The official journal of the Japan Atherosclerosis Society and
the Asian Pacific Society of Atherosclerosis and Vascular Diseases

Original Article

J Atheroscler Thromb, 2022; 29: 1835-1848. http://doi.org/10.5551/jat.63311

Association of Kidney Function with Serum Levels of Cholesterol Absorption and Synthesis Markers: The CACHE Study CKD Analysis

Tetsuo Shoji[1, 2], Yusuke Akiyama[3], Hisako Fujii[4], Mariko Harada-Shiba[5], Yutaka Ishibashi[6, 7], Tatsuro Ishida[8], Yasushi Ishigaki[9], Daijiro Kabata[10], Yasuki Kihara[11], Kazuhiko Kotani[12], Satoshi Kurisu[11], Daisaku Masuda[13], Tetsuya Matoba[14], Kota Matsuki[5, 15], Takeshi Matsumura[16], Kenta Mori[17], Tomoko Nakagami[18], Masamitsu Nakazato[19], Satsuki Taniuchi[10], Hiroaki Ueno[19], Shizuya Yamashita[13], Hisako Yoshida[10], and Hiroshi Yoshida[20]

[1]Department of Vascular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan
[2]Vascular Science Center for Translational Research, Osaka City University Graduate School of Medicine, Osaka, Japan
[3]Division of Cardiovascular Medicine, Oita Prefectural Hospital, Oita, Japan
[4]Department of Health and Medical Innovation, Osaka City University Graduate School of Medicine, Osaka, Japan
[5]Department of Molecular Innovation in Lipidology, National Cerebral and Cardiovascular Center Research Institute, Suita, Japan
[6]Department of General Medicine, Shimane University Faculty of Medicine, Izumo, Japan
[7]Jinjukai Education & Training Center for Healthcare Professionals, Shimane, Japan
[8]Division of Cardiovascular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan
[9]Division of Diabetes, Metabolism and Endocrinology, Department of Internal Medicine, Iwate Medical University, Iwate, Japan
[10]Department of Medical Statistics, Osaka City University Graduate School of Medicine, Osaka, Japan
[11]Department of Cardiovascular Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[12]Division of Community and Family Medicine, Jichi Medical University, Shimotsuke-City, Japan
[13]Rinku General Medical Center, Izumisano, Osaka, Japan
[14]Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[15]Department of Endocrinology and Metabolism, Hirosaki University Graduate School of Medicine, Hirosaki, Aomori, Japan
[16]Department of Metabolic Medicine, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan
[17]Department of General Internal Medicine, Kobe University Hospital, Kobe, Japan
[18]Division of Diabetology and Metabolism, Department of Internal Medicine, Tokyo Women's Medical University School of Medicine, Tokyo, Japan
[19]Division of Neurology, Respirology, Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan
[20]Department of Laboratory Medicine, The Jikei University Kashiwa Hospital, Kashiwa, Chiba, Japan

Aim: Serum levels of cholesterol absorption and synthesis markers are known to be associated with cardiovascular risk. Individuals with reduced kidney function or chronic kidney disease (CKD) are at an increased risk for cardiovascular disease. Hence, we examined the relationship between estimated glomerular filtration rate (eGFR) and serum markers of cholesterol absorption and synthesis.

Methods: The CACHE (Cholesterol Absorption and Cholesterol synthesis in High-risk patiEnts) Consortium, comprised of 13 research groups in Japan possessing data of lathosterol (Latho, synthesis marker) and campesterol (Campe, absorption marker) measured via gas chromatography, compiled the clinical data using the REDCap system. Among the 3597 records, data from 2944 individuals were utilized for five analyses including this CKD analysis.

Results: This study analyzed data from 2200 individuals including 522 hemodialysis patients; 42.3% were female, the median age was 58 years, and the median eGFR was 68.9 mL/min/1.73 m^2. Latho, Campe, and Campe/Latho ratio were significantly different when compared across CKD stages. When the associations of eGFR with these markers were assessed with multivariable nonlinear regression models, Latho, Campe, and Campe/Latho ratio showed positive, inverse, and inverse associations with eGFR. These associations were significantly modified by sex, the presence/absence of diabetes mellitus, and the presence/absence of statin use.

Conclusion: We showed that individuals with lower eGFR have lower cholesterol synthesis marker levels and higher cholesterol absorption marker levels in this large sample.

Key words: Lathosterol, Campesterol, Cholesterol metabolism, Kidney function, Chronic kidney disease

Introduction

Patients with reduced kidney function or chronic kidney disease (CKD) is at an elevated risk for cardiovascular disease (CVD). The risk of incidence of CVD is higher in those with lower estimated glomerular filtration (eGFR)[1]. Among patients with kidney failure treated with hemodialysis, the risk of death from CVD is 10–30 times higher than those with the general population[2]. The increased CVD risk in CKD has been attributed not only to traditional risk factors including hypertension, diabetes mellitus, and dyslipidemia but also to nontraditional risk factors, which include renal anemia, bone and mineral disorder, protein–energy wasting, inflammation, oxidative stress, and uremic toxins[3, 4].

Apart from serum lipids and lipoprotein levels, alterations in cholesterol metabolism may affect the risk of CVD. Dietary cholesterol is absorbed from the intestine, and the key intestinal cholesterol transporter is Nieman-Pick C1-like protein 1 (NPC1L1)[5]. Ezetimibe is a selective inhibitor of the NPC1L1-mediated cholesterol absorption and lowers plasma concentration of cholesterol[5]. Cholesterol absorption can be assessed by measuring the serum level of plant sterols such as campesterol (Campe)[6], which is not synthesized in humans but absorbed via the NPC1L1 in the intestine. Besides dietary cholesterol intake, cholesterol is synthesized mainly by the liver, and 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme in cholesterol biosynthesis. Statins selectively inhibit this enzyme and reduce cholesterol in plasma[7]. Cholesterol synthesis can be evaluated by measuring serum levels of the precursors of cholesterol such as lathosterol (Latho)[6]. Previous studies showed that high cholesterol absorbers had higher risks for all-cause death and cardiovascular death in a cohort of home-dwelling elderly individuals[8]. Similarly, in a cohort of patients with kidney failure undergoing hemodialysis, high cholesterol absorbers had a higher risk for all-cause mortality[9]. Importantly, these patients on hemodialysis had increased levels of cholesterol absorption markers and decreased levels of cholesterol synthesis markers[9, 10]. In a post hoc analysis of a randomized controlled trial with atorvastatin, the effect of atorvastatin on cardiovascular outcomes was modified by the baseline level of cholesterol absorption[11]. Thus, altered cholesterol metabolism may be one of the nontraditional risk factors that could affect not only prognosis but also the effectiveness of lipid-lowering medications to reduce cardiovascular risk in patients with low kidney function.

Thus far, little is known regarding the possible changes in cholesterol metabolism among patients with CKD not treated with dialysis. Sonoda *et al* [12] showed in patients with 146 patients with type 2 diabetes mellitus without taking lipid-lowering medications that a marker of cholesterol synthesis (Latho) was positively associated with eGFR and that a marker of cholesterol absorption (Campe) was inversely associated with eGFR. Campe/Latho ratio showed an inverse association with eGFR in their study. However, since the above-mentioned study by Sonoda *et al* [12] included only patients with diabetes mellitus, the associations between eGFR and cholesterol metabolism markers may be different in the absence of diabetes mellitus. Also, since healthy men and women have different levels of serum markers of cholesterol metabolism[13], sex-related differences should be considered in the relationship between kidney function and cholesterol metabolism. Additionally, many patients are treated with statin and other lipid-lowering medications that could affect cholesterol metabolism. Thus, to establish the association between eGFR and cholesterol metabolism markers, it is important to handle these factors as potential effect modifiers in the analysis of associations between eGFR and cholesterol metabolism markers.

Aim

In the present study, we addressed the following two research questions. First, is eGFR associated with cholesterol metabolism biomarkers in a wide range of kidney function from those with normal kidney function to patients with kidney failure needing hemodialysis? Second, are the associations between eGFR and cholesterol metabolism biomarkers modified by age, the presence of diabetes mellitus, and the use of statin medication?

Methods

Ethical Consideration

This study adhered to the latest version of the

Address for correspondence: Tetsuo Shoji, Department of Vascular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan, Vascular Science Center for Translational Research Center, Osaka City University Graduate School of Medicine, Osaka, Japan E-mail: t-shoji@med.osaka-cu.ac.jp

Received: October 10, 2021 Accepted for publication: January 6, 2022

Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects by the Ministry of Health, Labor and Welfare and Ministry of Education, Japan (the original version in 2016 which was modified in 2017). The study protocol was reviewed and approved by the Ethics Committee, Osaka City University Graduate School of Medicine, Osaka, Japan (Approval No. 3871), and was registered at UMIN-CTR (UMIN000030635). Also, the protocol of this study was approved by the review board of each participating institution prior to the study.

Clinical Data Collection

Thirteen research groups in Japan that possessed data of serum markers of cholesterol metabolism made up the CACHE consortium. CACHE stands for Cholesterol Absorption and Cholesterol synthesis in High-risk patiEnts. Clinical data including serum biomarkers of cholesterol metabolism were collected and compiled using the web-based system called Research Electronic Data Capture (REDCap)[14, 15] (https://projectredcap.org/about/) at Osaka City University (http://www.hosp.med.osaka-cu.ac.jp/self/hyokac/redcap/index.shtml).

Selection of the CACHE Population and Participants for this Analysis

From the total of 3597 records accumulated in the REDCap system, we selected the CACHE population for analysis by excluding (1) the second records of the same individuals and (2) participants with missing values of age, sex, or both height and weight. For this "CACHE-CKD analysis," individuals were further excluded if serum creatinine was missing.

Estimation of Kidney Function by eGFR

eGFR was calculated from age, sex, and serum creatinine using the equation for the Japanese by Matsuo *et al*[16].

Assays for Lathosterol and Campesterol Concentrations

Serum concentrations of Latho and Campe were measured as the biomarkers for cholesterol synthesis and absorption, respectively, by gas chromatography at SRL Inc., Tokyo, Japan. The procedure of gas chromatography has been described elsewhere in detail[13]. Besides concentrations of Latho and Campe, we calculated the Campe-to-Latho ratio (Campe/Latho ratio) for the assessment of the relative status of cholesterol absorption to cholesterol synthesis[12].

Other Variables

The CACHE study collected clinical data from medical records or data sets for research purpose regarding the following items: (1) clinical background including age, sex, smoking status, high-risk conditions [prior coronary artery disease (CAD), prior stroke, prior peripheral artery disease (PAD), diabetes mellitus, CKD including dialysis, and familial hypercholesterolemia], and comorbidity such as hypertension and hyperuricemia; (2) blood tests including total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose, hemoglobin A1c (HbA1c), serum creatinine, eGFR, uric acid, serum albumin, aspartate transaminase (AST), alanine transaminase (ALT), C-reactive protein, and red blood cells (RBC), hemoglobin, mean corpuscular volume (MCV), white blood cells (WBC), and platelet counts; (3) physical examination and vital signs including height, body weight, body mass index (BMI), systolic blood pressure, diastolic blood pressure, and pulse rate; (4) medication use including drugs for dyslipidemia [statin, fibrate, ezetimibe, resin, probucol, omega-3 polyunsaturated fatty acid (PUFA), nicotinic acid, Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor, and microsomal triglyceride transfer protein (MTP) inhibitor], hypertension, diabetes mellitus, and hyperuricemia; and (5) specific treatments including hemodialysis and LDL apheresis.

Diabetes mellitus was defined by either previous diagnosis of diabetes mellitus, use of any antidiabetic medication, fasting plasma glucose of 126 mg/dL or higher, or hemoglobin A1c (HbA1c) by the National Glycohemoglobin Standardization Program (NGSP) value of 6.5% or higher according to the diagnostic criteria by the American Diabetes Association and the Japan Diabetes Society (JDS)[17, 18]. If the previously used HbA1c value by the JDS was entered, it was converted to the NGSP value using a conversion formula provided by JDS[19].

Hypertension was defined either by use of any antihypertensive medication, systolic blood pressure of 140 mmHg or higher, or diastolic blood pressure of 90 mmHg or higher according to the criteria by the Japanese Society of Hypertension[20].

CKD was defined in this study by eGFR lower than 60 mL/min/1.73 m^2 using the equation for the Japanese[16]. Because the CACHE study did not collect data on proteinuria, proteinuria was not considered for the definition of CKD in this study. Patients with kidney failure treated with hemodialysis were included in patients with CKD. CKD stages were classified according to the KDIGO guideline[21]: Stage G3a if

eGFR was 45–59.9 mL/min/1.73 m^2, Stage G3b if eGFR was 30–44.9 mL/min/1.73 m^2, Stage G4 if eGFR was 15–29.9 mL/min/1.73 m^2, Stage G5ND if eGFR was <15 mL/min/1.73 m^2 and not on dialysis treatment, and stage G5D if the subject was on dialysis. All patients in stage G5D in this study were treated by hemodialysis. In this study, individuals with eGFR ≥ 60 mL/min/1.73 m^2 were classified as having no CKD.

Familial hypercholesterolemia was diagnosed by the criteria from Japan Atherosclerosis Society[22].

Regarding lipid parameters, we used the following rules: (1) TG and HDL-C values were used as entered. (2) Using total cholesterol (TC), TG, and HDL-C, non-HDL-C was calculated by subtracting HDL-C from TC, and low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula[23]. (3) If LDL-C measured by a homogenous assay was entered but TC was not available, the LDL-C by a homogenous assay was used for analysis, and non-HDL-C was calculated as LDL-C plus TG/5. (4) If LDL-C or non-HDL-C cannot be calculated because of a missing value of TG or HDL-C, it was handled as missing. In the CACHE-CKD analysis, we presented data of TG, HDL-C, non-HDL-C, and LDL-C thus determined.

Statistical Analysis

The clinical characteristics were presented by CKD stages for the participants selected for this analysis. Continuous and categorical variables were summarized by medians (interquartile ranges, IQR) and numbers (percentages), respectively, and compared via the Kruskal–Wallis test or Fisher's exact test, respectively.

The associations between CKD stages and the serum markers for cholesterol metabolism were examined using linear regression models without and with covariates' adjustment. Spearman's rank correlation analysis showed significant associations between serum lipids and the markers of cholesterol metabolism **(Supplemental Table 1)**. Then, the adjustment was made for non-HDL-C, HDL-C, and TG, besides age, sex, presence of any CVD, diabetes mellitus, hypertension, smoking status, BMI, use of statin, use of fibrates, and use of other lipid-lowering medications.

The associations of eGFR (exposure) with serum markers of cholesterol metabolism (outcomes) were examined using nonlinear regression models, which consider the restricted cubic spline term for eGFR with three knots (10th, 50th, and 90th percentile levels). The rms function was implemented in the rms and Hmisc packages in R. To meet the normal assumption of the regression model, we logarithmically transformed the objective variables and then used them in the regression models. In the above regression models, all missing values were complemented through the multiple imputation methods on the basis of the predictive mean matching approach.

Moreover, to examine whether the associations differ depending on the patients' characteristics, we performed similar analyses considering a cross-product term between eGFR and each candidate, separately.

All statistical inferences were conducted with a two-sided 5% significance level using R software version 4.0.3 (https://cran.r-project.org/).

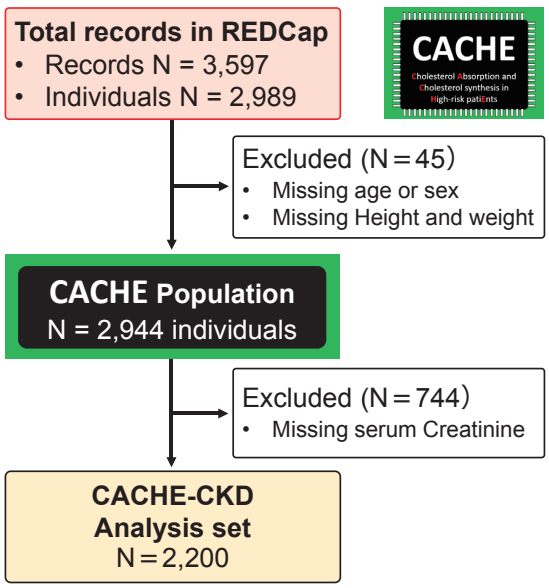


Fig. 1. Selection of participants for this CACHE-CKD analysis

The initial dataset contained 3597 records from 2989 independent individuals. We excluded the second records from the same individuals. We further excluded 45 individuals who had missing values of age, sex, and/or height and body weight. The remaining 2944 were defined as the CACHE population for further analyses including this CACHE-CKD analysis. We excluded 447 individuals with missing values of serum creatinine, and the remaining 2200 subjects were analyzed in this CACHE-CKD analysis.

Results

Selection of Participants for this Analysis

Fig. 1 shows the selection of participants for this CACHE-CKD analysis. We collected 3597 records for 2989 individuals, and the repeated records were not used. By excluding 45 subjects with missing data on age, sex, or both height and weight, the CACHE population (N = 2944) was determined. For the purpose of this CACHE-CKD analysis, 744 subjects were further excluded because of a missing value of

Table 1. Characteristics of participants of this analysis

Variables	Unit	Total subjects	Missing	CKD stages						*P* value
				No CKD	CKD-G3a	CKD-G3b	CKD-G4	CKD-G5ND	CKD-G5D	
Number of subjects	---	2200	---	1379	203	56	18	22	522	---
Serum creatinine	mg/dL	0.84 (0.66–2.38)	0 (0.0%)	0.70 (0.60–0.83)	1.02 (0.85– 1.10)	1.29 (1.14– 1.40)	2.00 (1.83–2.40)	6.60 (4.44– 7.63)	11.55 (9.87– 13.45)	<0.001
eGFR	mL/min/ 1.73m^2	68.9 (20.4–82.7)	0 (0.0%)	78.5 (70.5– 90.8)	55.5 (51.5– 57.9)	41.6 (37.1– 43.2)	23.2 (18.6– 27.2)	7.6 (5.8– 10.1)	3.6 (3.2– 4.2)	<0.001
Age	years	58 (49–66)	0 (0.0%)	55 (44–63)	65 (59–73)	72 (63–78)	70 (61–77)	65 (60–72)	61 (54–68)	<0.001
Sex (female)	N (%)	931 (42.3)	0 (0.0%)	365 (46.1%)	66 (32.5%)	19 (33.9%)	8 (44.4%)	9 (70.9%)	194 (37.2%)	<0.001
Current smoking	N (%)	410 (18.7%)	5 (0.23%)	158 (11.5%)	27 (13.3%)	10 (17.9%)	1 (5.6%)	1 (4.5%)	213 (40.8%)	<0.001
BMI	kg/m^2	22.6 (20.5–24.8)	13 (0.59%)	22.7 (20.8–25.1)	23.7 (21.6–26.0)	23.4 (21.8–25.2)	24.5 (23.7–26.2)	23.4 (21.1–24.0)	21.6 (19.6–23.5)	<0.001
HDL-C	mg/dL	53 (42–65)	0 (0.0%)	57 (47–69)	49 (41–63)	42 (37–52)	39 (33– 46)	33 (26–45)	44 (36–54)	<0.001
Non-HDL-C	mg/dL	140 (112–170)	5 (0.23%)	150 (125–177)	144 (115–178)	120 (88–155)	151 (120–231)	128 (108–164)	114 (91–138)	<0.001
TG	mg/dL	101 (72–143)	0 (0.0%)	97 (70–135)	113 (86–155)	104 (79–143)	167 (102–241)	129 (94–173)	103 (72–151)	<0.001
Comorbidities										
CAD	N (%)	328 (14.9%)	0 (0.0%)	152 (11.1%)	73 (36.0%)	34 (60.7%)	7 (38.9%)	4 (18.2%)	58 (11.1%)	<0.001
Stroke	N (%)	98 (4.5%)	0 (0.0%)	16 (1.2%)	6 (3.0%)	5 (8.9%)	1 (5.6%)	0 (0.0%)	70 (13.4%)	<0.001
PAD	N (%)	83 (3.8%)	0 (0.0%)	27 (2.0%)	15 (7.4%)	12 (21.4%)	2 (11.1%)	3 (13.6%)	24 (4.6%)	<0.001
Any CVD	N (%)	431 (19.6%)	0 (0.0%)	174 (12.6%)	77 (37.9%)	34 (60.7%)	7 (38.9%)	4 (18.2%)	135 (25.9%)	<0.001
Diabetes mellitus	N (%)	588 (34.5%)	498 (22.6%)	314 (32.6%)	76 (56.3%)	39 (90.7%)	16 (88.9%)	22 (100.0%)	121 (12.2%)	<0.001
Hypertension	N (%)	1081 (49.9%)	35 (1.59%)	431 (32.0%)	132 (65.7%)	42 (75.0%)	10 (55.6%)	16 (72.7%)	450 (86.2%)	<0.001
Lipid-lowering medications										
Statin	N (%)	365 (16.6%)	0 (0.0%)	231 (16.8%)	71 (35.0%)	28 (50.0%)	4 (22.2%)	4 (18.2%)	27 (5.2%)	<0.001
Fibrate	N (%)	14 (0.6%)	0 (0.0%)	6 (0.4%)	3 (1.5%)	1 (1.8%)	1 (5.6%)	0 (0.0%)	3 (0.6%)	0.044
Ezetimibe	N (%)	60 (2.7%)	0 (0.0%)	54 (3.9%)	4 (2.0%)	2 (3.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001
Resins	N (%)	15 (0.7%)	0 (0.0%)	14 (1.0%)	1 (0.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.251
Probucol	N (%)	18 (0.8%)	0 (0.0%)	15 (1.1%)	2 (1.0%)	1 (1.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.253
Omega-3 PUFA	N (%)	28 (1.3%)	0 (0.0%)	15 (1.1%)	9 (4.4%)	1 (1.8)	1 (5.6%)	1 (4.5%)	1 (0.2%)	<0.001
Nicotinic acid	N (%)	5 (0.2%)	0 (0.0%)	1 (0.1%)	3 (1.5%)	1 (1.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001
PCSK9 inhibitor	N (%)	4 (0.2%)	0 (0.0%)	3 (0.2%)	0 (0.0%)	1 (1.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.093
MTP inhibitor	N (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA

The table gives medians (interquartile ranges) for continuous variables and numbers (percentages) for categorical variables.

serum creatinine. Finally, 2200 individuals were selected for this analysis.

Table 1 gives characteristics of the study subjects by CKD stages. It was noted that the numbers of subjects in CKD stage G4 (N = 18, 0.8% of total) and G5ND (N = 22, 1.0% of total) were quite limited.

Markers of Cholesterol Metabolism in CKD Stages

The levels of serum Latho, Campe, and Campe/Latho ratio were compared among the CKD stages without adjustment **(Supplemental Fig. 1)**. When covariates' adjustment was done, Latho levels were the lowest in the CKD stage G4 and G5D, whereas Campe and Campe/Latho ratio were the highest in CKD stage G5D **(Supplemental Fig. 2)**.

Markers of Cholesterol Metabolism as Functions of eGFR

Restricted cubic spline curves were used to show the multivariable-adjusted relationship between eGFR and serum levels of Latho, Campe, and Campe/Latho ratio in the total subjects **(Fig. 2)**. Latho showed a positive association with eGFR in the range below 60–70 mL/min/1.73 m^2, whereas the association was not apparent in eGFR higher than this level. Campesterol and Campe/Latho showed inverse associations with eGFR, although these associations were less clear in eGFR ranges higher than 60–70 mL/min/1.73 m^2.

Effect Modification by Diabetes Mellitus

Fig. 3 shows the results stratified by the presence of diabetes mellitus. Latho was positively associated with eGFR in patients with diabetes mellitus, whereas Latho showed an inverted U-shaped association with eGFR in individuals without diabetes mellitus. The effect modification by diabetes mellitus was significant ($P = 0.010$). Diabetes mellitus was associated with

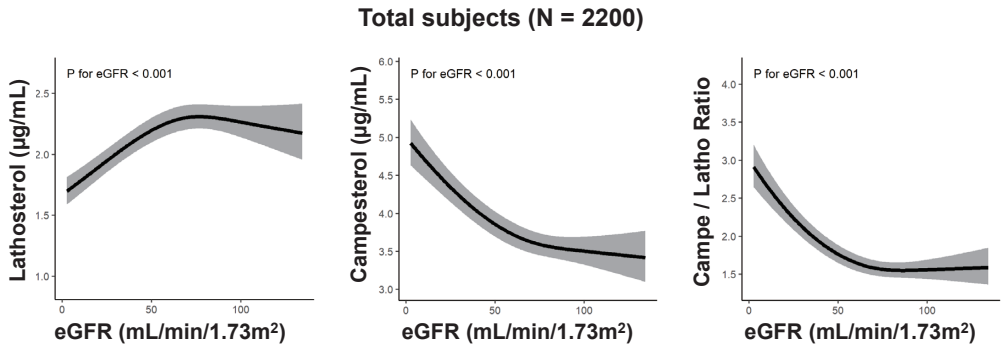


Fig. 2. Markers of cholesterol metabolism as functions of eGFR

The associations of eGFR with serum levels of Latho, Campe, and Campe/Latho ratio were analyzed with a multivariable-adjusted nonlinear regression model in the total subjects (N = 2200). The curves and shaded areas indicate means and 95% confidence intervals. The adjustment was done for age, sex, presence of any cardiovascular disease, diabetes mellitus, hypertension, smoking status, body mass index, non-high-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, use of statin, use of fibrates, and use of other lipid-lowering medications.

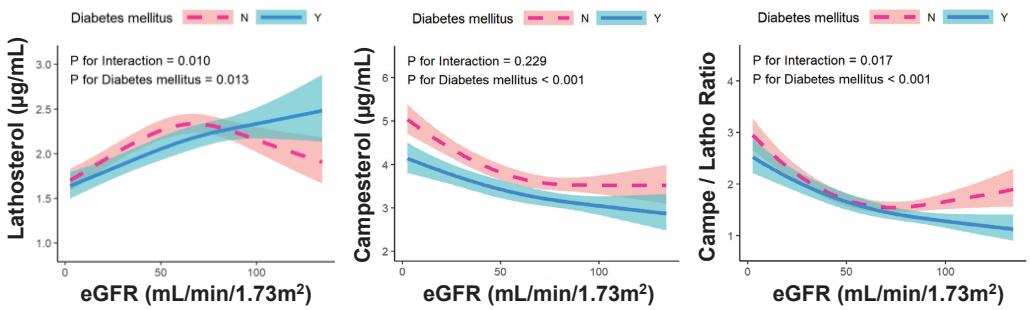


Fig. 3. Effect modification by the presence of diabetes mellitus

The association between eGFR and each marker of cholesterol metabolism was analyzed in the subgroups with and without diabetes mellitus, and the effect modification of the presence of diabetes mellitus was evaluated. The curves and shaded areas indicate means and 95% confidence intervals.

higher Latho levels in those with eGFR was higher than 80–90 mL/min/1.73 m^2, whereas diabetes mellitus was associated with similar or lower Latho levels in the eGFR range below this level.

Patients with diabetes mellitus showed lower Campe levels than those without diabetes mellitus ($P < 0.001$), although the association between eGFR and Campe was not significantly modified by the presence of diabetes mellitus ($P = 0.229$).

The Campe-to-Latho ratio was generally lower in patients with diabetes mellitus ($P < 0.001$). The Campe/Latho ratio showed an inverse association with eGFR in patients with diabetes mellitus, whereas such an inverse association was not apparent in individuals without diabetes mellitus having eGFR higher than 60–70 mL/min/1.73 m^2. The effect modification by the presence of diabetes mellitus was significant ($P = 0.017$).

Effect Modification by Sex

Fig. 4 presents the results stratified by sex. Women had lower levels of Latho than men ($P < 0.001$). Although both men and women showed a positive association between eGFR and Latho in the eGFR range below 60–70 mL/min/1.73 m^2, such an association was not apparent in the eGFR range higher than this level. The association between eGFR and Latho was modified by sex ($P = 0.038$).

Men had higher levels of Campe than women in the eGFR range between 20–30 and 100–110 mL/min/1.73 m^2, whereas the difference was less apparent outside this eGFR range ($P < 0.001$). Although the inverse association between eGFR and Campe was seen in both men and women, the association was modified by sex ($P = 0.016$).

Women had higher levels of Campe/Latho ratio regardless of eGFR ($p < 0.001$). Campe/Latho ratio showed an inverse association with eGFR, and this

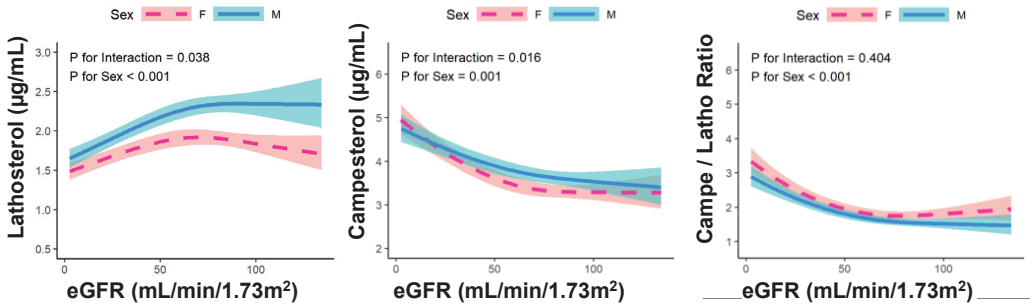


Fig. 4. Effect modification by sex

The association between eGFR and each marker of cholesterol metabolism was analyzed in the subgroups of men and women, and the effect modification of sex was evaluated. The curves and shaded areas indicate means and 95% confidence intervals.

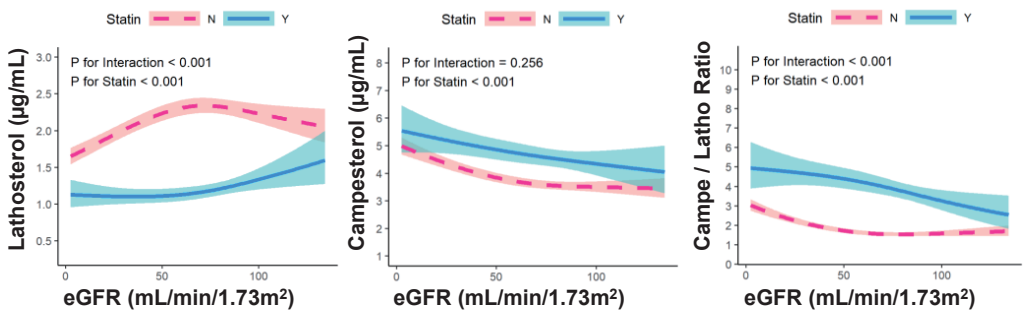


Fig. 5. Effect modification by the use of statin

The association between eGFR and each marker of cholesterol metabolism was analyzed in the subgroups with and without the use of statin, and the effect modification of the use of statin was evaluated. The curves and shaded areas indicate means and 95% confidence intervals.

association was not significantly modified by sex ($P=0.404$).

Effect Modifications by the Use of Statin

Fig. 5 presents the results stratified by the use of statin. The use of statin was associated with significantly lower levels of Latho ($P<0.001$). The individuals without statin use showed an inverted U-shaped association between eGFR and Latho, and a positive association was noticed in the eGFR ranges below 70–80 mL/min/1.73 m^2. By contrast, the association between eGFR and Latho was less remarkable in statin users, showing a significant effect modification by the use of statin ($P<0.001$).

Statin users showed higher levels of Campe ($P<0.001$). The inverse association between eGFR and Campe was found regardless of the use of statin, and the effect modification by the statin use was not significant ($P=0.256$).

Campe/Latho ratio was higher in statin users ($P<0.001$). An inverse association between eGFR and Campe/Latho ratio can be seen in statin users, whereas it was less apparent in individuals without statin use with eGFR higher than 60 mL/min/1.73 m^2. The effect modification by the use of statin was significant ($P<0.001$).

Additional Analysis Excluding Patients undergoing Hemodialysis

Additional analysis was done in 1678 individuals excluding those on hemodialysis. **Supplemental Fig. 3** shows the adjusted associations between eGFR and serum markers of cholesterol metabolism. Similar to the analysis in the total subjects, Latho was positively associated with eGFR in the eGFR range below 70–80 mL/min/1.73 m^2, whereas the association was inverse in the eGFR range higher than this level. The association between eGFR and Latho was significant ($P<0.001$). Unlike the results in the total subjects, the association between eGFR and Campe was not significant in the subjects excluding those on hemodialysis ($P=0.523$). Nevertheless, similar to the results in the total subjects, the inverse association between eGFR and Campe/Latho was significant ($P=0.008$), and the association was more apparent in the eGFR range below 60–70 mL/min/1.73 m^2.

Additional Analysis only in Patients undergoing Hemodialysis

Additional analysis was conducted in 522

patients undergoing hemodialysis. **Supplemental Fig. 4** shows the adjusted associations between eGFR and serum markers of cholesterol metabolism in this subgroup. Within the narrow range of eGFR (2.5–6.5 mL/min/1.73 m^2), both Latho and Campe were inversely associated with eGFR ($P<0.001$ and $P=0.034$, respectively), whereas the association between eGFR and Campe/Latho ratio was not significant ($P=0.109$).

Additional Analysis in Female Subjects Stratified by Age Category

The associations between eGFR and cholesterol metabolism markers were additionally analyzed only in female subjects stratified by age category (50 years or younger vs. over 50 years old). **Supplemental Fig. 5** shows that the younger age group showed lower Latho levels, higher Campe levels, and higher Campe/Latho ratios than the older counterpart in a wide range of eGFR, particularly in eGFR >60 mL/min/1.73 m^2. Also, we noted a marginally significant effect modification by age category on Latho.

Discussion

Using the relatively large sample of the CACHE, this study showed that eGFR was associated positively with Latho, inversely with Campe and Campe/Latho ratio in individuals with various kidney functions. Also, this study showed that some of these associations were modified by the presence of diabetes mellitus, sex, and the use of statin.

We confirmed previous results that the level of cholesterol absorption marker was elevated in patients with lower kidney function including those on hemodialysis[9, 10, 12]. The underlying mechanism for this association is unclear, but some explanations are possible. Intestinal cholesterol absorption is mediated by the function of NPC1L1 [24]. Transcription of NPC1L1 is suppressed by the action of a nuclear receptor peroxisome proliferator-activated receptor α (PPARα)[25]. PPARα activation by fenofibrate was shown to suppress intestinal transcription of NPC1L1 mRNA and fractional absorption of cholesterol in mice[25]. Pemafibrate is also known to activate PPARα and suppress NPC1L1 mRNA in mice[26]. Chronic renal failure was shown to reduce PPARα mRNA level in a rat model[27]. The role of PPARα in cholesterol synthesis is much less clear than that in cholesterol absorption[28]. Taken together, suppressed PPARα and subsequent increase in NPC1L1 is likely to explain the increased level of Campe in patients with decreased kidney function.

This study also confirmed previous results that the serum level of cholesterol synthesis marker was decreased in patients with lower kidney function including those on hemodialysis[9, 10, 12]. HMG-CoA reductase is the key enzyme in the biosynthesis of cholesterol, and it is upregulated by activation of sterol regulatory element-binding protein 2 (SREBP2) upon cell cholesterol depletion[29]. Inflammation and protein–energy wasting may contribute to it. Serum cholesterol level is known to decrease in these conditions, and it is included as one of the diagnostic criteria of protein–energy wasting[30]. A dietary weight loss intervention resulted in decreased Latho and increased Campe concentrations in moderately obese men[31]. Although a reduced availability of source nutrients for cholesterol biosynthesis may explain the decreased cholesterol synthesis, the precise mechanisms for it in patients with low kidney function are unknown at present.

A recent study by Zhou *et al* [32] revealed that hyperphosphatemia and excessive cellular uptake of phosphate can result in increased α-mannosidase II activity, increased SREBP cleavage activating protein (SCAP), overactivation of SERBP2, increased HMG-CoA reductase, and robust increase in *de novo* cholesterol synthesis. The experiments by Zhou *et al* used mouse aorta and primary human aortic smooth muscle cells, demonstrating that hyperphosphatemia could promote atherosclerotic vascular changes[32]. If similar changes occur in the intestine and the liver, overactivation of SREBP2 could increase the transcription of *NPC1L1* leading to an increased cholesterol absorption in patients with low kidney function and hyperphosphatemia. Nevertheless, the sequence of events starting from hyperphosphatemia does not explain the reduced level of serum Latho in patients with low eGFR. Presumably, the above-mentioned mechanism found in vascular smooth muscle cells does not play an important role, if any, in the cholesterol homeostasis in the liver.

This study revealed that the presence of diabetes mellitus modifies the associations of eGFR with Latho and Campe/Latho ratio. The association between eGFR and Latho was positive in patients with diabetes mellitus, whereas it was inverted U-shaped in individuals without diabetes mellitus. Also, the inverse association between eGFR and Campe/Latho ratio was more apparent in patients with diabetes mellitus. These findings in patients with diabetes mellitus are in line with the results by Sonoda *et al* [12] who analyzed only patients with type 2 diabetes mellitus. Nonetheless, a recent study by Emrich *et al* [33] reported that there was no significant association between CKD stages and Campe/Latho ratio in 251 patients with CKD not on dialysis. The majority (67.4%) of the

study subjects of Emrich *et al* [33] did not have diabetes mellitus. Thus, the discrepancy between studies may be explained by the effect modification by the presence of diabetes mellitus.

Our results indicated the sex-related difference in serum Latho and Campe in individuals with a wide range of kidney functions and comorbidities. The higher levels of Latho in men in our study agree with a recent report by Yoshida *et al* in healthy Japanese subjects [13] and the report by Dayspring *et al* from the United States [34], whereas Matthan *et al* [35] reported that Latho levels were comparable between men and women in the participants of Framingham Offspring Study. The positive effect of estradiol on cholesterol synthesis in human hepatoma cells (HepG2) does not fit the clinical observations [36], and the actual roles of sex steroids on cholesterol synthesis are largely unknown.

Regarding sex-related differences in cholesterol absorption, we found that male sex was associated with higher levels of Campe. This agrees with the result by Matthan *et al* [35] but is contrary to the above-mentioned studies by Yoshida *et al* [13] and Dayspring *et al* [34]. Dayspring *et al* showed that Campe levels were higher in females than males in individuals who were 50 years old or older, and they confirmed the higher cholesterol absorption using other markers such as serum sitosterol and cholestanol. Although we do not know the reason for the discrepancy among studies, differences in statistical methods may explain it. Our results were adjusted for many potential confounders including BMI and statin use, and Matthan *et al* made an adjustment for BMI, whereas the other two studies took no statistical adjustment. Additionally, menopause or estrogen status may be an important factor in female subjects. In our study, when we analyzed the female subjects stratified by age category (50 years or younger vs. over 50 years old), the younger group of female subjects had a lower Latho level and a higher Campe level than the older counterpart in a wide range of eGFR, particularly in eGFR >60 mL/min/1.73 m^2 **(Supplemental Fig. 5)**. Thus, the consideration of menopause or estrogen status in female subjects may explain the discrepancy among studies.

It was an expected result that statin use was associated with a lower Latho level and a higher Campe level because statin inhibits HMG-CoA reductase and Latho was downstream of this step. Also, intestinal cholesterol absorption was known to increase by statin [37]. What is the mechanism for the increased Campe level associated with the use of statin? Inhibition of HMG-CoA reductase activity by statin results in decreased intracellular cholesterol content, which stimulates SREBP2 to upregulate transcription of the HMG-CoA R gene on the one hand. On the other hand, SREBP2 activation also upregulates transcription of the NPC1L1 gene, increases the intestinal transport of sterols via NPC1L1, and increases the serum level of Campe. Thus, the so-called "compensatory increase" in cholesterol absorption during statin use can be explained by the activation of SREBP2.

In the additional analysis only in patients undergoing hemodialysis, eGFR was inversely associated with Latho level within a narrow eGFR range. This was in contrast to the positive association in individuals not on dialysis treatment in a wide range of eGFR below 60–70 mL/min/1.73 m^2. Since kidney function is almost completely lost in patients requiring hemodialysis, a variation in eGFR of the dialysis population reflects a variation of creatinine generation from muscle. Hence, careful consideration is needed in the interpretation of the association between eGFR and Latho and other markers of cholesterol metabolism in patients who need dialysis treatment. A higher serum creatinine level is associated with higher muscle strength and lean body mass [38] and lower risk of death among patients on hemodialysis [39]. Therefore, a higher serum Latho in hemodialysis patients with a higher serum creatinine concentration may be explained by a better nutritional condition.

This study has several limitations. First, although this analysis included 2200 individuals having various levels of kidney function, we had only a small number of patients in CKD stages G4 and G5ND. Thus, the confidence interval was larger in a lower eGFR range, and the association of eGFR with Latho was not statistically significant in the subgroup analysis excluding patients on hemodialysis. Further studies are needed in patients with advanced stages of CKD, not on dialysis. Second, since the information was lacking in the CACHE dataset, we could not assess the possible influence of proteinuria on the cholesterol metabolism markers. Third, we used creatinine-based eGFR for the estimation of kidney function. Since serum creatinine is affected by muscle mass regardless of dialysis treatment, careful interpretation of eGFR is needed. The relative influence of muscle mass may be larger in subjects with lower kidney function particularly in hemodialysis patients, although the absolute influence of muscle mass may be smaller in these patients based on the very high prevalence of sarcopenia in patients on hemodialysis [40]. Cystatin C-based eGFR may be better in this regard [41]. Additionally, eGFR of patients treated with hemodialysis may be inaccurate as the measure of patient's own kidney function, because it is affected by

hemodialysis treatment. However, as shown by the median (IQR) eGFR levels of CKD stage G5ND [7.6 (5.8–10.1) mL/min/1.73 m^2] and stage G5D [3.6 (3.2–4.2) mL/min/1.73 m^2], the replacement of renal function by standard hemodialysis treatment is too small to cause misclassification of kidney function of hemodialysis patients. Hence, the use of eGFR is valid in the regression analysis, which included hemodialysis patients. Fourth, the CACHE population was made of data from the experts of cardiology, lipidology, diabetology, endocrinology, nephrology, general internal medicine, and preventive medicine. Thus, the population was heterogeneous and included patients with various comorbidities and medications. To address this issue, we performed vigorous statistical adjustments for possible confounders, and we considered effect modifications. Conversely, the relatively large sample size was one of the strengths of this study. Another strength is the continuous analysis from subjects with normal kidney function to patients with kidney failure needing dialysis.

Conclusion

This study established the association of kidney function as assessed using eGFR with serum biomarkers of synthesis and absorption of cholesterol using the relatively large sample of the CACHE study. Patients with lower kidney function had lower levels of Latho, higher levels of Campe, and higher Campe/Latho ratios. Some of the associations between eGFR and these markers were found to be modified by the presence of diabetes mellitus, sex, and the use of statins. Further studies are needed to elucidate the mechanisms behind the observed associations and to examine whether the measurements of these markers are useful in the management and care of patients with CKD.

Acknowledgements

Part of this study was presented at the 53rd Annual Meeting of the Japan Atherosclerosis Society (October 22-23, 2021, Kyoto, Hybrid version) and at the 18th International Symposium of Atherosclerosis (October 24-27, 2021, Kyoto, Hybrid version).

Funding

This study was supported by a grant to TS from Bayer Yakuhin Ltd. The funder played no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Conflict of Interest

Tetsuo Shoji reported personal fee and research grant from Bayer Yakuhin Ltd. Tatsuro Ishida reported personal fee from Bayer Yakuhin Ltd and Kowa Inc. Yasushi Ishigaki reported personal fee from Bayer Yakuhin, Kowa Pharmaceutical Company, MSD, Novartis, Novo Nordisk, Ono Pharmaceutical, Sanofi K.K., and Takeda Pharmaceutica; research grant from Daiichi Sankyo, and Takeda Science Foundation; and Scholarship grant from MSD and Ono Pharmaceutical. Tetsuya Matoba reported personal fee from Bayer Yakuhin Ltd and MSD; and research grant from Amgen and Kowa. Tomoko Nakagami reported personal fee from Sanwa Kagaku Kenkyusho Co Ltd, Sumitomo Dainippon Pharma Co, Ltd, Novo Nordisk Pharma Ltd Japan, Eli Lily Japan KK, and Boehringer Ingelheim Japan Inc. Shizuya Yamashita reported personal fee from Kowa. Hiroshi Yoshida reported personal fee from Denka Company Ltd and Kowa Company Ltd. Other authors reported no financial conflict of interest relevant to this study.

References

1) Go AS, Chertow GM, Fan D, McCulloch CE and Hsu CY: Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med, 2004; 351: 1296-1305
2) Foley RN, Parfrey PS and Sarnak MJ: Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis, 1998; 32: S112-119
3) Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer M, Raij L, Spinosa DJ, Wilson PW, American Heart Association Councils on Kidney in Cardiovascular Disease HBPRCC, Epidemiology and Prevention: Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation, 2003; 108: 2154-2169
4) Wanner C, Amann K and Shoji T: The heart and vascular system in dialysis. Lancet, 2016; 388: 276-284
5) Altmann SW, Davis HR, Jr., Zhu LJ, Yao X, Hoos LM, Tetzloff G, Iyer SP, Maguire M, Golovko A, Zeng M, Wang L, Murgolo N and Graziano MP: Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. Science, 2004; 303: 1201-1204
6) Miettinen TA, Tilvis RS and Kesaniemi YA: Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. Am J Epidemiol, 1990; 131: 20-31

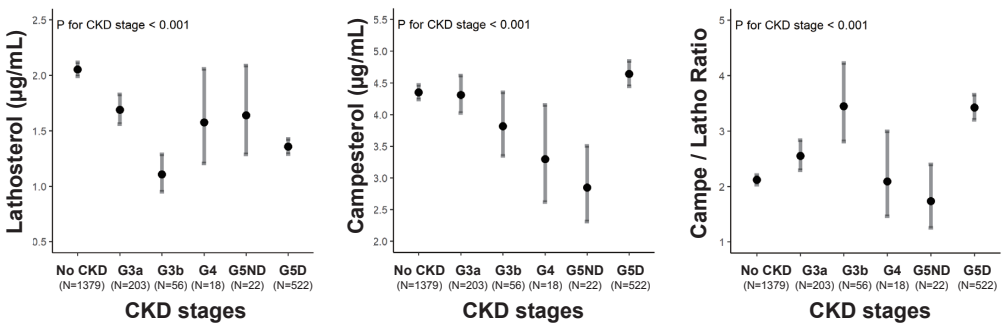
7) Brown MS and Goldstein JL: Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. J Lipid Res, 1980; 21: 505-517
8) Strandberg TE, Tilvis RS, Pitkala KH and Miettinen TA: Cholesterol and glucose metabolism and recurrent cardiovascular events among the elderly: a prospective study. J Am Coll Cardiol, 2006; 48: 708-714
9) Rogacev KS, Pinsdorf T, Weingartner O, Gerhart MK, Welzel E, van Bentum K, Popp J, Menzner A, Fliser D, Lutjohann D and Heine GH: Cholesterol synthesis, cholesterol absorption, and mortality in hemodialysis patients. Clin J Am Soc Nephrol, 2012; 7: 943-948
10) Fukushima M, Miura S, Mitsutake R, Fukushima T, Fukushima K and Saku K: Cholesterol metabolism in patients with hemodialysis in the presence or absence of coronary artery disease. Circ J, 2012; 76: 1980-1986
11) Silbernagel G, Fauler G, Genser B, Drechsler C, Krane V, Scharnagl H, Grammer TB, Baumgartner I, Ritz E, Wanner C and Marz W: Intestinal cholesterol absorption, treatment with atorvastatin, and cardiovascular risk in hemodialysis patients. J Am Coll Cardiol, 2015; 65: 2291-2298
12) Sonoda M, Shoji T, Kimoto E, Okute Y, Shima H, Naganuma T, Motoyama K, Morioka T, Mori K, Fukumoto S, Shioi A, Koyama H, Emoto M and Inaba M: Kidney function, cholesterol absorption and remnant lipoprotein accumulation in patients with diabetes mellitus. J Atheroscler Thromb, 2014; 21: 346-354
13) Yoshida H, Tada H, Ito K, Kishimoto Y, Yanai H, Okamura T, Ikewaki K, Inagaki K, Shoji T, Bujo H, Miida T, Yoshida M, Kuzuya M and Yamashita S: Reference Intervals of Serum Non-Cholesterol Sterols by Gender in Healthy Japanese Individuals. J Atheroscler Thromb, 2020; 27: 409-417
14) Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N and Conde JG: Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform, 2009; 42: 377-381
15) Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, McLeod L, Delacqua G, Delacqua F, Kirby J, Duda SN and Consortium RE: The REDCap consortium: Building an international community of software platform partners. J Biomed Inform, 2019; 95: 103208
16) Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H and Hishida A: Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis, 2009; 53: 982-992
17) American Diabetes A: Diagnosis and classification of diabetes mellitus. Diabetes Care, 2014; 37 Suppl 1: S81-90
18) Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus, Seino Y, Nanjo K, Tajima N, Kadowaki T, Kashiwagi A, Araki E, Ito C, Inagaki N, Iwamoto Y, Kasuga M, Hanafusa T, Haneda M and Ueki K: Report of the committee on the classification and diagnostic criteria of diabetes mellitus. J Diabetes Investig, 2010; 1: 212-228
19) Kashiwagi A, Kasuga M, Araki E, Oka Y, Hanafusa T, Ito H, Tominaga M, Oikawa S, Noda M, Kawamura T, Sanke T, Namba M, Hashiramoto M, Sasahara T, Nishio Y, Kuwa K, Ueki K, Takei I, Umemoto M, Murakami M, Yamakado M, Yatomi Y, Ohashi H and Committee on the Standardization of Diabetes Mellitus-Related Laboratory Testing of Japan Diabetes Society: International clinical harmonization of glycated hemoglobin in Japan: From Japan Diabetes Society to National Glycohemoglobin Standardization Program values. J Diabetes Investig, 2012; 3: 39-40
20) Shimamoto K, Ando K, Fujita T, Hasebe N, Higaki J, Horiuchi M, Imai Y, Imaizumi T, Ishimitsu T, Ito M, Ito S, Itoh H, Iwao H, Kai H, Kario K, Kashihara N, Kawano Y, Kim-Mitsuyama S, Kimura G, Kohara K, Komuro I, Kumagai H, Matsuura H, Miura K, Morishita R, Naruse M, Node K, Ohya Y, Rakugi H, Saito I, Saitoh S, Shimada K, Shimosawa T, Suzuki H, Tamura K, Tanahashi N, Tsuchihashi T, Uchiyama M, Ueda S, Umemura S and Japanese Society of Hypertension Committee for Guidelines for the Management of Hypertension: The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2014). Hypertens Res, 2014; 37: 253-390
21) Stevens PE, Levin A and Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members: Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. Ann Intern Med, 2013; 158: 825-830
22) Harada-Shiba M, Arai H, Ishigaki Y, Ishibashi S, Okamura T, Ogura M, Dobashi K, Nohara A, Bujo H, Miyauchi K, Yamashita S, Yokote K and Working Group by Japan Atherosclerosis Society for Making Guidance of Familial Hypercholesterolemia: Guidelines for Diagnosis and Treatment of Familial Hypercholesterolemia 2017. J Atheroscler Thromb, 2018; 25: 751-770
23) Friedewald WT, Levy RI and Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem, 1972; 18: 499-502
24) Davis HR, Jr., Zhu LJ, Hoos LM, Tetzloff G, Maguire M, Liu J, Yao X, Iyer SP, Lam MH, Lund EG, Detmers PA, Graziano MP and Altmann SW: Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. J Biol Chem, 2004; 279: 33586-33592
25) Valasek MA, Clarke SL and Repa JJ: Fenofibrate reduces intestinal cholesterol absorption via PPARalpha-dependent modulation of NPC1L1 expression in mouse. J Lipid Res, 2007; 48: 2725-2735
26) Sairyo M, Kobayashi T, Masuda D, Kanno K, Zhu Y, Okada T, Koseki M, Ohama T, Nishida M, Sakata Y and Yamashita S: A Novel Selective PPARalpha Modulator (SPPARMalpha), K-877 (Pemafibrate), Attenuates Postprandial Hypertriglyceridemia in Mice. J Atheroscler Thromb, 2018; 25: 142-152
27) Mori Y, Hirano T, Nagashima M, Shiraishi Y, Fukui T and Adachi M: Decreased peroxisome proliferator-activated receptor alpha gene expression is associated with dyslipidemia in a rat model of chronic renal failure.

Metabolism, 2007; 56: 1714-1718
28) Li T and Chiang JY: Regulation of bile acid and cholesterol metabolism by PPARs. PPAR Res, 2009; 2009: 501739
29) Brown MS, Radhakrishnan A and Goldstein JL: Retrospective on Cholesterol Homeostasis: The Central Role of Scap. Annu Rev Biochem, 2018; 87: 783-807
30) Fouque D, Kalantar-Zadeh K, Kopple J, Cano N, Chauveau P, Cuppari L, Franch H, Guarnieri G, Ikizler TA, Kaysen G, Lindholm B, Massy Z, Mitch W, Pineda E, Stenvinkel P, Trevino-Becerra A and Wanner C: A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. Kidney Int, 2008; 73: 391-398
31) Leichtle AB, Helmschrodt C, Ceglarek U, Shai I, Henkin Y, Schwarzfuchs D, Golan R, Gepner Y, Stampfer MJ, Bluher M, Stumvoll M, Thiery J and Fiedler GM: Effects of a 2-y dietary weight-loss intervention on cholesterol metabolism in moderately obese men. Am J Clin Nutr, 2011; 94: 1189-1195
32) Zhou C, He Q, Gan H, Zeng T, Liu Q, Moorhead JF, Varghese Z, Ouyang N and Ruan XZ: Hyperphosphatemia in chronic kidney disease exacerbates atherosclerosis via a mannosidases-mediated complex-type conversion of SCAP N-glycans. Kidney Int, 2021;
33) Emrich IE, Heine GH, Schulze PC, Rogacev KS, Fliser D, Wagenpfeil S, Bohm M, Lutjohann D and Weingartner O: Markers of cholesterol synthesis to cholesterol absorption across the spectrum of non-dialysis CKD: An observational study. Pharmacol Res Perspect, 2021; 9: e00801
34) Dayspring TD, Varvel SA, Ghaedi L, Thiselton DL, Bruton J and McConnell JP: Biomarkers of cholesterol homeostasis in a clinical laboratory database sample comprising 667,718 patients. J Clin Lipidol, 2015; 9: 807-816
35) Matthan NR, Zhu L, Pencina M, D'Agostino RB, Schaefer EJ and Lichtenstein AH: Sex-specific differences in the predictive value of cholesterol homeostasis markers and 10-year cardiovascular disease event rate in Framingham Offspring Study participants. J Am Heart Assoc, 2013; 2: e005066
36) Meng Y and Zong L: Estrogen stimulates SREBP2 expression in hepatic cell lines via an estrogen response element in the SREBP2 promoter. Cell Mol Biol Lett, 2019; 24: 65
37) Tremblay AJ, Lamarche B, Lemelin V, Hoos L, Benjannet S, Seidah NG, Davis HR, Jr. and Couture P: Atorvastatin increases intestinal expression of NPC1L1 in hyperlipidemic men. J Lipid Res, 2011; 52: 558-565
38) Noori N, Kovesdy CP, Bross R, Lee M, Oreopoulos A, Benner D, Mehrotra R, Kopple JD and Kalantar-Zadeh K: Novel equations to estimate lean body mass in maintenance hemodialysis patients. Am J Kidney Dis, 2011; 57: 130-139
39) Pupim LB, Caglar K, Hakim RM, Shyr Y and Ikizler TA: Uremic malnutrition is a predictor of death independent of inflammatory status. Kidney Int, 2004; 66: 2054-2060
40) Mori K, Nishide K, Okuno S, Shoji T, Emoto M, Tsuda A, Nakatani S, Imanishi Y, Ishimura E, Yamakawa T, Shoji S and Inaba M: Impact of diabetes on sarcopenia and mortality in patients undergoing hemodialysis. BMC Nephrol, 2019; 20: 105
41) Horio M, Imai E, Yasuda Y, Watanabe T and Matsuo S: GFR estimation using standardized serum cystatin C in Japan. Am J Kidney Dis, 2013; 61: 197-203

Supplemental Table 1. Correlation matrix between serum lipids and markers of cholesterol metabolism

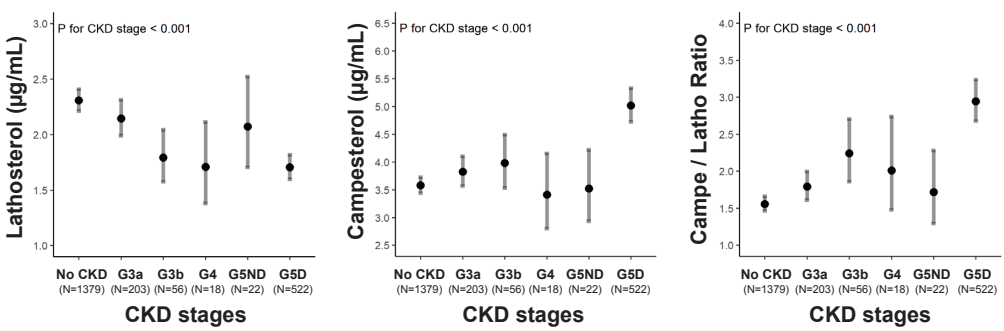
	Non-HD-C	HDL-C	TG	Lathosterol	Campesterol	Campe/Latho
Non-HDL-C	---	r = 0.066 *P* = 0.002	r = 0.362 *P* < 0.001	r = 0.485 *P* < 0.001	r = 0.222 *P* < 0.001	r = –0.243 *P* < 0.001
HDL-C	r = 0.066 *P* = 0.002	---	r = –0.429 *P* < 0.001	r = 0.138 *P* < 0.001	r = 0.315 *P* < 0.001	r = 0.096 *P* < 0.001
TG	r = 0.362 *P* < 0.001	r = –0.429 *P* < 0.001	---	r = 0.212 *P* < 0.001	r = –0.018 *P* = 0.392	r = –0.181 *P* < 0.001
Lathosterol	r = 0.485 *P* < 0.001	r = 0.138 *P* < 0.001	r = 0.212 *P* < 0.001	---	r = –0.029 *P* = 0.177	r = –0.783 *P* < 0.001
Campesterol	r = 0.222 *P* < 0.001	r = 0.315 *P* < 0.001	r = –0.018 *P* = 0.392	r = –0.029 *P* = 0.177	---	r = 0.606 *P* < 0.001
Campe/Latho	r = –0.243 *P* < 0.001	r = 0.096 *P* < 0.001	r = –0.181 *P* < 0.001	r = –0.783 *P* < 0.001	r = 0.606 *P* < 0.001	---

The table gives Spearman's correlation coefficients and *P* values in the 2200 subjects.
Abbreviation: r, Spearman's correlation coefficient.



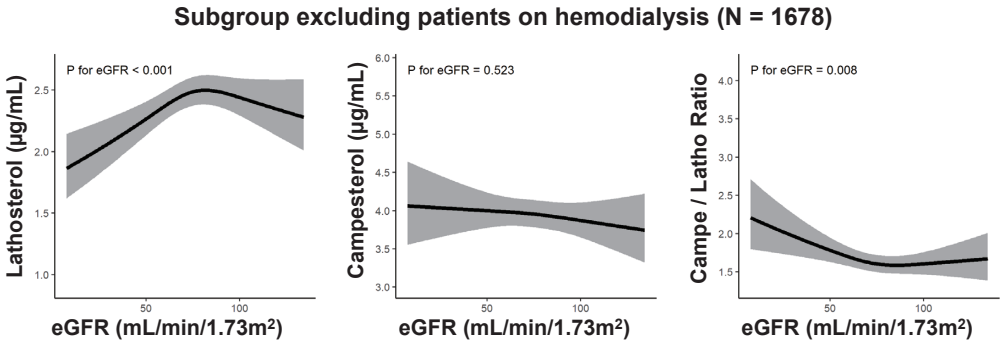
Supplemental Fig. 1. Unadjusted comparison of markers of cholesterol metabolism among CKD stages

The serum levels of lathosterol, campesterol, and campesterol/lathosterol ratio were compared among CKD stages. The points and vertical lines indicate means and 95% confidence intervals.



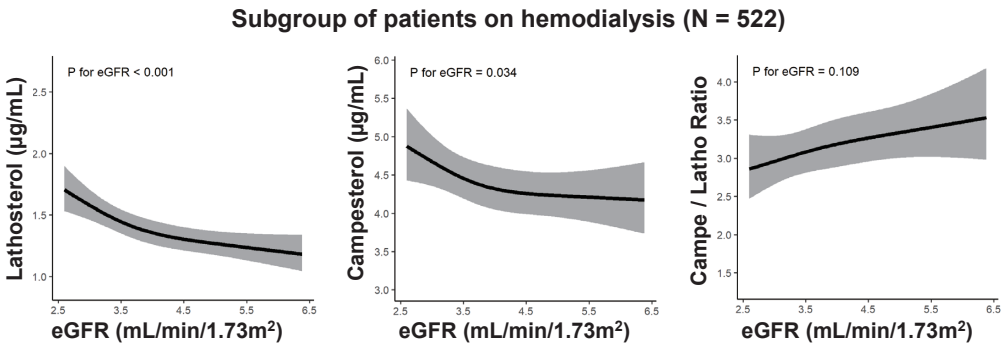
Supplemental Fig. 2. Adjusted comparison of markers of cholesterol metabolism among CKD stages

The serum levels of lathosterol, campesterol, and campesterol/lathosterol ratio were compared among CKD stages after adjustment for the same 13 factors as used for the nonlinear regression analysis shown in Figure 2. The points and vertical lines indicate means and 95% confidence intervals.



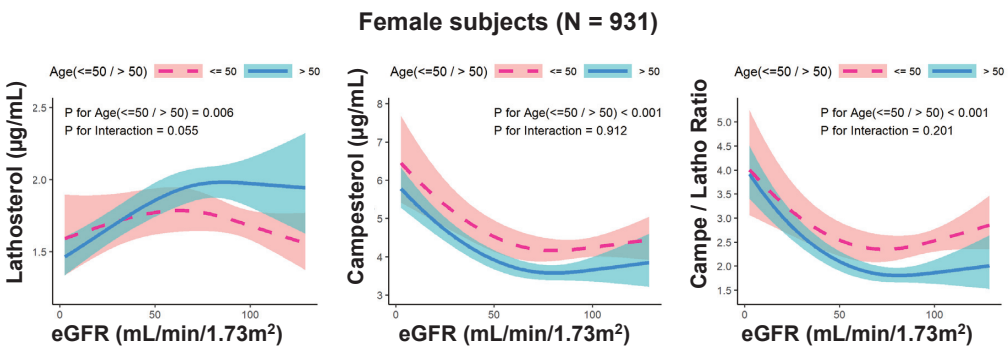
Supplemental Fig. 3. Additional analysis excluding patients undergoing hemodialysis

The association between eGFR and each marker of cholesterol metabolism was analyzed in the subgroup excluding patients undergoing hemodialysis (N = 1678). The adjustment was done for the same variables as used in the total 2200 subjects. The curves and shaded areas indicate means and 95% confidence intervals.



Supplemental Fig. 4. Additional analysis in only patients undergoing hemodialysis

The association between eGFR and each marker of cholesterol metabolism was analyzed in the subgroup including only patients undergoing hemodialysis (N = 522). The covariates' adjustment was made for the same variables as used in the total 2200 subjects. The curves and shaded areas indicate means and 95% confidence intervals. Note the very narrow range of eGFR in this subgroup.



Supplemental Fig. 5. Additional analysis in female subjects stratified by age category

The associations between eGFR and cholesterol metabolism marker were analyzed only in female subjects stratified by age category (50 years or younger vs. over 50 years old). The curves and shaded areas indicate means and 95% confidence intervals.