Temporal changes in secondary prevention and cardiovascular outcomes after revascularization for peripheral arterial disease in Denmark: a nationwide cohort study

Trinder, M.; Wang, Y.A.; Madsen, C.M.; Ponomarev, T.; Bohunek, L.; Daisely, B.A.; ... ; Brunham, L.R.

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ORIGINAL RESEARCH ARTICLE

Inhibition of Cholesteryl Ester Transfer Protein Preserves High-Density Lipoprotein Cholesterol and Improves Survival in Sepsis

BACKGROUND: The high-density lipoprotein hypothesis of atherosclerosis has been challenged by clinical trials of cholesteryl ester transfer protein (CETP) inhibitors, which failed to show significant reductions in cardiovascular events. Plasma levels of high-density lipoprotein cholesterol (HDL-C) decline drastically during sepsis, and this phenomenon is explained, in part, by the activity of CETP, a major determinant of plasma HDL-C levels. We tested the hypothesis that genetic or pharmacological inhibition of CETP would preserve high-density lipoprotein levels and decrease mortality in clinical cohorts and animal models of sepsis.

METHODS: We examined the effect of a gain-of-function variant in *CETP* (rs1800777, p.Arg468Gln) and a genetic score for decreased *CETP* function on 28-day sepsis survival using Cox proportional hazard models adjusted for age and sex in the UK Biobank (n=5949), iSPAAR (Identification of SNPs Predisposing to Altered Acute Lung Injury Risk; n=882), Copenhagen General Population Study (n=2068), Copenhagen City Heart Study (n=493), Early Infection (n=200), St Paul's Intensive Care Unit 2 (n=203), and Vasopressin Versus Norepinephrine Infusion in Patients With Septic Shock studies (n=632). We then studied the effect of the CETP inhibitor, anacetrapib, in adult female APOE*3-Leiden mice with or without human CETP expression using the cecal-ligation and puncture model of sepsis.

RESULTS: A fixed-effect meta-analysis of all 7 cohorts found that the *CETP* gain-of-function variant was significantly associated with increased risk of acute sepsis mortality (hazard ratio, 1.44 [95% CI, 1.22–1.70]; $P<0.0001$). In addition, a genetic score for decreased CETP function was associated with significantly decreased sepsis mortality in the UK Biobank (hazard ratio, 0.77 [95% CI, 0.59–1.00] per 1 mmol/L increase in HDL-C) and iSPAAR cohorts (hazard ratio, 0.60 [95% CI, 0.37–0.98] per 1 mmol/L increase in HDL-C). APOE*3-Leiden.CETP mice treated with anacetrapib had preserved levels of HDL-C and apolipoprotein-AI and increased survival relative to placebo treatment (70.6% versus 35.3%, Log-rank $P=0.03$), whereas there was no effect of anacetrapib on the survival of APOE*3-Leiden mice that did not express *CETP* (50.0% versus 42.9%, Log-rank $P=0.87$).

CONCLUSIONS: Clinical genetics and humanized mouse models suggest that inhibiting CETP may preserve high-density lipoprotein levels and improve outcomes for individuals with sepsis.

Mark Trinder, MSc
Yanan Wang, PhD
Christian M. Madsen, PhD
Tatjana Ponomarev, BSc
Lubos Bohunek, BSc
Brendan A. Daisely, BSc
HyeJin Julia Kong, BSc
Lisanne L. Blauw, PhD
Børge G. Nordestgaard, MD, DMSc
Anne Tybjærg-Hansen, MD, DMSc
Mark M. Wurfel, MD, PhD
James A. Russell, MD
Keith R. Walley, MD
Patrick C.N. Rensen, PhD
John H. Boyd, MD
Liam R. Brunham, MD, PhD

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Clinical Perspective

What Is New?

- Genetic variations in *CETP* that increase high-density lipoprotein cholesterol were associated with a reduced risk of 28-day mortality from sepsis.
- In mouse models of sepsis, inhibition of cholesteryl ester transfer protein with anacetrapib preserved high-density lipoprotein cholesterol levels, decreased the severity of endotoxemia, and improved survival after cecal-ligation and puncture.

What Are the Clinical Implications?

- High-density lipoprotein cholesterol, a commonly used biomarker for cardiovascular risk assessment, may also predict risk of death from sepsis.
- Cholesteryl ester transfer protein inhibitors that have been tested in clinical trials of cardiovascular disease could be repurposed and studied in clinical trials of sepsis.

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to an infection.[1] Sepsis is a major cause of in-hospital mortality, and may be responsible for as many as one-fifth of all in-hospital deaths globally.[2] Numerous clinical trials designed to evaluate therapeutic strategies beyond fluid resuscitation and antibiotics have failed to improve outcomes from sepsis,[3] suggesting the need for therapies that target pathways causal to the pathogenesis of sepsis.

High-density lipoprotein (HDL) particles, although best known for their inverse association with coronary artery disease, possess many properties that are relevant to sepsis. These include their ability to sequester and eliminate bacterial toxins such as lipopolysaccharide,[4,5] as well as their anti-inflammatory,[6] antithrombotic,[7] and vasoprotective properties, which may be exerted by specific apolipoproteins carried by HDL.[8] The quantity of HDL, as reflected by the concentration of HDL cholesterol (HDL-C), declines drastically during sepsis,[9–11] compared with other forms of systemic inflammation such as trauma,[12] and greater decline of HDL-C is associated with worse clinical outcomes.[9,10,13] We recently reported that a gain-of-function single-nucleotide polymorphism (SNP) in the *CETP* gene is an important contributor to the decline of HDL-C during sepsis.[9,14] CETP (cholesteryl ester transfer protein) is a plasma protein that mediates the bidirectional transfer of triglycerides and cholesteryl esters between triglyceride-rich lipoproteins and HDL particles.[15] This exchange depletes HDL particles of their cholesterol content, induces HDL catabolism by hepatic lipase, and reduces the plasma concentration of HDL particles, HDL-C, and apolipoprotein-AI, the main apolipoprotein component of HDL.

Mendelian randomization studies suggest that the associations between low levels of HDL-C and increased risk of infectious hospitalizations and acute mortality from sepsis may be causal in nature,[9,16] which supports the notion that HDL may represent a therapeutic target for sepsis. This concept is further supported by animal studies demonstrating that transgenic overexpression of apolipoprotein-AI, or transfusion of HDL, can reduce organ damage and mortality from sepsis.[17] However, such approaches are impractical for clinical translation, and whether acute pharmacological intervention after the onset of sepsis could prevent the decline in HDL-C is unknown. Small molecule pharmacological inhibitors of CETP exist, and have been shown in large clinical trials to be safe and well tolerated.[18–20] We hypothesized that pharmacological inhibition of CETP could blunt the reduction in HDL-C and apolipoprotein-AI that occurs during sepsis, and that this would lead to improved survival.

METHODS

Transparency and Openness Promotion Guidelines Statement

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Cohorts Used for Genetic Association Analyses

All clinical cohorts were approved by their appropriate research ethics review boards, and all individuals gave informed consent (Methods in the Data Supplement). The cohort characteristics and genotyping strategies of the UK Biobank,[21] iSPAAR (Identification of SNPs Predisposing to Altered Acute Lung Injury Risk), Copenhagen General Population Study,[22] Copenhagen City Heart Study,[22] Early Infection,[10] SPHICU2 (St Paul's Intensive Care Unit Version 2),[9] and VASST (Vasopressin Versus Norepinephrine Infusion in Patients With Septic Shock)[23] cohorts have been described previously (Methods in the Data Supplement). The characteristics of each cohort and the available clinical and genetic data are shown in the Table, as well as how these cohorts were used to perform genetic associations between variants in *CETP* and sepsis phenotypes. Enrollment into the iSPAAR, Early Infection, SPHICU2, and VASST cohorts was on the basis of clinical diagnosis of sepsis (Methods in the Data Supplement). In the UK Biobank, Copenhagen General Population Study,[22] and Copenhagen City Heart Study,[22] cases of sepsis were identified using International Statistical Classification of Diseases and Related Health Problems diagnosis codes related to sepsis (Methods in the Data Supplement).[16,22]

Construction of HDL-C Polygenic Scores Using Genetic Variants Related to the CETP Gene

We calculated a weighted polygenic score using genome-wide association study summary statistics for the association of HDL-C levels with SNPs found near the *CETP* gene for

Table. Clinical Data Used for Genetic Associations of *CETP* With Sepsis Phenotypes

Study cohort	Study design	Data available for analyses				
		Genetic	Clinical sepsis outcome	Plasma lipids	Longitudinal WBC count	Plasma cytokines
Copenhagen General Population	Sepsis cohort form a volunteer population study (n=1916)	rs1800777 genotype	28-d mortality	At study enrollment	N/A	N/A
Copenhagen City Heart	Sepsis cohort form a volunteer population study (n=493)	rs1800777 genotype	28-d mortality	At study enrollment	N/A	N/A
UK Biobank	Sepsis cohort form a volunteer population study (n=5949)	rs1800777 genotype, *CETP* genetic score*	28-d mortality	At study enrollment	N/A	N/A
Identification of SNPs Predisposing to Altered Acute Lung Injury Risk (iSPAAR)	Observational sepsis cohort (n=882)	rs1800777 genotype, *CETP* genetic score*	28-d mortality	N/A	N/A	N/A
Early Infection	Observational sepsis cohort (n=200)	rs1800777 genotype	28-d mortality	During sepsis	N/A	Yes
St Paul's Hospital Intensive Care Unit 2 (SPHICU2)	Observational sepsis cohort (n=203)	rs1800777 genotype	28-d mortality	N/A	N/A	N/A
Vasopressin Versus Norepinephrine in Severe Septic Shock Trial (VASST)	Sepsis cohort from a randomized controlled trial (n=632)	rs1800777 genotype	28-d mortality	N/A	Yes	Yes

N/A indicates not applicable; and WBC, white blood cell.
*Genotyping array data containing all single nucleotide variants needed to calculate the *CETP* genetic score available.

individuals from the UK Biobank and iSPAAR cohorts (Tables I and II in the Data Supplement). Weighted *CETP* polygenic scores were calculated using the effect sizes that describe the association of 8 independent SNPs with HDL-C levels (Table I in the Data Supplement).[24] Polygenic scores were calculated using the formula $\Sigma (\beta_x * SNP_x)$, where β_x is the effect size for the HDL-C-increasing allele and SNP_x is the number of HDL-C increasing alleles (0, 1, or 2) for SNP_x. This score has been described by Ference et al.[24]

Mouse Models

All animal studies were approved by the University of British Columbia animal ethics committee and performed in accordance with institutional guidelines. Female APOE*3-Leiden.CETP and APOE*3-Leiden transgenic mice[25] were bred at Leiden University Medical Center.[25,26] Mice were provided ad libitum access to chow diet and water and housed under a 12-hour light/dark cycle (6 AM to 6 PM Pacific Standard Time light, 6 PM to 6 AM Pacific Standard Time dark). All experimental sepsis procedures were performed when mice were 13 to 16 weeks of age and between 8 AM and 12 PM Pacific Standard Time. Individuals performing procedures and assessing mouse physiology were blinded to treatment allocation of the animals.

Immediately after euthanization, blood was collected using lithium heparin tubes, and plasma was isolated according to the manufacturer's protocol (Sarstedt, catalog No. 15.1673.100). Plasma was stored at –80°C until analyses.

Mouse Endotoxemia Studies

The CETP inhibitor, anacetrapib, was obtained from Selleck Chemicals (catalog No. S2748). Mice were treated with a semisynthetic cholesterol-rich diet, containing 15% cocoa butter (wt/wt), and 0.15% cholesterol (wt/wt) Western-type diet with or without anacetrapib (10 mg/kg body weight per day)[27] for 3 weeks before endotoxemia (TestDiet, modification of Semi-Purified Diet 58B0, catalog No. 1815730-204).

Untagged and Alexa Fluor 594–tagged lipopolysaccharide (LPS) from *Escherichia coli* O55:B5 were obtained from Sigma-Aldrich (catalog No. L2880) and Thermo Fisher Scientific (catalog No. L23353), respectively. LPS was diluted in sterile saline. APOE*3-Leiden.CETP mice were treated with 5 mg/kg of LPS (50% untagged, 50% Alexa Fluor 594–tagged) or vehicle intravenously by tail vein injection. Mice were monitored every 2 hours using core body temperature by rectal thermometer, Murine Sepsis Score, and Mouse Clinical Assessment Score for Sepsis (Tables III and IV in the Data Supplement).[28] Mice were euthanized at 6 hours after LPS injection.[29]

Mouse Cecal-Ligation and Puncture Studies

APOE*3-Leiden.CETP and APOE*3-Leiden mice were treated with the Western-type diet described above or chow diet for 3 weeks before the cecal-ligation and puncture (CLP) operations.[27] CLP operations were performed as previously described.[29] Briefly, mice were anesthetized with 1% to 3% isoflurane and kept warm (>35°C) using a heating pad. A midline laparotomy (2 cm) was performed, and the cecum was exposed and ligated using a 6-0 suture anterior to the ileocecal valve, without causing intestinal obstruction. The cecum was perforated once through and through in the midsection using an 18.5-gauge needle, after which the incision was closed. Sham procedures were performed identically but without ligation or puncturing the cecum.

A stock solution of CETP inhibitor, anacetrapib, was made by dissolving anacetrapib in polyethylene glycol 300 (Sigma-Aldrich, catalog No. 90878) with 0.5% Tween20 at 400 µg/

mL (Sigma-Aldrich, catalog No. P1379).[30] The stock solution of anacetrapib was diluted 2-fold with sterile double deionized H_2O to make a 200 µg/mL working solution of anacetrapib. At 6 hours after CLP, mice were treated with anacetrapib (on time via intravenous injection at 1 mg/kg and on time via subcutaneous injection at 1.5 mg/kg, respectively)[30] or vehicle. Anacetrapib has a relatively long half-life of 12 to 34 hours.[30] All mice were treated with subcutaneous imipenem/cilastatin at 6 hours after CLP (25 mg/kg; Sandoz Canada Inc, drug identification number 02358344)[29] to model standard-of-care treatment of human sepsis. To assess the acute changes in plasma lipid metabolism, mice were euthanized 24 hours after a CLP procedure of "moderate" severity (ligation midway between the ileocecal valve and distal cecum).

Alternatively, to assess the ability of CETP inhibition to attenuate sepsis mortality, mice were monitored to the terminal end point of 72 hours after CLP procedure.[29] A "severe" CLP procedure was performed by ligating just between the ileocecal valve and cecum just distal to the ileocecal valve. Mice were treated with subcutaneous imipenem/cilastatin twice daily (25 mg/kg doses).[29] Mice were humanely euthanized if core body temperature declined <32°C or weight loss was >20%.

Measurement of HDL-C, Apolipoprotein-AI, Low-Density Lipoprotein Cholesterol, CETP Activity, and Cytokines in Mouse Plasma

HDL-C (Crystal Chem, catalog No. 79990), apolipoprotein-AI (Abcam, catalog No. ab238260), low-density lipoprotein cholesterol (LDL-C; Crystal Chem, catalog No. 79980), exogenous CETP activity, which is independent of endogenous lipoproteins as donors and acceptors (Sigma-Aldrich, catalog No. MAK106), cytokines (Th1/Th2 Cytokine and Chemokine 20-Plex Mouse ProcartaPlex Panel 1, Invitrogen, catalog No. EPX200-26090-901; Mouse IL-1 beta/IL-1F2 Quantikine High-Sensitivity ELISA Kit, R&D Systems, catalog No. MHSLB00), blood urea nitrogen (Thermo Fisher Scientific, catalog No. EIABUN), and creatinine (Sigma-Aldrich, catalog No. MAK080) were measured in mouse plasma samples according to the manufacturers' instructions. Each sample was measured in technical duplicate.

Statistical Analyses

Analyses were performed using GraphPad Prism version 8.2.0 (GraphPad Software, Inc.), Stata 13.1, or R version 3.5.1 (R Core Team, 2013). For comparisons between 2 groups, data were analyzed using an unpaired t test, Mann-Whitney U test, or χ^2 test as appropriate. For comparisons between >2 groups, a 1-way ANOVA or Kruskal-Wallis test with Tukey or Dunn post hoc test was performed, respectively.

Unadjusted and age- and sex-adjusted linear regression models were used to test the association between HDL-C levels and *CETP* polygenic score. Time-to-event analyses for risk of sepsis mortality between genetic groupings were analyzed using Log-rank tests or Cox proportional hazards models with the "survival" package version 2.43 to 3 and "simPH" package version 1.3.10 for R. Cox proportional hazards models were adjusted for age and sex or Acute Physiology and Chronic Health Evaluation III score and sex as described. An inverse variance, weighted fixed-effect meta-analysis was performed with the "meta" package version 4.9 to 7. Heterogeneity was assessed using Cochran Q-statistic and Higgin and Thompson I^2 test.

Mouse and cytokine data were normalized by log-transformation. We used K-means clustering with k set to 2 to cluster mouse cytokine data from using the proinflammatory cytokines related to innate immunity including interleukin-1β, interleukin-6, tumor necrosis factor-α, interleukin-18, interleukin-12 p70, granulocyte-macrophage colony-stimulating factor, macrophage inflammatory protein-2, monocyte chemoattractant protein-1, monocyte chemoattractant protein-3, and macrophage inflammatory protein-1α.

All measurements presented were taken from distinct samples. Statistical significance was claimed when 2-sided *P* values were ≤0.05. When stated, *P* values were adjusted for multiple testing using the Benjamini-Hochberg procedure.

RESULTS

Genetic Variants in CETP Associate With Acute Mortality From Sepsis

The *CETP* gain-of-function variant, rs1800777, is associated with lower levels of HDL-C during sepsis and increased risk of organ failure and death.[9,14] Consistent with previous reports,[9,31] we found that carriers of the *CETP* gain-of-function variant from the general population had significantly lower levels of HDL-C relative to noncarriers in the UK Biobank cohort (1.30 versus 1.39 mmol/L, *P*=0.0001; Table V in the Data Supplement).

We validated the association between rs1800777 and death from sepsis by showing that carriers of this *CETP* gain-of-function variant had greater risk of acute sepsis mortality in the UK Biobank (Figure 1A) and the iSPAAR cohorts (Figure 1B). These associations were significant in Cox proportional hazards models that were adjusted for age and sex for UK Biobank (hazard ratio [HR], 1.29 [95% CI, 1.03–1.60]; *P*=0.03) and Acute Physiology and Chronic Health Evaluation III score and sex for iSPAAR (HR, 1.61 [95% CI, 1.03–2.51]; *P*=0.04). There were no significant differences in the enrollment characteristics of *CETP* gain-of-function carriers versus noncarriers in either the UK Biobank or iSPAAR cohorts other than levels of HDL-C (Tables V and VI in the Data Supplement).

We next performed a meta-analysis of the effect of rs1800777 on sepsis mortality in UK Biobank, iSPAAR, Copenhagen General Population Study (Table VII in the Data Supplement; Figure I in the Data Supplement), and Copenhagen City Heart Study cohorts (Table VIII in the Data Supplement, Figure I in the Data Supplement), as well as the previously reported Early Infection, SPHI-CU2, and VASST cohorts.[9] This demonstrated a significant association between *CETP* gain-of-function variant and 28-day sepsis mortality (age- and sex-adjusted HR,

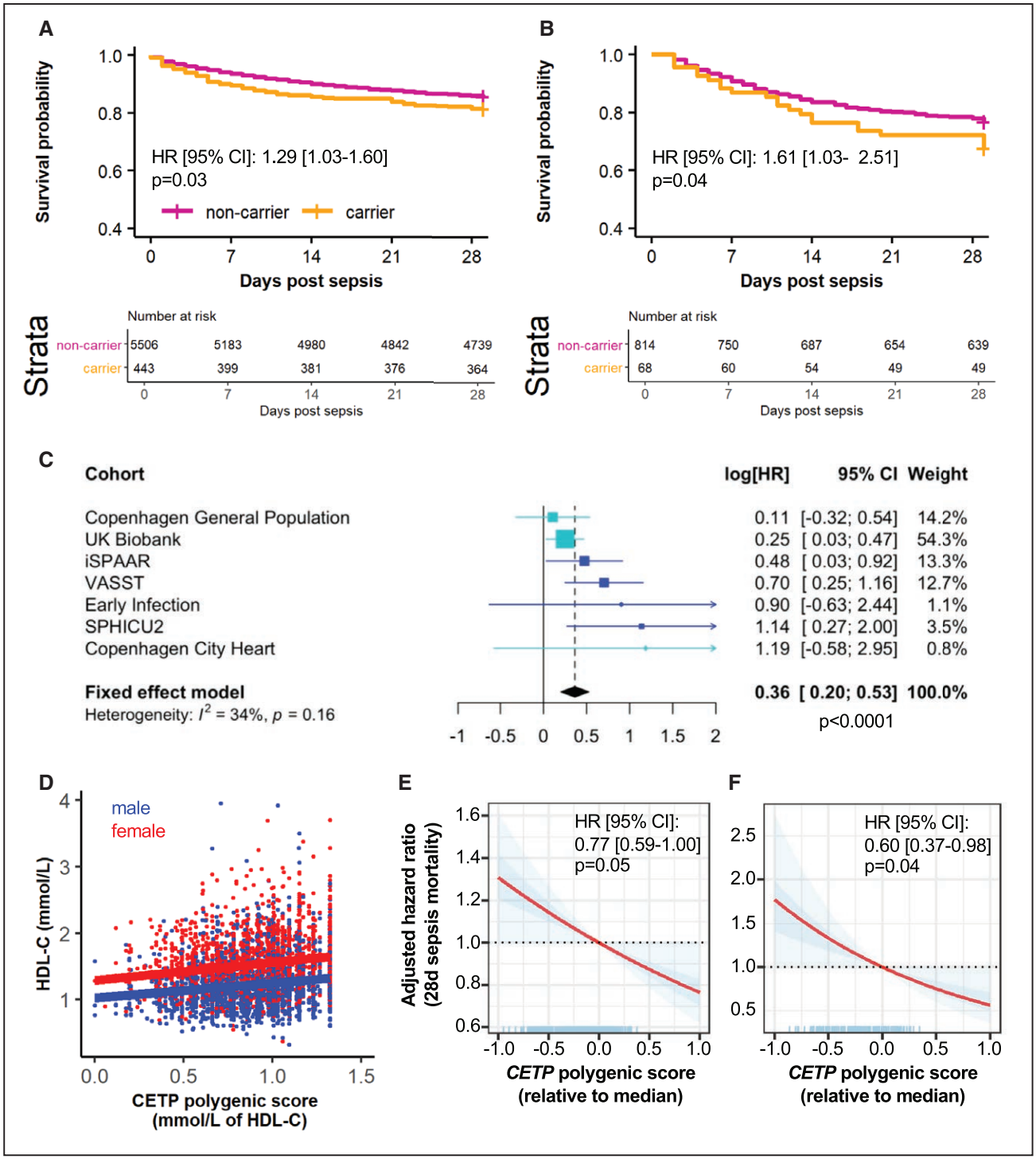

Figure 1. Genetic variants in CETP associate with acute mortality from sepsis.
The 28-day survival curves are displayed for individuals from the (**A**) UK Biobank and (**B**) iSPAAR (Identification of SNPs Predisposing to Altered Acute Lung Injury Risk) cohorts that experienced an episode of sepsis stratified by *CETP* gain-of-function carrier status (1800777-A). **C**, A fixed-effect meta-analysis displays the hazard ratio (HR) for the association between risk of 28-d sepsis mortality of CETP gain-of-function carrier status after adjustments for age and sex. **D**, The association between HDL-C levels and CETP polygenic score is shown for UK Biobank participants before their hospitalization for sepsis stratified by sex (female=red, blue=male). Adjusted HRs are displayed for risk of 28-d sepsis mortality vs *CETP* polygenic score for the (**E**) UK Biobank and (**F**) iSPAAR cohorts (light blue shading: 95% CI; darker blue shading: SE). CETP indicates cholesteryl ester transfer protein; HDL-C, high-density lipoprotein cholesterol; SPHICU2, St Paul's Intensive Care Unit version 2; and VASST, Vasopressin Versus Norepinephrine Infusion in Patients With Septic Shock.

1.44 [95% CI, 1.22–1.70]; *P*<0.0001; Figure 1C) and no evidence of interstudy heterogeneity (Q=9.16 with 6 degrees of freedom; *P*=0.16).

To investigate whether reduced CETP activity is associated with a reduction in sepsis mortality, we assessed the association between a *CETP* genetic score

(composed of genetic variants in the *CETP* gene that are associated with higher levels of HDL-C) and risk of acute mortality from sepsis.[24] Levels of HDL-C were significantly associated with *CETP* genetic score for individuals from the UK Biobank before their sepsis hospitalization when adjusted for age and sex (β [SE]: 0.25 [0.021]; $P<0.0001$; $R^2=0.17$; Figure 1D). We observed a significant inverse association between *CETP* genetic score and acute sepsis mortality for UK Biobank participants who were hospitalized for sepsis (age- and sex-adjusted HR, 0.77 [95% CI, 0.59–1.00] per 1 mmol/L of genetically predicted HDL-C; $P=0.05$; Figure 1E). This inverse association between *CETP* genetic score and sepsis mortality was replicated in the iSPAAR cohort (APACHE III score [Acute Physiology and Chronic Health Evaluation]- and sex-adjusted HR, 0.60 [95% CI, 0.37–0.98] per 1 mmol/L of genetically predicted HDL-C; $P=0.04$; Figure 1F).

Pharmacological Inhibition of CETP Reduces the Severity of Endotoxemia

To investigate the effect of pharmacological inhibition of CETP on sepsis, we used female APOE*3-Leiden.CETP mice, a well-established model of human-like lipoprotein metabolism that responds to the HDL-C–raising effects of CETP inhibition.[25,27,32] Given that torcetrapib was associated with increased risk of mortality from infection in humans,[33] increased proinflammatory atherosclerotic lesions in mice,[32] and could potentially interfere with the elimination of pathogen-associated lipids,[34] we first assessed whether 3-week, subchronic treatment with anacetrapib would result in adverse immune responses to infusion of LPS, the critical inflammatory mediator of Gram-negative sepsis (Figure 2A). After 2 weeks of treatment and before LPS exposure, mice treated with the CETP inhibitor had 72% higher levels of HDL-C relative to mice treated with placebo (mean±SD, 2.45±0.91 versus 1.31±0.68 mmol/L; unpaired t test, $P=0.0002$; Figure II in the Data Supplement). There was no significant difference in LDL-C levels between CETP inhibitor– and placebo-treated mice (Figure II in the Data Supplement; unpaired t test, $P=0.69$).

Six hours after administration of LPS, HDL-C levels declined substantially relative to baseline levels, which is consistent with human sepsis.[9–11] However, HDL-C levels were significantly higher in mice treated with CETP inhibitor relative to placebo at 6 hours after LPS exposure (mean±SD, 1.15±0.42 versus 0.63±0.41 mmol/L; unpaired t test, $P<0.001$; Figure 2B). Plasma apolipoprotein-AI levels were also significantly higher in mice treated with CETP inhibitor relative to placebo (median±SD, 44.3±24.6 versus 11.0±10.2 μg/mL; Mann-Whitney test, $P<0.0001$; Figure 2C). LDL-C levels did not differ between CETP inhibitor– and placebo-treated mice (Figure 2D). Mice treated with CETP inhibitor displayed significantly less severe symptoms of endotoxemia than placebo mice, as reflected by the core body temperature at 6 hours after LPS exposure (Figure 2E) and the Murine Clinical Assessment Score through the 6 hours of endotoxemia (Figure 2F).

CETP Inhibition Is Associated With a Less Proinflammatory Cytokine Profile After Endotoxemia

We assessed the levels of 20 cytokines related to T helper 1 versus T helper 2 inflammatory responses at 6 hours after LPS administration. Mice treated with CETP inhibitor had significantly lower levels of interleukin-12 p70 and macrophage inflammatory protein-1α than mice treated with placebo (Figure III in the Data Supplement). Placebo-treated mice that received intravenous saline instead of LPS had cytokine levels that were extremely low or below the limit of detection (data not shown).

To further explore the effect of CETP inhibition on levels of proinflammatory innate immune–related cytokines, we used cluster analysis to group mice on the basis of the severity of innate immune–mediated inflammation. Mice that were categorized into the "high" inflammation group had significantly higher levels of tumor necrosis factor-α, interleukin-18, interferon-γ, interferon-γ–induced protein 10, interleukin-12 p70, growth-regulated oncogene-α/chemokine C-X-C motif ligand 1, chemokine C-C motif ligand 5, macrophage inflammatory protein-1α, monocyte-chemotactic protein 3/chemokine C-C motif ligand 7, macrophage inflammatory protein-2, and macrophage inflammatory protein-1β relative to mice in the "low" inflammation group (Figure III in the Data Supplement). When cytokine cluster groups were stratified by CETP inhibitor versus placebo treatment groups, mice that were treated with CETP inhibitor were significantly more likely to have "low" inflammation compared with mice treated with placebo (73.6% versus 31.3%; χ^2 $P=0.01$; Figure III in the Data Supplement).

Genetic Increase in CETP Associates With Delayed Inflammation in Patients

To further investigate the relevance of these findings to human physiology, we measured the levels of proinflammatory cytokines in patients with sepsis. In the VASST cohort, carriers of the *CETP* gain-of-function variant had significantly lower levels of circulating white blood cells early in the course of septic shock episode relative to noncarriers (Figure 3A), which could indicate a delay in the initial immune recruitment to the source of infection. Consistent with this reasoning, carriers of the *CETP* gain-of-function variant also had significantly higher levels of interleukin-8, a proinflammatory

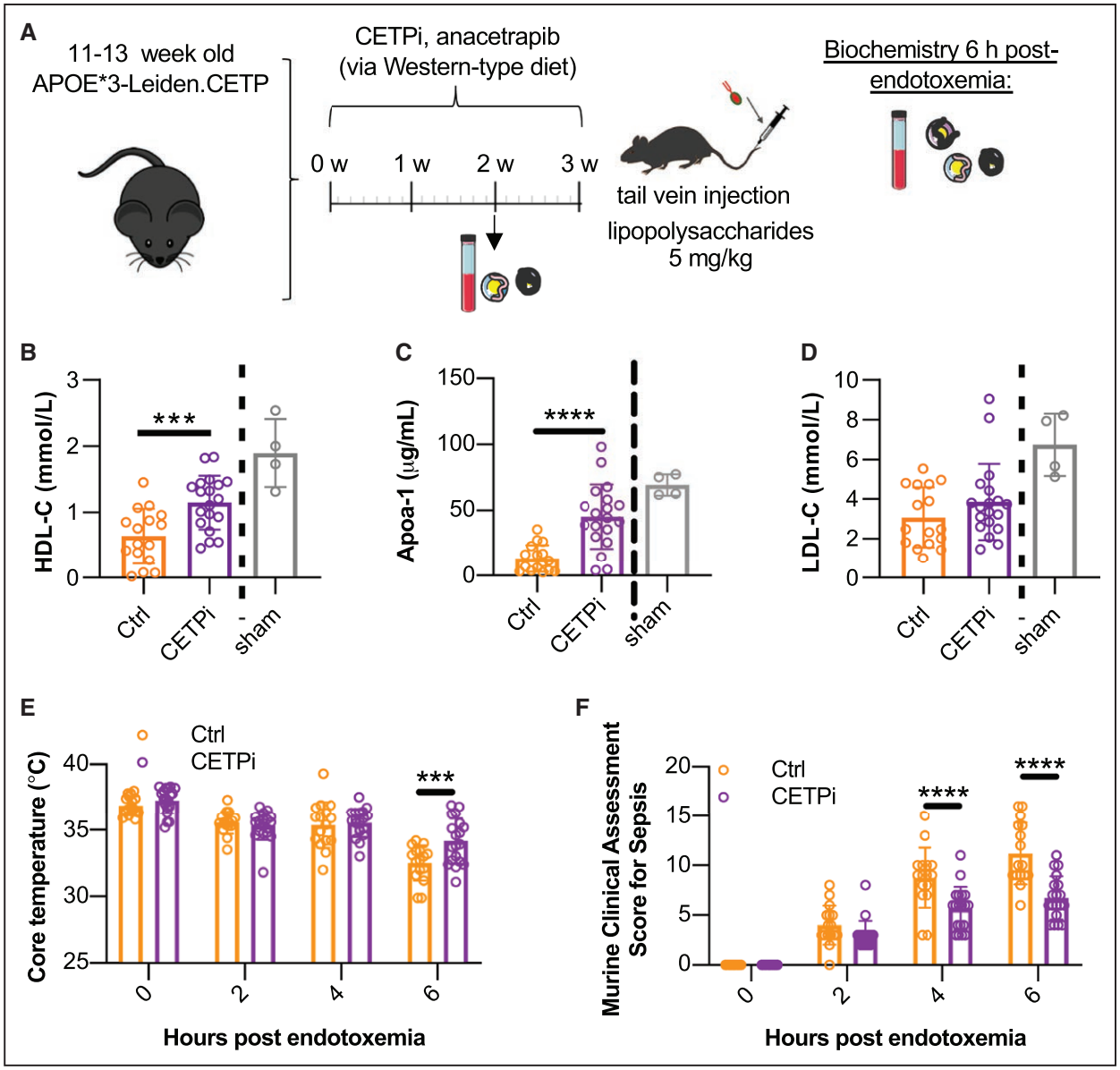

Figure 2. Pharmacological inhibition of CETP reduces the severity of endotoxemia.
A, Schematic of the endotoxemia model of sepsis used in this study. **B**, Female APOE*3-Leiden.CETP mice, fed a Western-type diet and exposed to CETP inhibitor (CETPi; n=19), anacetrapib, had significantly higher levels of plasma (**B**) HDL-C and (**C**) apolipoprotein-AI compared with placebo-treated (Ctrl, n=16) mice after 6 h of endotoxemia. **D**, There was no significant difference in plasma LDL-C levels between mice treated with CETP inhibitor and placebo after 6 h of endotoxemia. Mice exposed to CETPi displayed less severe sepsis symptoms as reflected by (**E**) core body temperature and (**F**) Murine Clinical Assessment Score for Sepsis. Data are displayed as mean±SD. ***P<0.001; ****P<0.0001. CETP indicates cholesteryl ester transfer protein; HDL-C, high-density lipoprotein cholesterol; and LDL-C, low-density lipoprotein cholesterol.

cytokine involved in recruiting neutrophils to the site of an infection and stimulating phagocytosis, compared with noncarriers in the VASST cohort (Figure 3B). The association between the *CETP* gain-of-function variant carrier status and elevated levels of plasma interleukin-8 at the time of admission for sepsis was replicated in the Early Infection cohort (Figure 3C). In addition, in the Early Infection cohort, which measured lipid levels at the time of emergency department admission for sepsis, levels of proinflammatory cytokines displayed a significant inverse association with continuous levels of HDL-C (interleukin-8: R^2=0.14, P<0.0001; interleukin-6: R^2=0.14, P<0.0001; and tumor necrosis factor-α: R^2=0.11, P<0.0001; Figure 3D). The levels of proinflammatory cytokines, interleukin-6, interleukin-8, and tumor necrosis factor-α were also significantly higher in patients who had HDL-C levels <0.65 mmol/L relative to ≥0.65 mmol/L, a clinically relevant cutoff that has been shown to predict sepsis mortality (Figure 3E).[10] These findings indicate that in both mice and humans, higher CETP activity during sepsis is associated with a greater proinflammatory response, and suggests that the results from mouse models of sepsis may have relevance to human physiology.

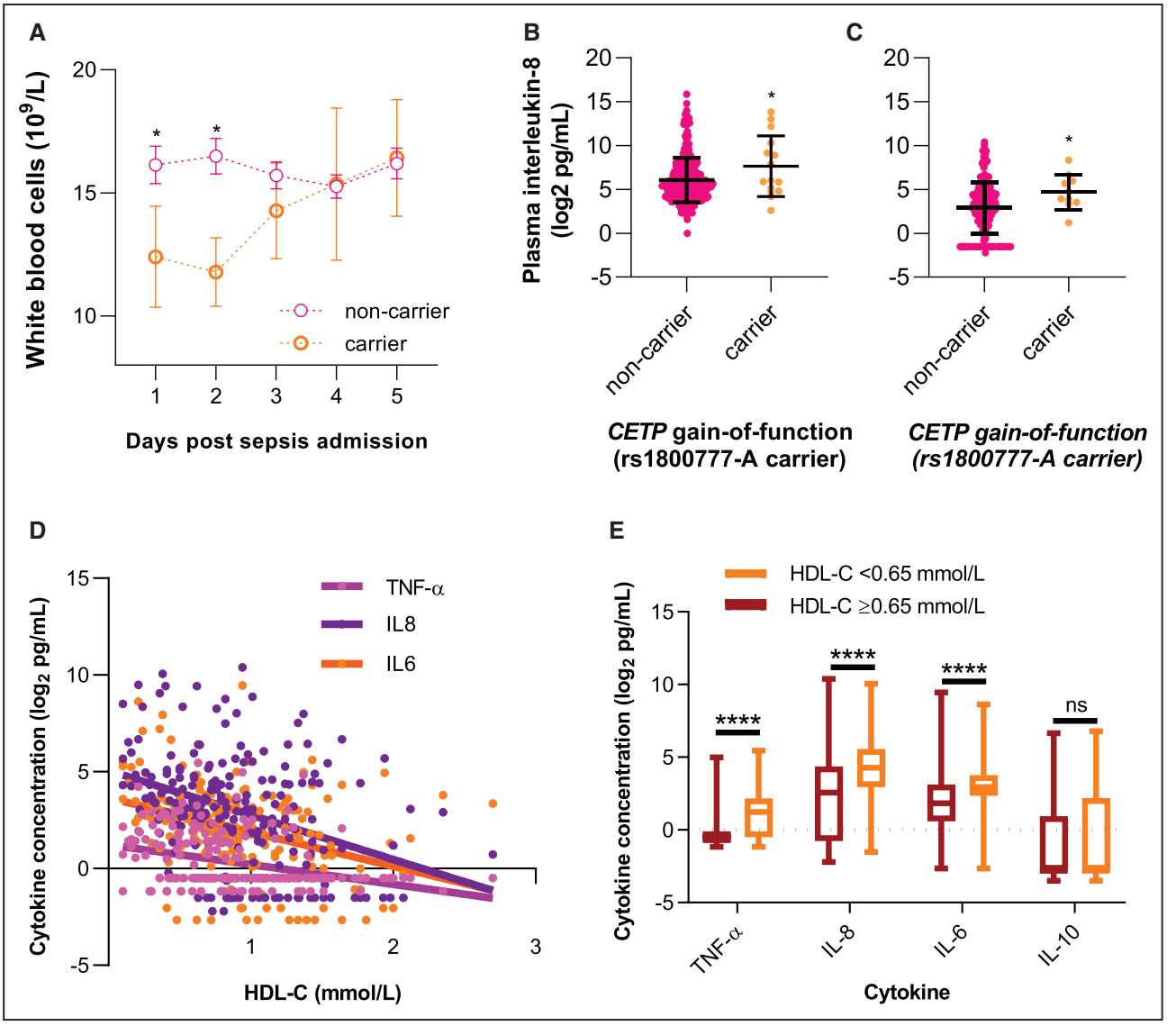

Figure 3. Genetic increase in CETP associates with delayed inflammation in individuals with sepsis.
A, The time-course of circulating white blood cells is displayed for carriers of the *CETP* gain-of-function variant, rs1800777-A, vs noncarriers in the VASST (Vasopressin in Septic Shock Trial) cohort. **B**, The levels of interleukin-8 in baseline plasma samples are displayed for carriers of the *CETP* gain-of-function variant vs noncarriers in the (**B**) VASST and (**C**) Early Infection cohorts. The levels of cytokines from baseline plasma samples of individuals from the Early Infection cohort are associated with (**D**) continuous levels of high-density lipoprotein cholesterol (HDL-C) and (**E**) a threshold of HDL-C previously determined to associate with clinical outcomes. Where applicable, data are displayed as mean±SD. **P*<0.05; *****P*<0.0001. CETP indicates cholesteryl ester transfer protein; IL, interleukin; ns, not significant; and TNF-α, tumor necrosis factor-α.

Acute Pharmacological Inhibition of CETP Reduces Sepsis Mortality

To investigate the therapeutic potential of CETP inhibition in sepsis, we tested the hypothesis that administration of anacetrapib after the onset of experimental sepsis would attenuate the decline in HDL-C and apolipoprotein-AI. To do so, we used the gold-standard CLP model, which better reflects the pathophysiology of human sepsis, including inflammation as well as infection, relative to LPS injection (Figure 4A). This experimental design mimics the "golden hour" approach of aggressive antimicrobial treatment and fluid resuscitation soon after the onset of sepsis in humans.[3,29,35] At 24 hours after CLP, mice treated with CETP inhibitor displayed significantly lower CETP activity, higher HDL-C levels, and higher apolipoprotein-AI levels compared with mice treated with placebo (HDL-C levels, mean±SD, 1.84±0.83 versus 1.00±0.18 mmol/L; Mann-Whitney test, *P*=0.008; Figure 4B–4D). There was no significant difference in LDL-C levels between CETP inhibitor– and placebo-treated mice (unpaired t test, *P*=0.55; Figure 4E). Mice treated with CETP inhibitor also displayed significantly lower levels of plasma creatinine and blood urea nitrogen than mice treated with placebo (Figure 4F and 4G).

To investigate the effect of CETP inhibition on sepsis mortality, we used a more severe CLP procedure,

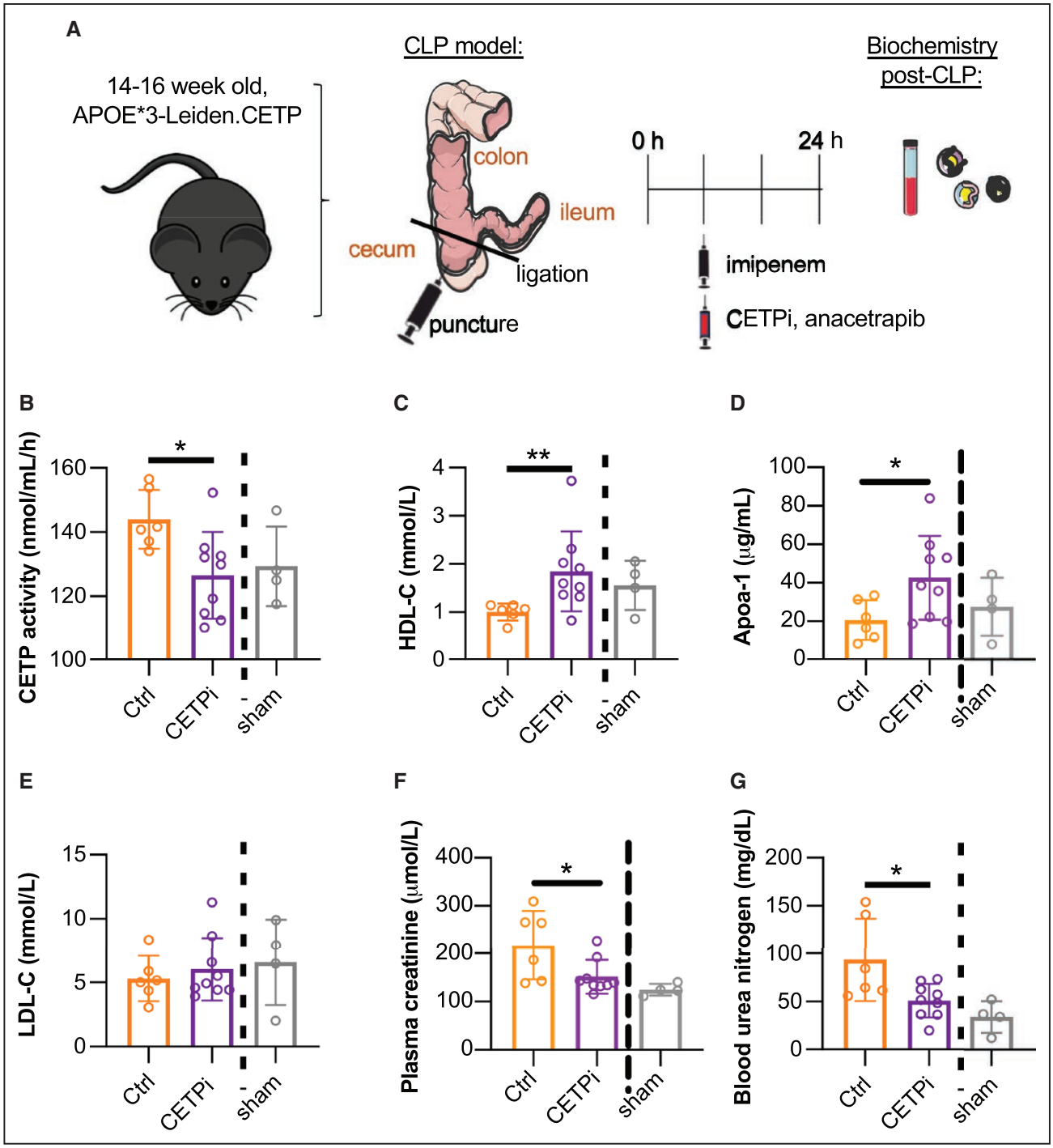

Figure 4. Acute pharmacological inhibition of CETP attenuates the decline in high-density lipoprotein cholesterol during sepsis.
A, Schematic of the cecal-ligation and puncture (CLP) model used in this acute biochemistry study in female APOE*3-Leiden.CETP mice fed a Western-type diet. Plasma (**B**) CETP activity, (**C**) HDL-C levels, (**D**) apolipoprotein-AI, (**E**) LDL-C levels, (**F**) plasma creatinine levels, and (**G**) blood urea nitrogen levels were measured in samples obtained at 24 h after CLP and were compared between mice treated with CETP inhibitor, anacetrapib (n=9), and placebo (Ctrl; n=6). Data are displayed as mean±SD. *P<0.05; **P<0.01. CETP indicates cholesteryl ester transfer protein; CETPi, cholesteryl ester transfer protein inhibitor; HDL-C, high-density lipoprotein cholesterol; and LDL-C, low-density lipoprotein cholesterol.

expected to result in higher mortality at 72 hours (Figure 5A).[29] APOE*3-Leiden.CETP mice treated with a single dose of CETP inhibitor at 6 hours after CLP had significantly reduced risk of death compared with mice treated with placebo (3-day survival: 70.6% versus 35.3%; Log-rank P=0.03; Figure 5B). We repeated this study in APOE*3-Leiden.CETP mice fed a chow rather than a cholesterol-rich, Western-type diet, which results in a less robust HDL-C response to CETP inhibitor.[25] Under these conditions, we observed a directionally similar improvement in survival after CETP inhibitor treatment (3-day survival: 68.8% versus 47.1%; Log-rank P=0.06;

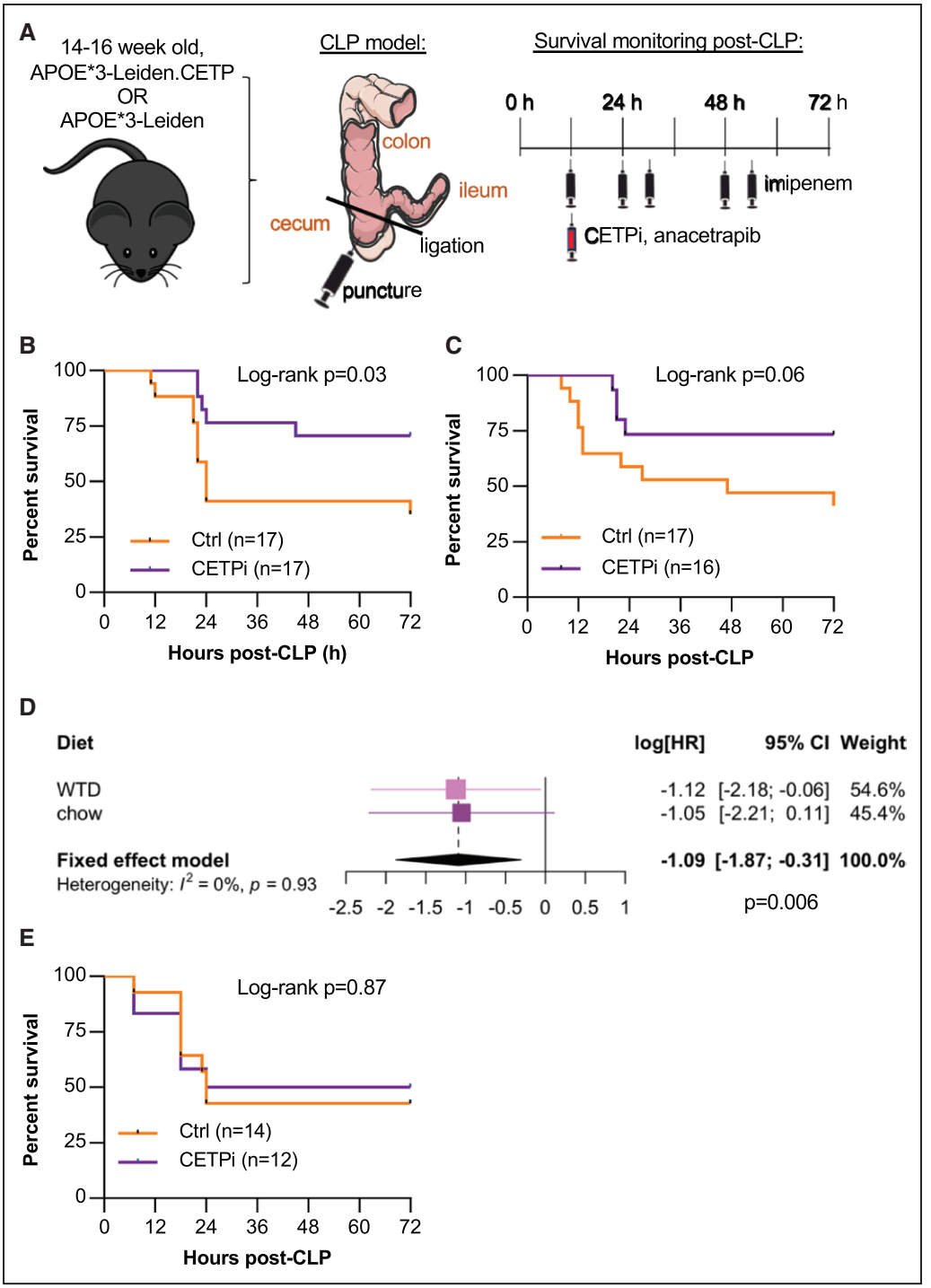

Figure 5. Acute pharmacological inhibition of CETP reduces sepsis mortality.
A, Schematic of the cecal-ligation and puncture (CLP) model used in the acute survival study. Female APOE*3-Leiden.CETP mice exposed to the CETP inhibitor, anacetrapib, had improved acute survival after the CLP procedure when previously fed a (**B**) Western-type diet (WTD) and (**C**) regular chow diet. **D**, The results of these 2 studies displayed minimal heterogeneity in an inverse variance fixed effect meta-analysis. **E**, After 3-week exposure to WTD, there was no trend toward improved survival in APOE*3-Leiden mice, which do not express *CETP*, when acutely treated with the CETP inhibitor, anacetrapib, after the CLP procedure. CETP indicates cholesteryl ester transfer protein; CETPi, cholesteryl ester transfer protein inhibitor; Ctrl, control; and HR, hazard ratio.

Figure 5C). When the results of these separate studies (3-day survival on Western-type versus chow diet) were combined in an inverse-variance fixed-effect meta-analysis to test the heterogeneity of study results, the results were highly statistically significant (HR, 0.34 [95% CI, 0.15–0.74]; *P*=0.006) and displayed little heterogeneity (Q=0.01 with 1 degree of freedom, *P*=0.93; Figure 5D).

To confirm that the effect of anacetrapib on sepsis mortality was dependent on the ability of anacetrapib to raise HDL-C by inhibiting CETP (rather than being

a result of potential off-target effects of anacetrapib), we repeated the CLP experiment in APOE*3-Leiden mice, which do not express the *CETP* transgene. When these mice were treated with a single dose of CETP inhibitor at 6 hours after CLP, there was no significant difference in survival relative to placebo-treated animals (3-day survival: 50.0% versus 42.9%; Log-rank *P*=0.87; Figure 5E), indicating that the survival-enhancing effect of anacetrapib is dependent on the presence of CETP.

DISCUSSION

Here we provide evidence that CETP influences survival from sepsis in humans, and that pharmacological inhibition of CETP reduces mortality in mouse models of sepsis. Specifically, we show that, among individuals with sepsis, genetic variation in *CETP* that increases CETP activity and leads to lower levels of HDL-C is associated with increased sepsis mortality, whereas genetic variation leading to lower CETP activity leads to decreased sepsis mortality. Mouse endotoxemia and CLP models of sepsis mimicked the clinical phenotype of decreased HDL-C during sepsis. Moreover, both subchronic and acute inhibition of CETP were able to attenuate the decline of HDL-C that occurs during sepsis and improve survival in mouse models of sepsis. Our results indicate that HDL is a critical modulator of immunity and that HDL levels can effectively be targeted in the acute phases of severe infection with existing CETP inhibitors.

Our findings of reduced sepsis severity in mice receiving the CETP inhibitor, anacetrapib, are consistent with the previous animal studies using genetically increased HDL-C or infusions of HDL or apolipoprotein-AI mimetics.[17,36] An important advance from this study is the demonstration that the decline in both HDL-C and apolipoprotein-AI that occurs during sepsis can be blunted, and mortality can be reduced, by administering a small molecule after the onset of sepsis. Anacetrapib preserved both apolipoprotein-AI and HDL-C levels during sepsis, suggesting that CETP inhibition mitigated the accelerated catabolism of HDL particles and prevented the generation of dysfunctional acute-phase HDL that occurs during systemic inflammation and sepsis. From the perspective of clinical translation, contemporary CETP inhibitors are attractive in that they increase HDL-C and preserve HDL particle number via a mechanism relevant to the metabolic changes that occur during sepsis,[9,37,38] and have been shown to be safe and well tolerated in large clinical trials of patients with cardiovascular disease.[18–20]

Several studies suggest that the proinflammatory response to LPS is attenuated in mice and humans treated with reconstituted HDL or apolipoprotein-AI mimetic peptides relative to those treated with placebo.[17,36,39] Similarly, humans with genetically low HDL-C have a greater in vivo inflammatory and procoagulation response to LPS relative to matched controls.[7] Mechanistic work suggests that HDL suppresses the activity of Toll-like receptors and downstream proinflammatory cytokines such as interleukin-6, interleukin-12p40, and tumor necrosis factor-α by upregulating the activating-transcription factor 3[6], a critical negative regulator of the transcription of genes involved in innate immunity.[40] It is also possible that apolipoprotein-AI–mediated removal of cholesterol from the cell membrane of immune cells, such as macrophages, modulates the cellular responses to proinflammatory stimuli.[41] Apolipoprotein-AI–mediated removal of membrane cholesterol may also promote the proinflammatory response and immune cell recruitment to the local site of infection needed to contain and eliminate invading pathogens.[41,42] Our study and the work of others provide evidence for HDL playing an important role in modulating the immune response during a severe infection, but the spatial and temporal dynamics of these interactions remain to be clarified in vivo.

Our findings contrast with previous work reporting that mice transgenic for only human CETP had improved survival when subjected to the CLP[43] and intraperitoneal endotoxemia[44] models of sepsis relative to wild-type mice. A key difference between these studies and our work is the use of APOE*3-Leiden.CETP mice, which display a more "human-like" plasma lipid profile composed of lower levels of HDL-C and higher levels of very-low-density lipoprotein cholesterol and LDL-C.[45] Mice expressing CETP alone have modest changes in HDL-C in response to CETP inhibition, because they lack an appreciable concentration of very-low-density lipoprotein and LDL cholesterol as donors for the CETP-mediated bidirectional exchange of neutral lipids.[46] In our study, CETP was also inhibited for an acute to subchronic time span, which mimics what could occur in a clinical trial in humans.[43,44] The results of our study are also consistent with clinical studies observing that higher levels and activity of CETP during the early phases of sepsis associate with future organ damage and death.[9,14,47] CETP expression and plasma CETP activity are decreased in the presence of LPS,[37,38,48] a physiological response that is hypothesized to attenuate the decline in HDL during sepsis and enable HDL to sequester pathogen lipids or perform other immunomodulatory effects.[6,48] Therefore, on the basis of previous literature and the experimental observations from this study, the association between CETP with sepsis outcomes appears to be dependent on changes to HDL[9,14,16] rather than CETP itself having innate anti-infectious properties.[34] Consistent with this, we found that treatment with the CETP inhibitor anacetrapib had no effect on sepsis survival in APOE*3-Leiden mice that lacked the *CETP* transgene.

HDL-C is inversely associated with risk of infectious disease hospitalization[16,22] and mortality from sepsis[10,11] in several observation studies, and Mendelian randomization suggests that these relationships may be causal.[16] The association between the *CETP* gain-of-function variant, rs1800777, and poor sepsis outcomes has been described previously.[9,14] Here, we expand on those previous findings by showing that both human genetic and preclinical mouse studies suggest that CETP inhibition could be a viable therapeutic strategy for clinical sepsis. However, 1 CETP inhibitor (torcetrapib) significantly increased the risk of infection mortality in the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events trial of secondary prevention for patients with high cardiovascular risk,[33] which would appear to contradict our observations. The other CETP inhibitors, dalcetrapib,[19] evacetrapib,[20] and anacetrapib,[18] did not result in increased risk of severe infection in large randomized controlled clinical trials of secondary prevention of major adverse cardiovascular events. The evidence from this study and clinical trials[18–20] suggests that the increased risk of infectious deaths with torcetrapib is likely not a class effect of CETP inhibitors and may represent an off-target effect that is unique to torcetrapib.[32,34] Whether CETP inhibition can rapidly prevent the decline of HDL during human sepsis and improve clinical outcomes will require further study in randomized controlled trials of CETP inhibitor(s).

Our study has some limitations that are worthy of consideration. First, HDLs are heterogenous particles composed of numerous particle subpopulations that vary in size and both lipid and protein composition. Here, we assessed apolipoprotein-AI and the cholesterol content of HDL, which does not reflect other protein or lipid components of HDL that may be critical to their anti-infectious activity. Second, the clinical cohorts used for the association of *CETP* genetic variants with sepsis outcomes were predominantly composed of individuals of White ethnicity, and future studies will be essential to assess the generalizability of these findings in other populations. For instance, in contrast to White individuals, Black individuals are known to have higher HDL-C, which is paradoxically associated with increased risk of coronary artery disease, potentially because of dysfunctional HDL.[49,50] Given these ethnic differences in HDL biology, it will be necessary for future studies to evaluate whether the effect of CETP inhibition on sepsis is similar between different ethnic groups. Third, the precise mechanism(s) by which preservation of HDL particles results in improved sepsis outcomes remain to be conclusively determined.

In summary, in individuals with sepsis, gain- and loss-of-function variants in *CETP* are associated with increased and reduced mortality, respectively. Inhibition of CETP in mouse models of sepsis reduces proinflammatory cytokine responses, preserves HDL-C and apolipoprotein-AI levels, and promotes survival. These findings suggest that therapeutic modulation of blood lipids with existing CETP inhibitors may target pathways that are causal to the pathogenesis of severe infections and sepsis.

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Correspondence

Liam R. Brunham, MD, PhD, Centre for Heart Lung Innovation, Room 166, 1081 Burrard Street, Vancouver, BC V6Z 1Y6, Canada. Email liam.brunham@ubc.ca

Affiliations

Centre for Heart Lung Innovation (M.T., T.P., L.B., H.J.K., J.A.R., K.R.W., J.H.B., L.R.B.), Experimental Medicine Program (M.T., J.H.B., L.R.B.), and Department of Medicine (J.H.B., L.R.B.), University of British Columbia, Vancouver, Canada. Department of Medicine, Division of Endocrinology (Y.W., L.L.B., P.C.N.R.) and Einthoven Laboratory for Vascular and Regenerative Medicine (Y.W., L.L.B., P.C.N.R.), Leiden University Medical Center, The Netherlands. Department of Clinical Biochemistry (C.M.M., B.G.N., J.A.R.), The Copenhagen General Population Study (C.M.M., B.G.N., A.T.-H.), Herlev Gentofte Hospital, Department of Clinical Biochemistry, Rigshospitalet (A.T.-H.), and The Copenhagen City Heart Study, Frederiksberg Hospital (B.G.N., A.T.-H.), Copenhagen University Hospital, Denmark. Faculty of Health and Medical Sciences, University of Copenhagen, Denmark (C.M.M., B.G.N., A.T.-H.). Department of Microbiology and Immunology, The University of Western Ontario, London, Canada (B.A.D.). Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of Washington, Seattle (M.M.W., K.R.W.).

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Disclosures

None.

Supplemental Materials

Expanded Methods
Data Supplement Figures I–III
Data Supplement Tables I–VIII
References 51–66

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