study
13 treatment in Cox models. We also performed Cox analyses stratified by sex and analyses
14 including an interaction term between sterol variables and sex.
15 The prognostic value of sterol variables on top of clinical variables was evaluated by the
16 increase Harrell's c-index (14, 15), continuous Net Reclassification Improvement (cNRI) (16),
17 and Integrated Discrimination Improvement (IDI) (16) indexes. cNRI and IDI were calculated
18 at 3.5 years. This timepoint was slightly earlier than the median of follow-up time (3.9 years).
19 All analyses were performed using R software (the R foundation for Statistical Computing).
20 The two-tailed significance level was set at $p < 0.05$. All academic authors received the analyses
21 results and confirm completeness of the reported analyses.

1 **Results**

2 **Baseline characteristics according to randomization groups**

3 Patients in the rosuvastatin and placebo groups had similar age, sex and racial distribution, body
4 mass index, blood pressure, and dialysis modalities. There were also no differences in
5 haemoglobin, albumin, calcium, phosphate, and C-reactive protein. The proportion of current
6 smokers was slightly higher in the placebo group. No significant differences were registered
7 for history of diabetes, history of atherosclerotic vascular disease and current medication. The
8 concentrations of standard lipid, cholestanol, lathosterol, and the cholestanol and lathosterol to
9 cholesterol ratios did not differ between the 2 groups either (*table 1*). The mean values for the
10 cholestanol and lathosterol to cholesterol ratios were 1.39 and 0.82 $\mu\text{mol}/\text{mmol}$, respectively.

11
12 **Baseline characteristics according to tertiles of non-cholesterol sterol to cholesterol ratios**

13 Tertiles of the cholestanol to cholesterol ratio

14 Age, sex distribution, proportion of current smokers, history of diabetes or hypertension did not
15 differ among the tertiles of the cholestanol to cholesterol ratio. Whereas troponin and galectin
16 3 did not differ among the tertiles of the cholestanol to cholesterol ratio, brain natriuretic peptide
17 was positively related to the tertiles of the cholestanol to cholesterol ratio. In contrast, the body
18 mass index, total cholesterol, LDL cholesterol, non-HDL cholesterol, triglycerides, and
19 apolipoprotein B were inversely related to the tertiles of the cholestanol to cholesterol ratio
20 (*table 2*).

21
22 Tertiles of the lathosterol to cholesterol ratio

23 Age, sex distribution, proportion of current smokers, and history of diabetes or hypertension
24 did not differ among the tertiles of the lathosterol to cholesterol ratio. The proportion of males
25 and the body mass index were positively related to tertiles of the lathosterol to cholesterol ratio.
26 Whereas troponin and galectin 3 did not differ among the tertiles of the lathosterol to cholesterol
27 ratio, brain natriuretic peptide was inversely related to the tertiles of the lathosterol to

1 cholesterol ratio. Total cholesterol, LDL cholesterol, non-HDL cholesterol, and apolipoprotein
2 B were inversely related to the lathosterol to cholesterol ratio. In contrast, triglycerides were
3 positively related to the lathosterol to cholesterol ratio (*table 3*).

5 **Association of cholesterol variables with outcome**

6 We found a post-hoc power of 42.8 % to find a significant effect on cardiovascular mortality
7 for the tertiles of lathosterol to cholesterol ratio and 84.5 % for the tertiles of cholestanol to
8 cholesterol ratio.

9 The 3rd vs. the 1st tertile of the cholestanol to cholesterol ratio was significantly associated with
10 increased risk of cardiovascular death in univariate (*central figure*) and multivariate models. In
11 contrast, the 3rd vs. the 1st tertile of the lathosterol to cholesterol ratio was significantly
12 associated decreased risk of cardiovascular death (*table 4*). Consistent results were obtained for
13 major adverse cardiovascular events (*supplementary table 1*).

14 There were 502 patients with cardiovascular death at 3.5 years, 1442 without cardiovascular
15 death at 3.5 years (including 92 with cardiovascular death after 3.5 years), and 401 patients
16 were excluded because they were lost to follow-up at 3.5 years. The cNRI (17.2 [5.1 to 26.6] p
17 = 0.006) and the IDI at 3.5 years (0.4 [0.1 to 1.1]; p = 0.004) were significant for the cholestanol
18 to cholesterol ratio using model 2 with borderline significance using model 3 (16.7 [-1.1 to
19 25.1], p = 0.058; and 0.2 [0.0 to 0.8], p = 0.064; respectively). The c-index was not significantly
20 improved after inclusion of the cholestanol to cholesterol ratio. The cNRI at 3.5 years (17.3 [-
21 0.4 to 22.5], p = 0.056) was at borderline significance and the IDI at 3.5 years (0.2 [0.0 to 0.8];
22 p = 0.028) was significant for the lathosterol to cholesterol ratio using model 2. Both, the cNRI
23 (10.8 [0.2 to 21.6], 0.046) and the IDI (0.3 [0.0 to 0.9], p = 0.026) were significant for the
24 lathosterol to cholesterol ratio using model 3. The c-index did not significantly increase for the
25 lathosterol to cholesterol ratios using both models of adjustment.

1 **Interaction with treatment effect**

2 There was no significant interaction between the cholestanol and lathosterol to cholesterol
3 tertiles and treatment group in predicting cardiovascular death (*table 4*). There was also no
4 significant interaction between the cholestanol and lathosterol to cholesterol tertiles and
5 treatment group in predicting major adverse cardiovascular events (*supplementary table 1*).

7 **Analyses stratified by sex**

8 The associations of the cholestanol and lathosterol to cholesterol ratios with cardiovascular
9 death were similar in females and males (*supplementary table 2*), with no significant
10 interactions (all $p > 0.4$). The associations of the cholestanol and lathosterol to cholesterol ratios
11 with major adverse cardiovascular events were also similar in females and males
12 (*supplementary table 3*), with no significant interactions (all $p > 0.2$).

1 **Discussion**

2 The present findings in the AURORA Study confirm that hemodialysis patients with high
3 cholesterol absorption and low cholesterol synthesis have increased cardiovascular risk, with
4 no differences between in females and males. Hence, the results are supportive of the suggestion
5 to use ezetimibe in hemodialysis patients. In accordance, the “Study of Heart and Renal
6 Protection” (SHARP Study) with 9,270 participants showed, that 20 mg of simvastatin plus 10
7 mg of ezetimibe reduced the risk of the first major atherosclerotic event during a median follow-
8 up of 4.9 years in patients with a wide range of advanced chronic kidney disease. The results
9 did not reach statistical significance for the subgroup of patients with hemodialysis but there
10 was no interaction between hemodialysis treatment and lipid lowering therapy with regard to
11 the primary endpoint (17). Of relevance, ezetimibe has also been demonstrated to reduce
12 cardiovascular events in the “The Improved Reduction of Outcomes: Vytorin Efficacy
13 International Trial” (IMPROVE-IT Study) (18) and has been found particularly beneficial in
14 the elderly (19). The reason for the association of lowest lathosterol to cholesterol tertile with
15 the highest risk of cardiovascular death may be explained by relatively low cholesterol synthesis
16 rates in patients with high cholesterol absorption (9). However, residual confounding by frailty
17 in those with low cholesterol synthesis cannot be completely ruled out (20).

18 According to the 4D Study, triglycerides, VLDL cholesterol, and apolipoprotein C-III were not
19 associated with outcomes (21). Hence, it appears unclear, whether increasing lipoprotein lipase
20 activity for example by blocking angiopoietin-like proteins 3 or 4 (22) will be instrumental to
21 further reduce cardiovascular risk in hemodialysis patients. Rather, future efforts to reduce
22 cardiovascular risk in hemodialysis patients may address hepatic lipase activity. Importantly,
23 low hepatic lipase activity is a feature of uraemia (23) and has been suggested to be a causal
24 cardiovascular risk factor (24).

25 The positive association of brain natriuretic peptide with the cholestanol to cholesterol tertiles
26 and the inverse relationship with the lathosterol to cholesterol tertiles may be a reflection of

1 ischaemic cardiomyopathy secondary to detrimental effects of high cholesterol absorption (9,
2 25). Another possibility may be that the association of high cholesterol absorption and low
3 cholesterol synthesis with increased brain natriuretic peptide is a feature of cardiac cachexia
4 (26).

5 In contrast to the 4D Study (11), the present data did not support that the use of the cholestanol
6 to cholesterol ratio may help to decide on treatment initiation with statins in hemodialysis
7 patients. The difference between the AURORA and 4D results may be due to differences in
8 patient characteristics such as the considerably lower body mass index and the lower proportion
9 of patients with diabetes in the AURORA study. Importantly, type 2 diabetes and the metabolic
10 syndrome are both associated with increased cholesterol synthesis and low cholesterol
11 absorption (25). Paradoxically, IMPROVE-IT trial patients with diabetes experienced a greater
12 reduction of LDL cholesterol and cardiovascular events compared to those without diabetes by
13 treatment with ezetimibe (27). This may partly be due to premedication with statins causing a
14 compensatory increase of cholesterol absorption (28). In addition, the results may due to higher
15 baseline cardiovascular risk in patients with diabetes compared to those without (27). The
16 difference in the results between the 4D and AURORA results with regard to the predictive
17 value of cholesterol absorption on the effectiveness of statin treatment to reduce cardiovascular
18 risk may also be due to power issues. Last but not least, the differences in outcomes between
19 the 4D and AURORA studies may be due to unequal effects of rosuvastatin and atorvastatin on
20 the microinflammatory state in patients with hemodialysis (29).

21 Based on the present observations it appears reasonable to focus on LDL cholesterol as a
22 biochemical parameter to decide on initiation of treatment with statins in hemodialysis patients.
23 Of relevance, post-hoc analyses in the 4D Study showed that atorvastatin treatment was
24 effective in reducing the risk of reaching the primary endpoint in hemodialysis patients with
25 high LDL cholesterol but not in those with low LDL cholesterol (30). Although this interaction
26 was not significant in the AURORA trial, the results appeared to indicate a similar trend

1 (*supplementary table 4*). In addition, baseline cardiovascular risk, general physical condition
2 (e.g. potential frailty), and age should be considered. Recently, a scoring system was introduced
3 to predict the effectiveness of statin treatment to reduce cardiovascular events in hemodialysis
4 patients. This scoring system, which also included the cholestanol to cholesterol ratio, was
5 based on results from the 4D study (31). The current findings indicate that it would be useful
6 to generate an improved algorithm that is based on data from both, the 4D and the AURORA
7 studies.

8 The AURORA Study is the largest randomized, placebo-controlled, multi-center statin trial in
9 hemodialysis patients. Hence, the present report on the role of cholesterol absorption and
10 synthesis in this patient group is also the largest so far. The AURORA Study is also the only
11 cohort in which the hypothesis generated based on the results from the 4D Study could be tested.
12 Moreover, there was a very precise clinical and biochemical characterization of the study
13 participants and a comprehensive, adjudicated, clinical follow-up. Another strong aspect of the
14 present investigation is the measurement of non-cholesterol sterols using an established method
15 by a single technician who was blinded to all clinical and biochemical data of the study
16 participants.

17 It may be a limitation of the present investigation that it was a post-hoc analysis. In addition,
18 the dietary habits of the participants, which may affect cholesterol absorption and associated
19 risk, were not evaluated systematically (32). Furthermore, measurement of non-cholesterol
20 sterols, unlike isotope methods (33), represents an indirect method to evaluate individual
21 cholesterol metabolism. Finally, the findings from the AURORA Study are restricted to patients
22 with 50 to 80 years of age and patients, who expected kidney transplantation within 1 year, had
23 also been excluded according to the AURORA study protocol.

24 In conclusion, the present data add to the evidence that hemodialysis patients with high
25 cholesterol absorption have increased cardiovascular risk. Assessment of the individual

- 1 cholesterol absorption rate to guide initiation of statin treatment is not supported by the findings
- 2 from the AURORA Study.

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1 **Authorship**

2 GS and HS drafted the manuscript. KD performed the statistical analysis. SS and GS performed
3 measurement on non-cholesterol sterols. WM, NG, and PR critically revised the manuscript.
4 RES, AGJ, ZAM, BF, and FZ designed the study. All gave final approval and agree to be
5 accountable for all aspects of work ensuring integrity and accuracy.

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Table 1

Title: Baseline characteristics of the cohort according to randomization group

Characteristic	Overall (n=2375)	Placebo (n=1164)	Rosuvastatin (n=1181)
Age (years)	64.3 ± 8.7	64.4 ± 8.8	64.1 ± 8.6
Female	889 (37.9 %)	429 (36.9 %)	460 (39.0 %)
Race			
Caucasian	1988 (84.8 %)	990 (85.1 %)	998 (84.5 %)
Black	78 (3.3 %)	40 (3.4 %)	38 (3.2 %)
Asian	128 (5.5 %)	62 (5.3 %)	66 (5.6 %)
Hispanic	93 (4.0 %)	46 (4.0 %)	47 (4.0 %)
Other race	58 (2.5 %)	26 (2.2 %)	32 (2.7 %)
Body mass index (kg/m ²)	25.3 ± 4.9	25.3 ± 5.2	25.2 ± 4.7
Systolic Blood Pressure (mmHg)	137.3 ± 24.4	136.9 ± 24.1	137.6 ± 24.7
Diastolic Blood Pressure (mmHg)	75.9 ± 12.7	75.6 ± 12.6	76.2 ± 12.7
Current smoker	362 (15.4 %)	199 (17.1 %)	163 (13.8 %)
Total cholesterol (mmol/L)	4.52 ± 1.09	4.49 ± 1.09	4.55 ± 1.10
LDL cholesterol (mmol/L)	2.57 ± 0.89	2.55 ± 0.88	2.58 ± 0.90
HDL cholesterol (mmol/L)	1.17 ± 0.40	1.16 ± 0.42	1.17 ± 0.38
Non-HDL cholesterol (mmol/L)	3.35 ± 1.08	3.33 ± 1.07	3.38 ± 1.10
Triglycerides (mmol/L)	1.72 ± 1.06	1.72 ± 1.07	1.73 ± 1.05
Apolipoprotein B (mg/dL)	81.4 ± 24.2	81.3 ± 24.4	81.4 ± 23.9
Lathosterol (µmol/L)	2.29 (1.26 - 4.35)	2.31 (1.28 - 4.43)	2.26 (1.25 - 4.24)
Cholestanol (µmol/L)	4.42 (2.55 - 7.41)	4.56 (2.59 - 7.58)	4.33 (2.52 - 7.34)
Ratio Lathosterol/Cholesterol (µmol/mmol)	0.52 (0.29 - 0.99)	0.54 (0.29 - 1.02)	0.51 (0.28 - 0.95)
Ratio Cholestanol/Cholesterol (µmol/mmol)	1.01 (0.59 - 1.72)	1.03 (0.59 - 1.75)	0.99 (0.59 - 1.69)
High sensitivity C-reactive protein (mg/dL)	0.51 (0.21 - 1.43)	0.54 (0.22 - 1.50)	0.48 (0.20 - 1.37)
Hemoglobin (g/dL)	11.7 ± 1.6	11.7 ± 1.6	11.7 ± 1.6
Albumin (g/L)	39.6 ± 3.5	39.6 ± 3.5	39.7 ± 3.5

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Characteristic	Overall (n=2375)	Placebo (n=1164)	Rosuvastatin (n=1181)
Calcium (mmol/L)	2.34 ± 0.22	2.34 ± 0.21	2.35 ± 0.22
Phosphate (mmol/L)	1.78 ± 0.55	1.78 ± 0.53	1.79 ± 0.57
Duration of treatment with hemodialysis (years)	3.5 ± 3.9	3.6 ± 3.9	3.4 ± 3.9
Duration of dialysis sessions (hr/week)	11.8 ± 1.8	11.9 ± 1.7	11.8 ± 1.8
Cause of end-stage renal disease			
Nephrosclerosis	468 (20.0 %)	235 (20.2 %)	233 (19.7 %)
Glomerulonephritis or vasculitis	423 (18.0 %)	211 (18.1 %)	212 (18.0 %)
Diabetes	432 (18.4 %)	202 (17.4 %)	230 (19.5 %)
Tubulointerstitial disease	362 (15.4 %)	174 (14.9 %)	188 (15.9 %)
Hereditary	301 (12.8 %)	158 (13.6 %)	143 (12.1 %)
Other	359 (15.3 %)	184 (15.8 %)	175 (14.8 %)
Clinical history			
Diabetes	604 (25.8 %)	284 (24.4 %)	320 (27.1 %)
Cardiovascular disease	924 (39.4 %)	463 (39.8 %)	461 (39.0 %)
Myocardial infarction	239 (10.2 %)	118 (10.1 %)	121 (10.2 %)
Coronary revascularization	139 (5.9 %)	70 (6.0 %)	69 (5.8 %)
Peripheral vascular disease	346 (14.8 %)	173 (14.9 %)	173 (14.6 %)
Drug therapy			
Angiotensin-converting-enzyme inhibitor or angiotensin-receptor blocker	853 (36.4 %)	428 (36.8 %)	425 (36.0 %)
Calcium-channel blocker	828 (35.3 %)	420 (36.1 %)	408 (34.5 %)
Beta-blocker	867 (37.0 %)	411 (35.3 %)	456 (38.6 %)
Diuretic	696 (29.7 %)	338 (29.0 %)	358 (30.3 %)
Platelet inhibitor	992 (42.3 %)	484 (41.6 %)	508 (43.0 %)
Vitamin D	1088 (46.4 %)	544 (46.7 %)	544 (46.1 %)
Calcium substitution	1753 (74.8 %)	873 (75.0 %)	880 (74.5 %)
Sevelamer	678 (28.9 %)	323 (27.7 %)	355 (30.1 %)
Erythropoietin	2070 (88.3 %)	1031 (88.6 %)	1039 (88.0 %)

Data are shown as mean ± standard deviation, median (inter-quartile range) or frequency (percentage). HDL high density lipoprotein; LDL low density lipoprotein.

Table 2**Title:** Risk factors of cardiovascular disease according to tertiles of the cholestanol/cholesterol ratio

	First tertile (n=778) ≤ 0.71 μmol/mmol	Second tertile (n=777)]0.71 ; 1.39] μmol/mmol	Third tertile (n=777) > 1.39 μmol/mmol	p-value^a
Cholestanol/Cholesterol (μmol/mmol)	0.71	1.01	1.39	-
Age (years)	64.1 ± 8.6	64.2 ± 8.9	64.5 ± 8.6	0.60
Male	459 (59.0 %)	500 (64.4 %)	490 (63.1 %)	0.076
Body mass index (kg/m ²)	25.5 ± 4.9	25.5 ± 5.0	24.8 ± 4.8	0.004
Current smoker	116 (14.9 %)	127 (16.3 %)	117 (15.1 %)	0.69
Systemic hypertension	382 (49.1 %)	387 (49.9 %)	420 (54.1 %)	0.11
Diabetes	195 (25.1 %)	196 (25.2 %)	208 (26.8 %)	0.70
Total cholesterol (mmol/L)	4.74 ± 1.14	4.55 ± 1.04	4.27 ± 1.04	<0.0001
LDL cholesterol (mmol/L)	2.70 ± 0.94	2.60 ± 0.85	2.41 ± 0.85	<0.0001
HDL cholesterol (mmol/L)	1.16 ± 0.41	1.17 ± 0.39	1.17 ± 0.41	0.86
Non-HDL cholesterol (mmol/L)	3.58 ± 1.15	3.38 ± 1.04	3.10 ± 1.01	<0.0001
Triglycerides (mmol/L)	1.90 ± 1.11	1.73 ± 1.15	1.54 ± 0.86	<0.0001
Apolipoprotein B (mg/dL)	85.6 ± 25.2	82.1 ± 23.3	76.3 ± 23.2	<0.0001
Fasting glucose (mmol/L)	5.65 ± 2.02	5.58 ± 1.97	5.46 ± 2.00	0.19
High sensitivity C-reactive protein (mg/dL)	0.48 (0.21 - 1.35)	0.46 (0.19 - 1.40)	0.57 (0.25 - 1.62)	0.002
Troponin I (pg/mL)	17.6 (10.8 - 33.2)	16.8 (9.7 - 30.9)	17.9 (9.9 - 34.2)	0.21
Galectin 3 (ng/mL)	68.9 ± 25.0	68.6 ± 23.5	70.1 ± 25.4	0.48
Brain natriuretic peptide (pg/mL)	101 (34 - 283)	111 (41 - 266)	142 (44 - 461)	<0.0001

Data are shown as mean ± standard deviation, median (inter-quartile range) or frequency (percentage).

^a p-values for means data were calculated with the use of ANalysis Of VAriance test, for medians with the use of Kruskal-Wallis test, and for percentages with the use of Fisher's exact test. HDL high density lipoprotein; LDL low density lipoprotein.

Table 3**Title:** Risk factors of cardiovascular disease according to tertiles of the lathosterol/cholesterol ratio

	First tertile (n=778) ≤ 0.36 μmol/mmol	Second tertile (n=777)]0.36 ; 0.77] μmol/mmol	Third tertile (n=777) > 0.77 μmol/mmol	p-value ^a
Age (years)	64.1 ± 8.8	64.7 ± 8.5	64.0 ± 8.8	0.20
Male	427 (54.9 %)	506 (65.1 %)	516 (66.4 %)	<0.0001
Body mass index (kg/m ²)	24.1 ± 4.3	25.4 ± 4.8	26.3 ± 5.3	<0.0001
Current smoker	127 (16.3 %)	118 (15.2 %)	115 (14.8 %)	0.69
Systemic hypertension	408 (52.4 %)	379 (48.8 %)	402 (51.8 %)	0.32
Diabetes	184 (23.7 %)	210 (27.0 %)	205 (26.4 %)	0.27
Total cholesterol (mmol/L)	4.68 ± 1.11	4.50 ± 1.04	4.38 ± 1.11	<0.0001
LDL cholesterol (mmol/L)	2.78 ± 0.91	2.56 ± 0.83	2.37 ± 0.88	<0.0001
HDL cholesterol (mmol/L)	1.17 ± 0.39	1.19 ± 0.42	1.13 ± 0.39	0.019
Non-HDL cholesterol (mmol/L)	3.50 ± 1.09	3.31 ± 1.02	3.25 ± 1.12	<0.0001
Triglycerides (mmol/L)	1.60 ± 0.83	1.65 ± 0.92	1.93 ± 1.33	<0.0001
Apolipoprotein B (mg/dL)	83.5 ± 24.1	81.3 ± 23.7	79.3 ± 24.6	0.003
Fasting glucose (mmol/L)	5.51 ± 1.93	5.67 ± 2.10	5.51 ± 1.95	0.21
High sensitivity C-reactive protein (mg/dL)	0.48 (0.20 - 1.56)	0.53 (0.22 - 1.43)	0.51 (0.21 - 1.31)	0.75
Troponin I (pg/mL)	18.9 (10.9 - 34.9)	17.3 (10.1 - 33.5)	16.4 (9.6 - 29.1)	0.019
Galectin 3 (ng/mL)	71.2 ± 25.6	68.4 ± 24.0	68.0 ± 24.2	0.020
Brain natriuretic peptide (pg/mL)	136 (46 - 370)	108 (39 - 320)	104 (30 - 242)	<0.0001

Data are shown as mean ± standard deviation, median (inter-quartile range) or frequency (percentage).

^a p-values for means data were calculated with the use of ANalysis Of VAriance test, for medians with the use of Kruskal-Wallis test, and for percentages with the use of Fisher's exact test. HDL high density lipoprotein; LDL low density lipoprotein.

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Table 4

Title: Association of the cholestanol and lathosterol to cholesterol ratios with cardiovascular death and interactions with treatment

		Model 1 ^a		Model 2 ^b		Model 3 ^c		
	N _{CV death} /N (%)	HR (CI 95 %)	p-value	HR (CI 95 %)	p-value	HR (CI 95 %)	p-value	p-value for interaction ^a
Lathosterol/Cholesterol			0.12		0.085		0.064	0.075
First tertile (ref.)	213/778 (27.4%)	1.00	-	1.00	-	1.00	-	
Second tertile	200/777 (25.7%)	0.92 (0.76 - 1.11)	0.38	0.89 (0.73 - 1.08)	0.24	0.88 (0.72 - 1.07)	0.20	
Third tertile	182/777 (23.4%)	0.81 (0.67 - 0.99)	0.041	0.80 (0.65 - 0.97)	0.027	0.78 (0.64 - 0.96)	0.019	
Cholestanol/Cholesterol			0.005		0.008		0.083	0.45
First tertile (ref.)	183/778 (23.5%)	1.00	-	1.00	-	1.00	-	
Second tertile	189/777 (24.3%)	1.06 (0.87 - 1.30)	0.55	1.05 (0.86 - 1.29)	0.64	1.06 (0.86 - 1.30)	0.60	
Third tertile	223/777 (28.7%)	1.36 (1.11 - 1.65)	0.002	1.33 (1.09 - 1.62)	0.004	1.24 (1.02 - 1.52)	0.034	

^a unadjusted.

^b adjusted for age and sex.

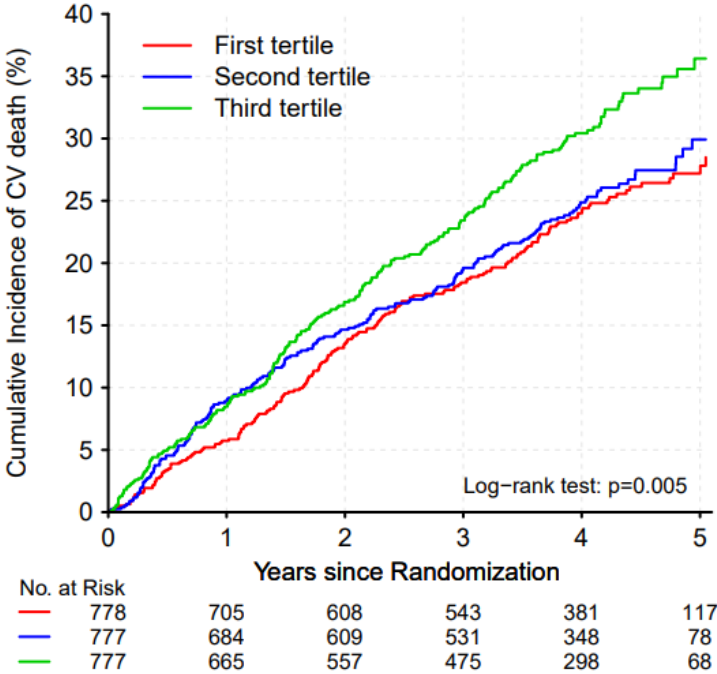
^c adjusted for age, sex, body mass index, systemic hypertension, diabetes, smoking, log high sensitivity C-reactive protein, and treatment group (rosuvastatin vs placebo).

N_{CV death} number of cardiovascular deaths; N total number; HR hazard ratio; CI confidence interval; ref. reference

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Central figure

Title: High cholesterol absorption as reflected by the cholestanol to cholesterol ratio is associated with increased cardiovascular risk in hemodialysis patients



CV cardiovascular

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Supplemental Data File (.doc, .tif, pdf, etc.)

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