European Heart Journal (2015) **36**, 3007–3016
doi:10.1093/eurheartj/ehv352

CLINICAL RESEARCH
Lipids

Serum amyloid A: high-density lipoproteins interaction and cardiovascular risk

Stephen Zewinger[1], Christiane Drechsler[2], Marcus E. Kleber[3], Alexander Dressel[3], Julia Riffel[1], Sarah Triem[1], Marlene Lehmann[1], Chantal Kopecky[4], Marcus D. Säemann[4], Philipp M. Lepper[5], Günther Silbernagel[6], Hubert Scharnagl[7], Andreas Ritsch[8], Barbara Thorand[9], Tonia de las Heras Gala[9], Stefan Wagenpfeil[10], Wolfgang Koenig[11,12,13], Annette Peters[9], Ulrich Laufs[14], Christoph Wanner[2], Danilo Fliser[1], Thimoteus Speer[1†], and Winfried März[3,7,15*†]

[1]Department of Internal Medicine IV, Saarland University Medical Centre, Homburg/Saar, Germany; [2]Division of Nephrology, Department of Medicine, University of Würzburg, Würzburg, Germany; [3]Mannheim Medical Faculty, Medical Clinic V (Nephrology, Hypertensiology, Endocrinology, Diabetology, Rheumatology), University of Heidelberg, Theodor-Kutzer-Ufer 1-3, Mannheim 68167, Germany; [4]Division of Nephrology and Dialysis, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; [5]Department of Internal Medicine V, Saarland University Medical Centre, Homburg/Saar, Germany; [6]Department of Angiology, Swiss Cardiovascular Center, Inselspital Bern, Bern, Switzerland; [7]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria; [8]Department of Internal Medicine I, Innsbruck Medical University, Innsbruck, Austria; [9]Helmholtz Center Munich, German Research Center for Environmental Health, Institute of Epidemiology II, Munich, Germany; [10]Institute for Medical Biometry, Epidemiology, and Medical Informatics, Saarland University, Homburg/Saar, Germany; [11]Department of Internal Medicine II—Cardiology, University of Ulm Medical Center, Ulm, Germany; [12]Deutsches Herzzentrum München, Technische Universität München, Munich, Germany; [13]DZHK (German Centre of Cardiovascular Research), Partner Site Munich Heart Alliance, Munich, Germany; [14]Department of Internal Medicine III, Saarland University Medical Centre, Homburg/Saar, Germany; and [15]Synlab Academy, Mannheim, Germany

Received 4 February 2015; revised 7 July 2015; accepted 10 July 2015; online publish-ahead-of-print 6 August 2015

See page 3017 for the editorial comment on this article (doi:10.1093/eurheartj/ehv465)

Aims High-density lipoproteins (HDLs) are considered as anti-atherogenic. Recent experimental findings suggest that their biological properties can be modified in certain clinical conditions by accumulation of serum amyloid A (SAA). The effect of SAA on the association between HDL-cholesterol (HDL-C) and cardiovascular outcome remains unknown.

Methods and results We examined the association of SAA and HDL-C with mortality in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study, which included 3310 patients undergoing coronary angiography. To validate our findings, we analysed 1255 participants of the German Diabetes and Dialysis study (4D) and 4027 participants of the Cooperative Health Research in the Region of Augsburg (KORA) S4 study. In LURIC, SAA concentrations predicted all-cause and cardiovascular mortality. In patients with low SAA, higher HDL-C was associated with lower all-cause and cardiovascular mortality. In contrast, in patients with high SAA, higher HDL-C was associated with increased all-cause and cardiovascular mortality, indicating that SAA indeed modifies the beneficial properties of HDL. We complemented these clinical observations by *in vitro* experiments, in which SAA impaired vascular functions of HDL. We further derived a formula for the simple calculation of the amount of biologically 'effective' HDL-C based on measured HDL-C and SAA from the LURIC study. In 4D and KORA S4 studies, we found that measured HDL-C was not associated with clinical outcomes, whereas calculated 'effective' HDL-C significantly predicted better outcome.

Conclusion The acute-phase protein SAA modifies the biological effects of HDL-C in several clinical conditions. The concomitant measurement of SAA is a simple, useful, and clinically applicable surrogate for the vascular functionality of HDL.

Keywords High-density lipoprotein • Serum amyloid A • Cardiovascular risk • Mortality • Dysfunctional HDL

Introduction

Cardiovascular disease (CVD) represents the major cause of death in Western populations. In Europe, more than four million deaths were caused by clinical complications of atherosclerosis such as myocardial infarction or stroke in 2000.[1] A variety of risk factors including arterial hypertension, smoking, dyslipidaemia, obesity,

* Corresponding author. Tel: +49 621 383 5172, Fax: +49 621 383 3804, Email: winfried.maerz@medma.uni-heidelberg.de

† Both authors contributed equally.

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2015. For permissions please email: journals.permissions@oup.com.

Downloaded from http://eurheartj.oxfordjournals.org/ by guest on April 14, 2016

source: https://d

diabetes mellitus, and chronic kidney disease (CKD) contribute to the development of atherosclerotic vascular lesions.[1–3]

In general, high-density lipoproteins (HDLs) are considered anti-atherogenic.[4,5] HDLs from healthy individuals have been shown to stimulate endothelial nitric oxide (NO) production, to reduce endothelial reactive oxygen species (ROS) production, and to prevent pro-inflammatory responses of the endothelium. *In vivo* administration of reconstituted HDL to hypercholesterolaemic men has been demonstrated to improve endothelial function.[6] Accordingly, observational trials have linked high concentrations of HDL-cholesterol (HDL-C) to improve the cardiovascular outcome.[7]

We and others have previously shown that HDLs may lose their vasoprotective properties in disease conditions such as diabetes mellitus, coronary artery disease (CAD), and CKD.[8–12] Studies examining the protein composition of HDL found that the acute-phase protein serum amyloid A (SAA) may incorporate into the HDL particle in disease conditions such as CAD or CKD.[13–16] As an acute-phase protein, SAA is also increased in patients with prevalent CVD.[17,18]

Although experimental evidence suggests that SAA may interfere with vasoprotective properties of HDL,[14] it is not clear whether incorporation of SAA into HDL may represent a common mechanism by which the biological function of HDL is modified. Therefore, in this study, we evaluated the effect of increasing concentrations of SAA on the long-term prognostic value of HDL in one large cohort of patients undergoing coronary angiography[19] and validated our findings in two additional cohorts with distinctly different subject characteristics.[20,21]

Methods

Study populations

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study enrolled 3316 patients undergoing coronary angiography between 1997 and 2000.[19] 3310 patients with all variables complete for current analyses were included. The study design and the examinations at baseline have been described previously.[19] The median follow-up time was 9.9 (8.5–10.7) years. All information on death during follow-up was obtained from local Health Departments. Cardiovascular mortality was defined as sudden cardiac death, fatal myocardial infarction, death due to congestive heart failure, or death immediately after intervention to treat CAD and fatal stroke.

The German Diabetes and Dialysis (4D) study is a prospective, randomized, multicentre trial enrolling 1255 patients with type 2 diabetes on haemodialysis for <2 years between 1998 and 2002. The study design has been described in detail previously.[20] The primary endpoint of the 4D study was defined as the composite of cardiac death, non-fatal myocardial infarction, and stroke, whichever occurred first (combined cardiovascular events). Here, we analysed the primary endpoint of the 4D study as well as all-cause mortality.

The Cooperative Health Research in the Region of Augsburg (KORA) S4 study is a population-based survey of inhabitants of Augsburg, Germany including 4261 participants between 1999 and 2001. We excluded subjects with previous myocardial infarction ($n = 77$), stroke ($n = 48$) or with missing values for HDL-C or SAA ($n = 102$), or those who were lost to follow-up ($n = 7$). Thus, 4027 participants were included in the present analyses. Details of the study protocol have been described.[21]

LURIC, 4D, and KORA S4 studies were approved by the local Ethics Committees and performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Procedures

Detailed description is presented in Supplementary material.

Endothelial assays

The effect of HDL from healthy subjects (50 μg/mL) supplemented with increasing concentrations of SAA on distinct endothelial functions (NO and ROS production, vascular cell adhesion molecule-1 (VCAM-1) expression, and endothelial mononuclear cell adhesion) was determined by incubating human aortic endothelial cells (HAEC) with HDL-SAA preparations. For a detailed description, see Supplementary material.

Statistical analysis

The association among SAA, HDL-C, and their interaction (SAA × HDL-C) with all-cause and cardiovascular mortality in the LURIC study has been assessed using Cox regression analyses adjusted for age, sex, acute coronary syndrome (ACS) at presentation, Friesinger coronary score, body mass index, glycated haemoglobin, smoking, lipid-lowering therapy, cystatin C, mean systolic blood pressure, and high-sensitivity (hs) C-reactive protein. General linear models were used to estimate the marginal means (adjusted) of SAA for several cardiovascular risk factors and disease states. A formula was built to calculate biologically effective HDL-C' on the basis of measured HDL-C and SAA. Survival analyses to examine the associations between calculated HDL-C' and distinct outcomes as indicated were performed in the 4D and KORA S4 studies. Detailed information on the statistical methods is given in Supplementary material.

Results

Baseline characteristics of the LURIC study are presented in Supplementary material online, *Table S1*. During a median follow-up time of 9.9 years, 995 participants (30.0%) died. About 66.5% ($n = 622$) of all deaths were caused by cardiovascular events.

We assessed the impact of increasing SAA on all-cause and cardiovascular mortality. Supplementary material online, *Figure S1* shows survival curves for all-cause and cardiovascular mortality, according to quintiles of SAA. Higher SAA levels were significantly associated with all-cause and cardiovascular mortality (both $P < 0.001$). By Cox regression analyses (Supplementary material online, *Table S2*), patients in the highest quintile of SAA had a significantly increased risk for all-cause [hazard ratio (HR) 1.58; 95% confidence interval (CI): 1.26–1.97] and cardiovascular mortality (HR 1.72; 95% CI: 1.30–2.28) after adjustment for potential confounders.

Recent experimental evidence suggests that SAA may be incorporated into HDL particles rendering HDL dysfunctional.[13,14] To examine the clinical relevance of these experimental findings, we assessed the association of HDL-C with all-cause and cardiovascular mortality at SAA concentrations above or below the 80th percentile (16.9 mg/L) (*Figure 1*). HDL-C was inversely, dose-dependently, and significantly associated with all-cause and cardiovascular mortality below the 80th percentile of SAA after adjustment for potential confounders. In marked contrast, at SAA above the 80th percentile, HDL-C not only lost its 'protective' association with mortality, but was also significantly and positively associated with all-cause and

Downloaded from http://eurheartj.oxfordjournals.org/ by guest on April 14, 2016

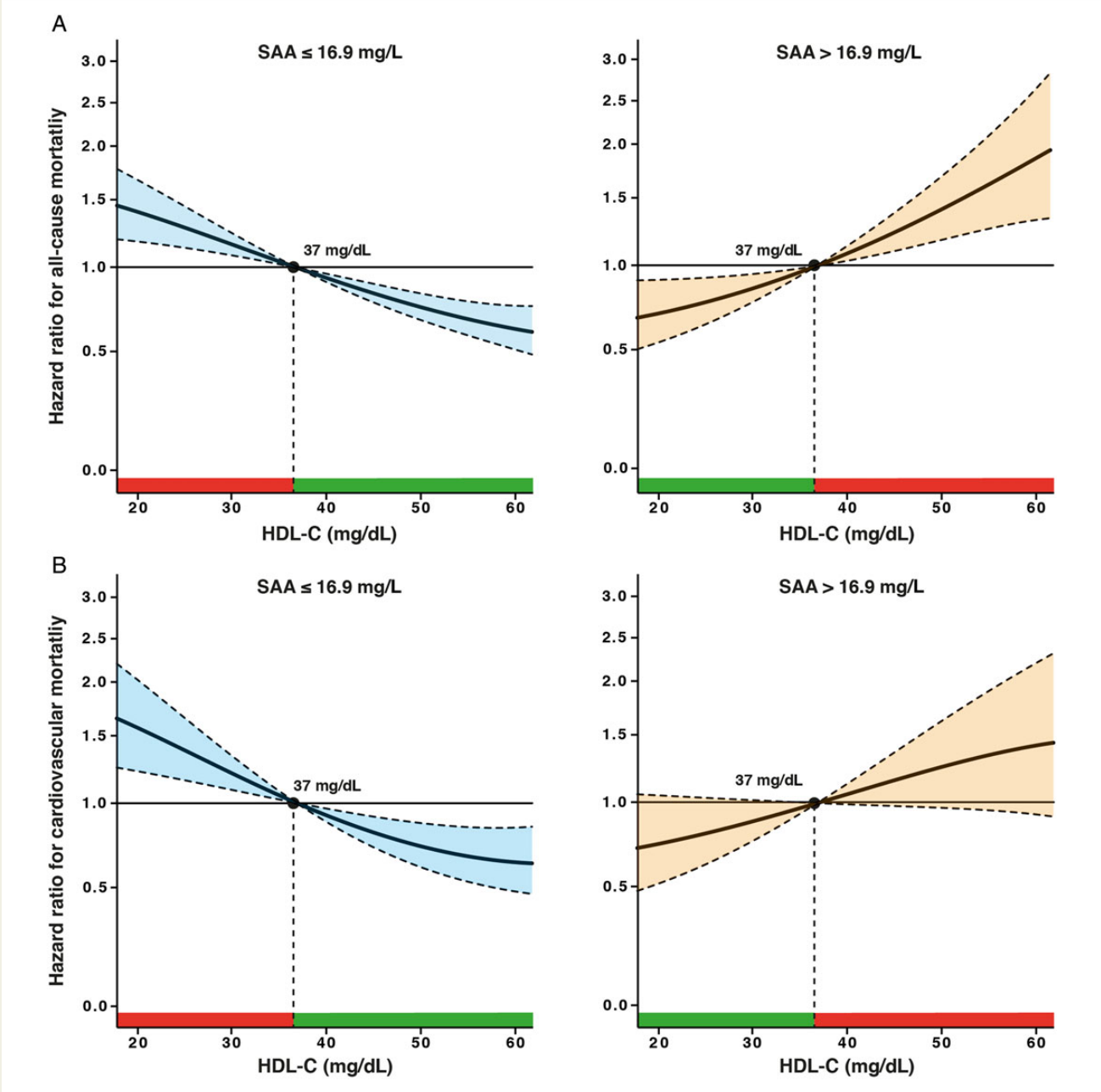

Figure 1 HRs of all-cause and cardiovascular mortality according to HDL-C levels at high and low SAA concentrations in the LURIC study. Multivariable-adjusted hazard functions for (*A*) all-cause and (*B*) cardiovascular mortality according to HDL-C concentrations at SAA below and above 16.9 mg/L (80th percentile). Solid lines represent the hazard functions and dashed lines the respective 95% CIs. The median of HDL-C (37 mg/dL) was chosen as reference (HR = 1.0). Adjusted for age, sex, ACS, Friesinger score, body mass index, glycated haemoglobin, smoking, lipid-lowering therapy, cystatin C, hs C-reactive protein, and mean systolic blood pressure. Green ranges represent the range of HDL-C with a HR $<$ 1 and red ranges with a HR $>$ 1.

cardiovascular mortality. To analyse the interaction between HDL-C and SAA, we performed Cox regression analyses including the interaction term between HDL-C divided in quartiles and SAA divided into two groups at the 80th (16.9 mg/L) or the 90th percentile (42.9 mg/L) (Supplementary material online, *Table S3*). These analyses revealed a significant interaction especially between HDL-C in the highest quartile and SAA, suggesting that SAA may modulate the biological effects of HDL.

Using the procedure described in Supplementary material, an equation was derived, which allows for calculation of the 'biologically effective' HDL-C′ concentration based on concomitantly measured HDL-C and SAA concentrations:

$$\mathrm{HDL}-C' = 20\cdot 14\big(0\cdot 213 * \ln(\ln(SAA)+1) + (0\cdot 073 * ln(ln(SAA)+1) - 0\cdot 283)\sqrt{\mathrm{HDL}-C} - 0\cdot 176\big)^2$$

Downloaded from http://eurheartj.oxfordjournals.org/ by guest on April 14, 2016

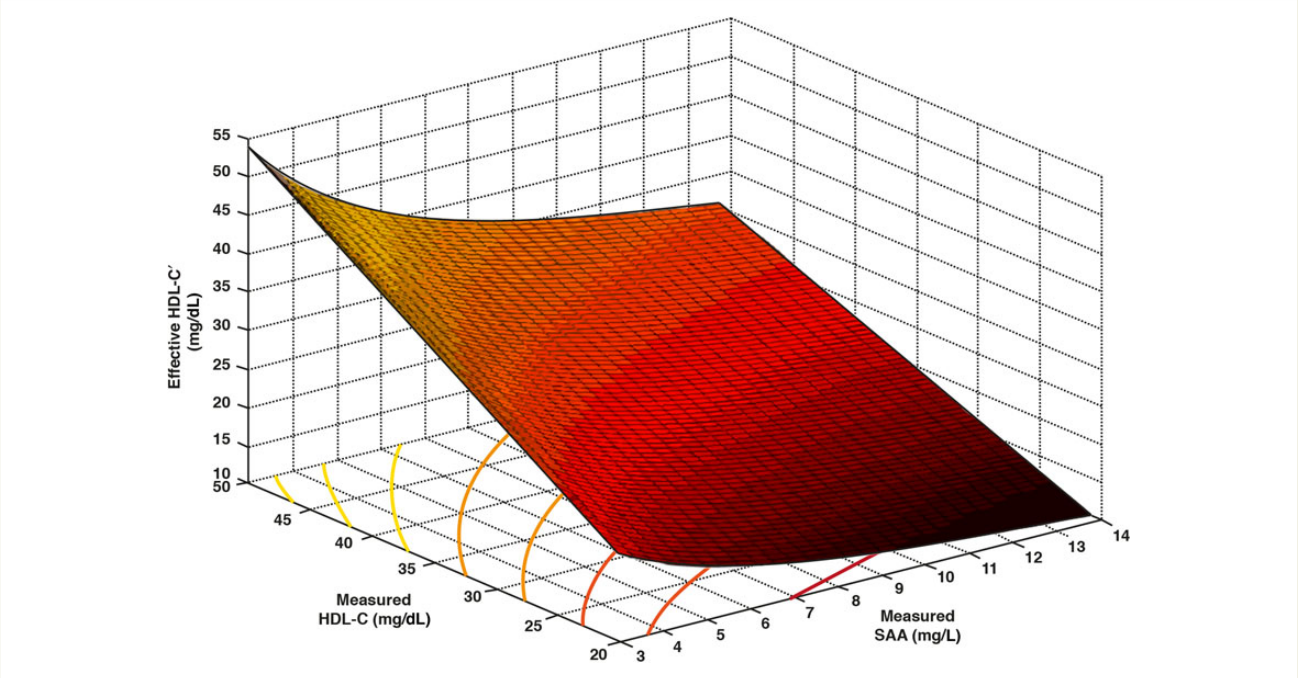

Figure 2 Calculation of biologically effective HDL-C′ by using HDL-C and SAA in the LURIC study. Graph of the function for the effective HDL-C concentration hdlch′ = hdlch′(saa,hdlch); isolines for hdlch′ = 15, 20, 25, . . . ,50 mg/dL are projected onto the hdlch-saa plane; the effective HDL-C concentration hdlch′ = f(saa,hdlc) (with $f(x,y) = 20.14(0.213\ln(\ln(x)+1) + (0.073\ln(\ln(x)+1)-0.283)y^{1/2}-0.176)^2$) is evaluated on the grid points (x_i,y_j) ($I = 0, \ldots, 28$ and $j = 0, \ldots, 75$), where $x_i = 2.8 + 0.4i$ and $y_j = 20 + 0.4j$.

The graph of the underlying function indicates that already slightly elevated SAA is sufficient to substantially reduce the proportion of biologically effective HDL-C (*Figure 2* and Supplementary material online, *Table S4*).

The acute-phase protein SAA can be associated with different cardiovascular risk factors. Therefore, we next examined clinical conditions associated with SAA (*Table 1*). Indeed, many cardiovascular risk factors (e.g. diabetes, smoking, age, and renal impairment) and manifest CVD (e.g. CAD and ACS) are related to significantly elevated SAA after adjustment for heterogeneity. Patients with angiographically proved CAD had more than two-fold higher SAA levels than those without CAD, and in ACS patients SAA was even increased more.

As renal impairment represents a potent cardiovascular risk factor and a clinical condition characterized by increased levels of SAA, we validated the aforementioned algorithm in participants of the 4D study. Baseline characteristics of the 4D study are shown in Supplementary material online, *Table S5*. During a mean follow-up of 3.9 years, 37.3% of the study participants ($n = 469$) reached the primary endpoint and 49.1% ($n = 617$) died from any cause. Using Cox regression analyses, we determined the impact of measured HDL-C and calculated biologically effective HDL-C′ on cardiovascular outcomes (Supplementary material online, *Table S6* and *Figure 3*). HDL-C did neither associate with the primary composite endpoint nor all-cause mortality. In contrast, calculated HDL-C′ was associated with a significantly reduced risk for occurrence of the primary composite endpoint as well as all-cause mortality.

Additionally, we analysed participants of the KORA S4 study. Baseline characteristics are shown in Supplementary material online, *Table S7*. During a mean follow-up of 11.0 ($\pm$1.7) years, 7.3% ($n = 294$) of the study participants died, including 2.6% ($n = 105$) deaths due to cardiovascular events. In survival analyses, measured HDL-C was not significantly associated with all-cause and cardiovascular mortality. However, in marked contrast, calculated biologically effective HDL-C′ significantly predicted all-cause and cardiovascular mortality (*Figure 4*).

Finally, we addressed the mechanisms underlying these findings on the cellular level by incubating cultured HAECs with HDL alone and with HDL supplemented with different concentrations of SAA. Although HDL alone significantly increased endothelial production of NO, an important regulator of the vascular tone, HDL supplemented with SAA not only lost its stimulatory effect, but also significantly inhibited endothelial NO production in a concentration-dependent fashion (*Figure 5A*). Vice versa, HDL supplemented with SAA significantly increased endothelial production of ROS, whereas HDL without SAA supplementation did not affect basal endothelial ROS production (*Figure 5B*). HDL also significantly reduced the expression of VCAM-1 on the surface of endothelial cells in contrast to SAA-supplemented HDL (*Figure 5C*). Accordingly, HDL significantly decreased the adhesion of mononuclear cells to tumour necrosis factor alpha (TNF-α)-treated endothelial cells (*Figure 5D*), whereas HDL supplemented with SAA lost this inhibitory activity.

Discussion

We found a statistically significant association between the SAA concentration and all-cause and cardiovascular mortality in a large

Downloaded from http://eurheartj.oxfordjournals.org/ by guest on April 14, 2016

Table 1 Association of cardiovascular risk factors and markers of disease severity with SAA concentrations in the LURIC study

	SAA (mg/L)[a]	Mean difference, %[b]	*P*-value[b]	*P*-value[c]	*P*-value[d]
Acute coronary syndrome					
No	17.1 (12.2–22.0)				
Yes	57.1 (50.0–64.3)	+234.1	<0.001		
Coronary artery disease					
No	14.8 (6.1–23.5)				
Yes	34.3 (29.4–39.2)	+132.3	<0.001		
Chronic kidney disease					
eGFR > 90 mL/min	24.3 (17.0–31.7)				
eGFR 60–90 mL/min	28.8 (23.0–34.5)	+10.3	0.353		
eGFR ≤ 60 mL/min	43.0 (32.1–53.9)	+43.4	0.008	0.019	
Smoking					
No	21.7 (15.3–28.2)				
Yes	35.1 (29.5–40.7)	+61.5	0.003		
Diabetes					
No	24.9 (19.3–30.6)				
Yes	36.1 (28.7–43.4)	+44.5	0.028		
Sex					
Female	26.4 (19.4–33.4)				
Male	32.2 (27.6–36.8)	+21.7	0.182		
Age (years)					
≤56.3	15.7 (7.3–24.0)				
56.3–63.5	24.5 (16.6–32.4)	+56.3	0.117		
63.6–70.5	31.6 (23.7–39.4)	+101.5	0.006	0.204	
≥70.6	45.6 (37.7–53.5)	+190.9	<0.001	<0.001	0.012

[a]Estimated marginal means and 95% CIs as calculated in a general linear model, adjusted for age, sex, lipid-lowering therapy, ACS, body mass index, mean systolic blood pressure, cystatin C, glycated haemoglobin, Friesinger score, smoking, and hs C-reactive protein (where appropriate).
[b]Comparison with the first category of each variable.
[c]Comparison with the second category of each variable.
[d]Comparison with the third category of each variable.

cohort of patients with high cardiovascular risk. Even more important, our data provide strong evidence both *in vivo* and *in vitro* that SAA transforms HDL from a protective into a noxious lipoprotein. In subjects with elevated SAA, higher HDL-C was associated with increased mortality, revealing that SAA significantly modifies vascular properties of HDL.

Atherosclerotic CVD is characterized by inflammatory reactions of the vessel wall including accumulation of leucocytes.[22] Consistently, several acute-phase proteins such as C-reactive protein, interleukin-6, or TNF-α have emerged as predictors for adverse outcomes in patients with clinically manifest or subclinical CVD.[18,23] Previous studies have also linked elevated SAA to poorer outcomes in patients with prevalent CVD.[24,25] Interestingly, in this study, higher SAA was not only associated with manifest CVD, but also with several clinical conditions associated with increased cardiovascular risk including CAD, diabetes mellitus, smoking, ageing, and renal impairment.

Exactly in these conditions, HDL has been suggested to be dysfunctional, whereas HDL from healthy subjects preserves vascular integrity and endothelial function.[8,26] The molecular mechanisms leading to dysfunctional HDL are only incompletely understood. Alterations of the protein and lipid composition of HDL have documented to be crucially related to abnormal vascular function of HDL.[13,14] Indeed, we recently showed that HDL from patients with CKD inhibits endothelial NO production and, thereby, increases arterial blood pressure. It is in line with the current findings that SAA accumulates in dysfunctional HDL particles of patients with CAD and/or CKD,[13,14] although the clinical relevance of these findings has been elusive so far.

Therefore, we examined the association between HDL-C and all-cause and cardiovascular mortality in patients with low and high SAA. In patients with low concentrations of SAA, HDL-C was related to reduced all-cause and cardiovascular mortality. In marked contrast, HDL-C was significantly and positively associated with all-cause and cardiovascular mortality at high SAA, even after adjustment for other inflammatory markers such as C-reactive protein. These results suggest that even moderately elevated SAA (>16.9 mg/L) is sufficient to substantially interfere with the beneficial vascular properties of HDL. These findings may not only be relevant to patients with CAD, as recent studies suggest,[27] but to all conditions that are associated with subclinical inflammation and, concomitantly, elevated SAA such as diabetes, CKD, or smoking.

Downloaded from http://eurheartj.oxfordjournals.org/ by guest on April 14, 2016

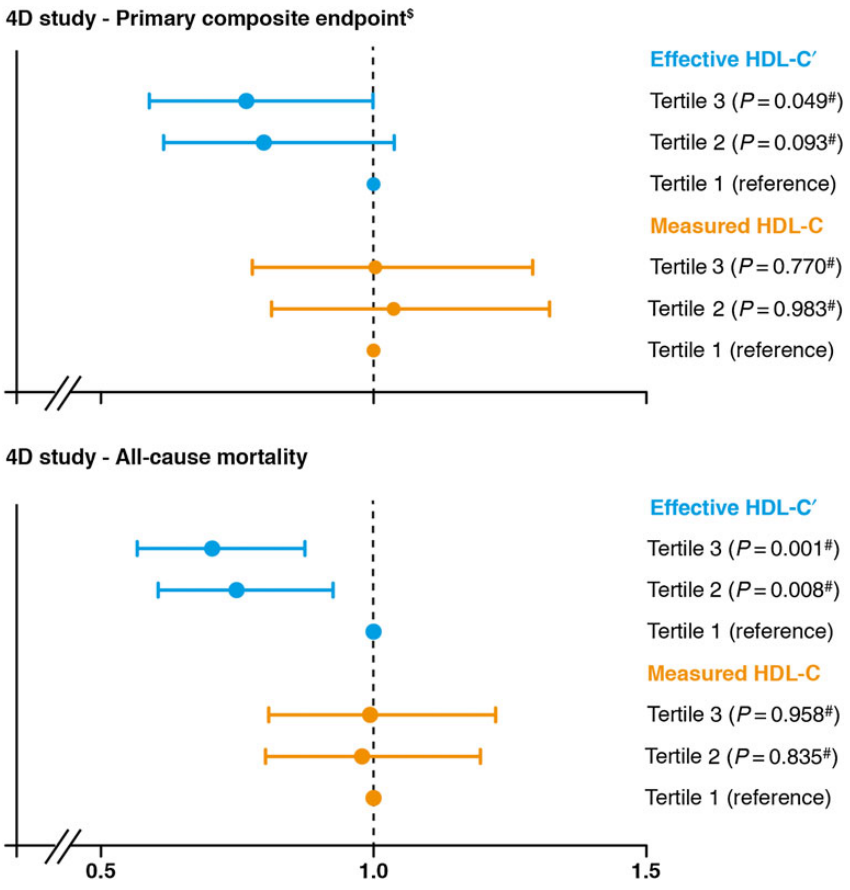

Figure 3 HRs of tertiles of measured and calculated biologically effective HDL-C for the primary composite endpoint as well as all-cause mortality in the 4D study. HRs derived from multivariable Cox regression analyses adjusted for age, sex, body mass index, glycated haemoglobin, smoking status, hs C-reactive protein, calcium, phosphate, duration of diabetes, hypertension, low-density lipoprotein, and medical treatment (placebo or atorvastatin). $The primary composite endpoint comprises cardiac death, non-fatal myocardial infarction, and stroke. #Comparison with tertile 1.

Notably, our results led to the development of an algorithm that can be used to calculate biologically effective HDL-C′. By using the well-described 4D cohort of dialysis patients as well as the KORA S4 cohort, the clinical relevance of this formula was proved.[20] Although measured HDL-C had no significant impact on cardiovascular outcomes as reported earlier,[28] higher levels of the calculated biologically effective HDL-C′ were associated with reduced risk all-cause mortality as well as cardiovascular endpoints. Although the LURIC study includes patients at intermediate-to-high risk of CVD, KORA represents the general population and 4D is a cohort with a very high risk. Thus, our formula has been validated over a very broad range of CVD risks from standard to extraordinary high.

Earlier studies have suggested that SAA might replace the main structural protein of HDL apolipoprotein A–I, which may mediate some of the vasoprotective properties of HDL.[29] Additionally, the accumulation of SAA in HDL was linked to reduced cholesterol efflux capacity and anti-inflammatory activity of HDL.[13,14] Moreover, SAA-enriched HDL may be associated with the development of atherosclerotic lesions in mice.[30] In experimental studies, we observed that SAA-supplemented HDL reduced endothelial NO production and concomitantly increased endothelial production of ROS. Thus, SAA turned HDL into a pro-inflammatory particle. These findings underscore the important role of SAA in inverting the beneficial vascular properties of HDL.

Our findings have several important clinical implications. Currently, all assays to measure the biological properties of HDL require time-consuming isolation of HDL by ultracentrifugation or immunological methods. Our data suggest that concomitantly measuring SAA and HDL may reflect the vascular function of HDL. This may be of importance as a plethora of clinical conditions are associated with inflammation and increased SAA. SAA, therefore, may aid in the identification of patients with increased risk for cardiovascular events, despite high HDL-C concentrations. Our algorithm to calculate the effective HDL-C′ can be easily implemented in routine diagnostics and can be used as a convenient tool to estimate the functionality of HDL without time-consuming isolation of HDL and demanding functional assays.

Efforts have recently been made to increase HDL-C by pharmacological means, e.g. using inhibitors of the cholesteryl-ester transfer protein (CETP). Large trials using the CETP inhibitors torcetrapib and dalcetrapib did not show a reduction of cardiovascular outcomes.[31,32] In these study programmes, a substantial proportion of

Downloaded from http://eurheartj.oxfordjournals.org/ by guest on April 14, 2016

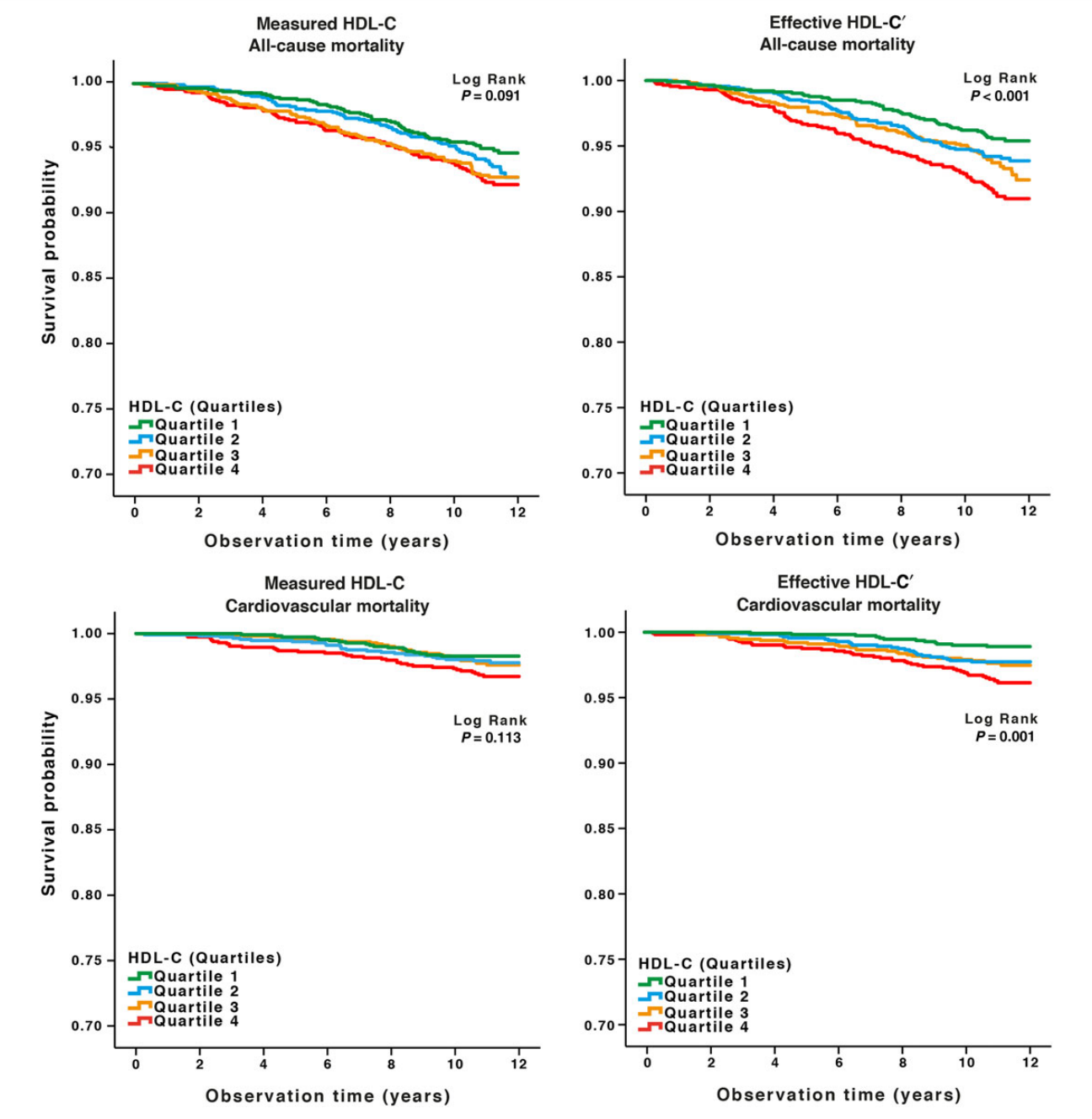

Figure 4 Univariate survival analyses for all-cause and cardiovascular mortality in the KORA S4 study according to quartiles of measured and calculated biologically effective HDL-C′. Quartiles for measured HDL-C: quartile 1, ≤45.3 mg/dL; quartile 2, 45.3–55.7 mg/dL; quartile 3, 55.7–68.2 mg/dL; and quartile 4, >68.2 mg/dL. Quartiles for calculated biologically effective HDL-C′: quartile 1, ≤46.3 mg/dL; quartile 2, 46.3–61.3 mg/dL; quartile 3, 61.3–82.2 mg/dL; and quartile 4, >82.2 mg/dL).

the patients had ACS, diabetes mellitus, and/or renal impairment, i.e. conditions with increased SAA.[32] Based on our findings, we speculate that raising HDL concentrations in patients with high SAA may be detrimental. Measuring SAA could potentially help to identify patients in whom HDL-increasing therapies may be advantageous. Although speculative, the decrease of SAA seen during statin treatment[33] may contribute to their atheroprotective effects and lowering may turn out a useful approach to restore HDL functionality.

The main strengths of the present study are the large study populations with long-term follow-up. Accurate and comprehensive clinical characterization of the participants made it possible to adjust for potential confounding variables. Confirmation of our results in the 4D study, including patients on dialysis treatment with a high risk for cardiovascular events, as well as in the KORA S4 study, comprising apparently healthy subjects in a population-based setting, further supports our conclusions. The clinical

Downloaded from http://eurheartj.oxfordjournals.org/ by guest on April 14, 2016

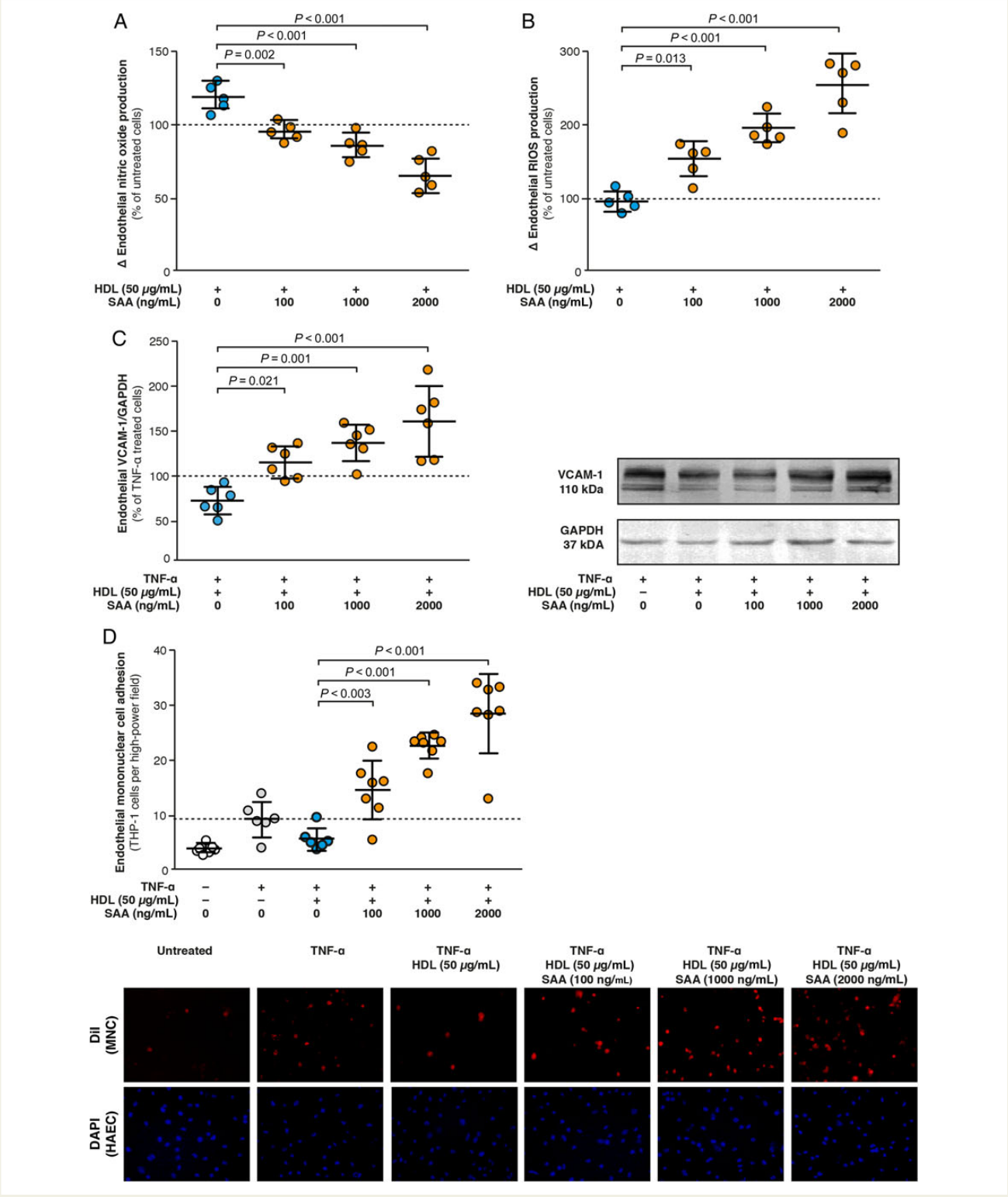

Figure 5 Endothelial effects of HDL supplemented with increasing concentrations of SAA. (*A*) Endothelial NO production as determined by electron spin resonance (ESR) spectroscopy in HAECs incubated with HDL (50 μg/mL) or HDL supplemented with SAA (100, 1000, or 2000 ng/mL) for 1 h. (*B*) Endothelial ROS production as determined by ESR spectroscopy in HAECs incubated with HDL (50 μg/mL) or HDL supplemented with SAA (100, 1000, or 2000 ng/mL) for 1 h. (*C*) Endothelial VCAM-1 expression as determined by western blot in HAECs incubated with HDL (50 μg/mL) or HDL supplemented with SAA (100, 1000, or 2000 ng/mL) for 5 h and TNF-α (0.1 ng/mL) for 4 h. (*D*) Adhesion of DiI-labelled THP-1 cells to HAECs counterstained with DAPI and incubated with HDL (50 μg/mL) or HDL supplemented with SAA (100, 1000, or 2000 ng/mL) for 5 h and TNF-α (0.1 ng/mL) for 4 h. $n = 5–7$ per group. Respective means and standard deviation are shown. *P*-values have been adjusted for multiple comparisons.

Downloaded from http://eurheartj.oxfordjournals.org/ by guest on April 14, 2016

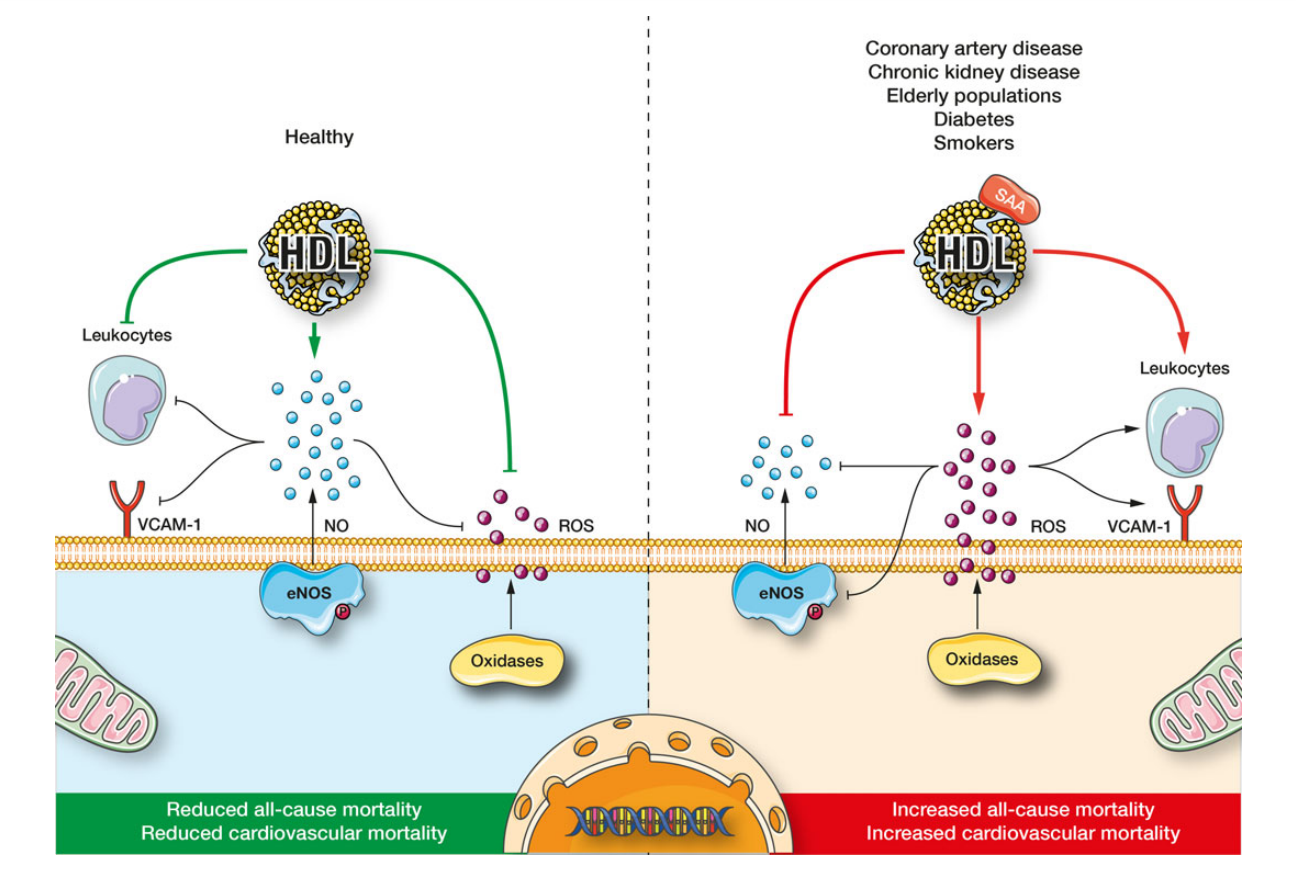

Figure 6 Summarizing figure.

observations are further strongly supported by the mechanistic cell culture studies. In summary, these findings indicate that the concept of calculating biologically effective HDL-C′ may be applicable to patients with manifest, prevalent, and without CVD. Limitations of our study are that the LURIC, 4D, and KORA S4 studies primarily included patients of Caucasian ancestry, which limits the extension of the present findings to other ethnicities. In addition, despite careful analysis, confounding due to differences of the baseline variables across SAA concentrations cannot be fully excluded in a *post hoc* analysis. Additional studies are necessary to determine the effect of different treatment strategies on the interaction between SAA and HDL-C and to assess the performance of calculated biologically effective HDL-C′ in currently available scoring algorithms to predict cardiovascular risk. Moreover, the time course and reversibility of SAA accumulation in HDL have to be determined.

In conclusion, SAA is a strong marker for cardiovascular and all-cause mortality, interacting with the functionality of HDL. SAA transforms HDL from a vasoprotective into a pro-atherosclerotic lipoprotein, which is associated with the substantially worsened cardiovascular outcome at least partially mediated by promoting endothelial dysfunction (*Figure 6*). Concomitantly measuring SAA concentrations may represent a convenient method to estimate HDL functionality in clinical routine.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Funding

The LURIC study was supported by unrestricted grants from Sanofi, Roche, Dade Behring, and AstraZeneca and has received funding from the 7th Framework Program (Atheroremo, grant agreement number_201668 and RiskyCAD, grant agreement number_305739) of the European Union and from the INTERREG IV Oberrhein Program (Project A28, Genetic mechanisms of cardiovascular diseases) with support from the European Regional Development Fund (ERDF) and the Wissenschaftsoffensive TMO. The KORA study was initiated and financed by the Helmholtz Zentrum München—German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ. SAA measurements in KORA S4 were possible through additional funds from the University of Ulm. S.Z. was supported by Deutsche Nierenstiftung. T.S. was supported by Deutsche Forschungsgemeinschaft, Deutsche Hochdruckliga, and Else-Kroener Fresenius Foundation.

Downloaded from http://eurheartj.oxfordjournals.org/ by guest on April 14, 2016

Conflict of interest: W.K. has received consulting fees from the Medicines Company and was member of the Steering Committee of Dal-Outcomes.

References

1. Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, Dallongeville J, De Backer G, Ebrahim S, Gjelsvik B, Herrmann-Lingen C, Hoes A, Humphries S, Knapton M, Perk J, Priori SG, Pyorala K, Reiner Z, Ruilope L, Sans-Menendez S, Op Reimer WS, Weissberg P, Wood D, Yarnell J, Zamorano JL, Walma E, Fitzgerald T, Cooney MT, Dudina A, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Funck-Brentano C, Filippatos G, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Altiner A, Bonora E, Durrington PN, Fagard R, Giampaoli S, Hemingway H, Hakansson J, Kjeldsen SE, Larsen ML, Mancia G, Manolis AJ, Orth-Gomer K, Pedersen T, Rayner M, Ryden L, Sammut M, Schneiderman N, Stalenhoef AF, Tokgozoglu L, Wiklund O, Zampelas A, European Society of Cardiology, European Association for Cardiovascular Prevention and Rehabilitation, Council on Cardiovascular Nursing, European Association for Study of Diabetes, International Diabetes Federation Europe, European Stroke Initiative, Society of Behavioural Medicine, European Society of Hypertension, WONCA Europe, European Heart Network, European Atherosclerosis Society. European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). *Eur J Cardiovasc Prev Rehabil* 2007;**14**(Suppl. 2): S1–S113.
2. Ortiz A, Covic A, Fliser D, Fouque D, Goldsmith D, Kanbay M, Mallamaci F, Massy ZA, Rossignol P, Vanholder R, Wiecek A, Zoccali C, London GM, Board of the E-mWGoERAE. Epidemiology, contributors to, and clinical trials of mortality risk in chronic kidney failure. *Lancet* 2014;**383**:1831–1843.
3. Speer T, Owala FO, Holy EW, Zewinger S, Frenzel FL, Stahli BE, Razavi M, Triem S, Cvija H, Rohrer L, Seiler S, Heine GH, Jankowski V, Jankowski J, Camici GG, Akhmedov A, Fliser D, Luscher TF, Tanner FC. Carbamylated low-density lipoprotein induces endothelial dysfunction. *Eur Heart J* 2014;**35**:3021–3032.
4. Mineo C, Shaul PW. Novel biological functions of high-density lipoprotein cholesterol. *Circ Res* 2012;**111**:1079–1090.
5. Luscher TF, Landmesser U, von Eckardstein A, Fogelman AM. High-density lipoprotein: vascular protective effects, dysfunction, and potential as therapeutic target. *Circ Res* 2014;**114**:171–182.
6. Spieker LE, Sudano I, Hurlimann D, Lerch PG, Lang MG, Binggeli C, Corti R, Ruschitzka F, Luscher TF, Noll G. High-density lipoprotein restores endothelial function in hypercholesterolemic men. *Circulation* 2002;**105**:1399–1402.
7. Miller NE, Thelle DS, Forde OH, Mjos OD. The Tromso Heart-study. High-density lipoprotein and coronary heart-disease: a prospective case–control study. *Lancet* 1977;**1**:965–968.
8. Sorrentino SA, Besler C, Rohrer L, Meyer M, Heinrich K, Bahlmann FH, Mueller M, Horvath T, Doerries C, Heinemann M, Flemmer S, Markowski A, Manes C, Bahr MJ, Haller H, von Eckardstein A, Drexler H, Landmesser U. Endothelial-vasoprotective effects of high-density lipoprotein are impaired in patients with type 2 diabetes mellitus but are improved after extended-release niacin therapy. *Circulation* 2010;**121**: 110–122.
9. Speer T, Rohrer L, Blyszczuk P, Shroff R, Kuschnerus K, Kränkel N, Kania G, Zewinger S, Akhmedov A, Shi Y, Martin T, Perisa D, Winnik S, Müller MF, Sester U, Wernicke G, Jung A, Gutteck U, Eriksson U, Geisel J, Deanfield J, von Eckardstein A, Lüscher TF, Fliser D, Bahlmann FH, Landmesser U. Abnormal high-density lipoprotein induces endothelial dysfunction via activation of toll-like receptor-2. *Immunity* 2013;**38**:754–768.
10. Speer T, Zewinger S, Fliser D. Uraemic dyslipidaemia revisited: role of high-density lipoprotein. *Nephrol Dial Transpl* 2013;**28**:2456–2463.
11. Zewinger S, Speer T, Kleber ME, Scharnagl H, Woitas R, Lepper PM, Pfahler K, Seiler S, Heine GH, Marz W, Silbernagel G, Fliser D. HDL cholesterol is not associated with lower mortality in patients with kidney dysfunction. *J Am Soc Nephrol* 2014;**25**:1073–1082.
12. Charakida M, Besler C, Batuca JR, Sangle S, Marques S, Sousa M, Wang G, Tousoulis D, Delgado Alves J, Loukogeorgakis SP, Mackworth-Young C, D'Cruz D, Luscher T, Landmesser U, Deanfield JE. Vascular abnormalities, paraoxonase activity, and dysfunctional HDL in primary antiphospholipid syndrome. *JAMA* 2009;**302**:1210–1217.
13. Holzer M, Birner-Gruenberger R, Stojakovic T, El-Gamal D, Binder V, Wadsack C, Heinemann A, Marsche G. Uremia alters HDL composition and function. *J Am Soc Nephrol* 2011;**22**:1631–1641.
14. Weichhart T, Kopecky C, Kubicek M, Haidinger M, Doller D, Katholnig K, Suarna C, Eller P, Tolle M, Gerner C, Zlabinger GJ, van der Giet M, Horl WH, Stocker R, Saemann MD. Serum amyloid A in uremic HDL promotes inflammation. *J Am Soc Nephrol* 2012;**23**:934–947.
15. Kopecky C, Genser B, Drechsler C, Krane V, Kaltenecker CC, Hengstschlager M, Marz W, Wanner C, Saemann MD, Weichhart T. Quantification of HDL proteins, cardiac events, and mortality in patients with type 2 diabetes on hemodialysis. *Clin J Am Soc Nephrol* 2015;**10**:224–231.
16. Kopecky C, Haidinger M, Birner-Grunberger R, Darnhofer B, Kaltenecker CC, Marsche G, Holzer M, Weichhart T, Antlanger M, Kovarik JJ, Werzowa J, Hecking M, Saemann MD. Restoration of renal function does not correct impairment of uremic HDL properties. *J Am Soc Nephrol* 2015;**26**:565–575.
17. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, Gallimore JR, Pepys MB. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ* 2000;**321**:199–204.
18. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000;**342**:836–843.
19. Winkelmann BR, Marz W, Boehm BO, Zotz R, Hager J, Hellstern P, Senges J. Rationale and design of the LURIC study—a resource for functional genomics, pharmacogenomics and long-term prognosis of cardiovascular disease. *Pharmacogenomics* 2001;**2**:S1–S73.
20. Wanner C, Krane V, Marz W, Olschewski M, Mann JF, Ruf G, Ritz E. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. *N Engl J Med* 2005; **353**:238–248.
21. Holle R, Happich M, Lowel H, Wichmann HE; MONICA/KORA Study Group. KORA—a research platform for population based health research. *Gesundheitswesen* 2005;**67**(Suppl. 1):S19–S25.
22. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999;**340**: 115–126.
23. Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. *Circulation* 2000;**101**:2149–2153.
24. Johnson BD, Kip KE, Marroquin OC, Ridker PM, Kelsey SF, Shaw LJ, Pepine CJ, Sharaf B, Bairey Merz CN, Sopko G, Olson MB, Reis SE, National Heart Lung and Blood Institute. Serum amyloid A as a predictor of coronary artery disease and cardiovascular outcome in women: the National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). *Circulation* 2004;**109**:726–732.
25. Morrow DA, Rifai N, Antman EM, Weiner DL, McCabe CH, Cannon CP, Braunwald E. Serum amyloid A predicts early mortality in acute coronary syndromes: a TIMI 11A substudy. *J Am Coll Cardiol* 2000;**35**:358–362.
26. Speer T, Rohrer L, Blyszczuk P, Shroff R, Kuschnerus K, Krankel N, Kania G, Zewinger S, Akhmedov A, Shi Y, Martin T, Perisa D, Winnik S, Muller MF, Sester U, Wernicke G, Jung A, Gutteck U, Eriksson U, Geisel J, Deanfield J, von Eckardstein A, Luscher TF, Fliser D, Bahlmann FH, Landmesser U. Abnormal high-density lipoprotein induces endothelial dysfunction via activation of Toll-like receptor-2. *Immunity* 2013;**38**:754–768.
27. Silbernagel G, Schottker B, Appelbaum S, Scharnagl H, Kleber ME, Grammer TB, Ritsch A, Mons U, Holleczek B, Goliasch G, Niessner A, Boehm BO, Schnabel RB, Brenner H, Blankenberg S, Landmesser U, Marz W. High-density lipoprotein cholesterol, coronary artery disease, and cardiovascular mortality. *Eur Heart J* 2013;**34**:3563–3571.
28. Silbernagel G, Genser B, Drechsler C, Scharnagl H, Grammer TB, Stojakovic T, Krane V, Ritz E, Wanner C, Marz W. HDL cholesterol, apolipoproteins, and cardiovascular risk in hemodialysis patients. *J Am Soc Nephrol* 2014;**26**:484–492.
29. Artl A, Marsche G, Lestavel S, Sattler W, Malle E. Role of serum amyloid A during metabolism of acute-phase HDL by macrophages. *Arterioscler Thromb Vasc Biol* 2000;**20**:763–772.
30. Lewis KE, Kirk EA, McDonald TO, Wang S, Wight TN, O'Brien KD, Chait A. Increase in serum amyloid a evoked by dietary cholesterol is associated with increased atherosclerosis in mice. *Circulation* 2004;**110**:540–545.
31. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, Lopez-Sendon J, Mosca L, Tardif JC, Waters DD, Shear CL, Revkin JH, Buhr KA, Fisher MR, Tall AR, Brewer B, for the ILLUMINATE Investigators. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med* 2007;**357**: 2109–2122.
32. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J, Chaitman BR, Holme IM, Kallend D, Leiter LA, Leitersdorf E, McMurray JJ, Mundl H, Nicholls SJ, Shah PK, Tardif JC, Wright RS, for the dal-OUTCOMES Investigators. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med* 2012;**367**:2089–2099.
33. Kinlay S, Schwartz GG, Olsson AG, Rifai N, Leslie SJ, Sasiela WJ, Szarek M, Libby P, Ganz P, Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering Study I. High-dose atorvastatin enhances the decline in inflammatory markers in patients with acute coronary syndromes in the MIRACL study. *Circulation* 2003; **108**:1560–1566.

Downloaded from http://eurheartj.oxfordjournals.org/ by guest on April 14, 2016