Effect of Anacetrapib on Cholesterol Efflux Capacity: A Substudy of the DEFINE Trial

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BACKGROUND: Anacetrapib is the only cholesteryl ester transfer protein inhibitor proven to reduce coronary heart disease (CHD). However, its effects on reverse cholesterol transport have not been fully elucidated. Macrophage cholesterol efflux (CEC), the initial step of reverse cholesterol transport, is inversely associated with CHD and may be affected by sex as well as haptoglobin copy number variants among patients with diabetes mellitus. We investigated the effect of anacetrapib on CEC and whether this effect is modified by sex, diabetes mellitus, and haptoglobin polymorphism.

METHODS AND RESULTS: A total of 574 participants with CHD were included from the DEFINE (Determining the Efficacy and Tolerability of CETP Inhibition With Anacetrapib) trial. CEC was measured at baseline and 24-week follow-up using J774 macrophages, boron dipyrromethene difluoride–labeled cholesterol, and apolipoprotein B–depleted plasma. Haptoglobin copy number variant was determined using an ELISA assay. Anacetrapib increased CEC, adjusted for baseline CEC, risk factors, and changes in lipids/apolipoproteins (standard β, 0.23; 95% CI, 0.05–0.41). This CEC-raising effect was seen only in men (P interaction=0.002); no effect modification was seen by diabetes mellitus status. Among patients with diabetes mellitus, anacetrapib increased CEC in those with the normal 1-1 haptoglobin genotype (standard β, 0.42; 95% CI, 0.16–0.69) but not the dysfunctional 2-1/2-2 genotypes (P interaction=0.02).

CONCLUSIONS: Among patients with CHD, anacetrapib at a dose linked to improved CHD outcomes significantly increased CEC independent of changes in high-density lipoprotein cholesterol or other lipids, with effect modification by sex and a novel pharmacogenomic interaction by haptoglobin genotype, suggesting a putative mechanism for reduced risk requiring validation.

Key Words: cholesteryl ester transfer protein ▪ coronary heart disease ▪ diabetes mellitus ▪ haptoglobin genotype ▪ sex

Low high-density lipoprotein (HDL) cholesterol (HDL-C) is a significant risk factor for atherosclerotic cardiovascular disease (ASCVD).³ However, HDL has many important functions that are not reflected by the cholesterol content and that may serve as potential therapeutic targets.³ The main antiatherosclerotic function ascribed to HDL is reverse cholesterol transport (RCT), the process by which cholesterol moves from the periphery to the liver and out of the body.⁴–⁶ Impaired RCT is inversely associated with atherosclerosis in both human and animal studies.⁷ Macrophage-specific cholesterol efflux capacity (CEC) is the key first step of RCT. CEC is inversely associated with both prevalent and incident ASCVD independent of HDL-C level.⁸–¹²

However, studies assessing the effects of interventions on CEC have been mixed, involved heterogeneous populations, and often included medications not yet proven to improve ASCVD risk.¹³–¹⁵ Anacetrapib is the only CETP (cholesteryl ester transfer protein) inhibitor that has been shown to reduce ASCVD.¹⁶,¹⁷ Its effects on lipids, not unlike
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Other CETP inhibitors, are diverse, including marked increases in HDL-C and apolipoprotein A-I (ApoA-I) as well as decreases in apolipoprotein B (ApoB) and lipoprotein (a) (Lp[a]). In a small study, anacetrapib 300 mg was shown to improve CEC\(^1\); however, its effect on CEC at the dose proven to reduce ASCVD (100 mg) among patients with coronary heart disease (CHD) is not well described.

Intriguingly, recent observational studies have suggested that CEC levels may not only differ by sex but that sex may modify the association between CEC and ASCVD.\(^1\) Whether this is relevant to anacetrapib’s effects on CEC is unknown. Beyond sex, haptoglobin is emerging as an HDL-associated protein that may impact CEC.\(^1\) Normally, haptoglobin binds free and glycosylated hemoglobin, preventing oxidative damage. However, in the context of hyperglycemia, a common structural variant (“2” allele) leads to increased haptoglobin-hemoglobin complexes with impaired clearance. These complexes bind to ApoA-I and other HDL-associated enzymes, enhancing their oxidation and reducing CEC and overall RCT.\(^1\) In addition, among those with diabetes mellitus (DM), the “2” allele has been linked to impaired coronary endothelial function\(^2\) and modifies the efficacy of intensive glucose control in reducing the risk for CHD.\(^3\) It remains unknown whether such pharmacogenomic interaction is relevant to a CETP inhibitor such as anacetrapib.

Thus, we sought to test the effect of anacetrapib at a dose proven to reduce ASCVD outcomes on CEC within the context of a randomized controlled trial (DEFINE [Determining the Efficacy and Tolerability of CETP Inhibition With Anacetrapib]) and whether this effect is modified by sex, DM, and haptoglobin polymorphism status.

METHODS

This study was conducted via an agreement with Merck through an investigator-initiated proposal. The data will be made available to any investigator after request and approval by both the senior author and Merck subject to the terms of the agreement. As previously reported in the DEFINE trial, the institutional review boards at each participating center approved the trial protocol. The UT Southwestern Medical Center institutional review board determined that this substudy was exempt.

Study Population

The DEFINE trial was a double-blind, randomized, placebo-controlled trial of anacetrapib 100 mg in participants at high risk on statin therapy. It enrolled 1623 participants with CHD or CHD risk equivalents from 153 centers in 20 countries from 2008 to 2010. Participants were treated with either anacetrapib 100 mg or placebo.

**CLINICAL PERSPECTIVE**

What Is New?
- We demonstrate that among patients with coronary heart disease, anacetrapib increases cholesterol efflux capacity independent of changes in high-density lipoprotein cholesterol or other lipids.
- This increase in cholesterol efflux capacity is modified by sex along with a pharmacogenomic interaction by haptoglobin genotype in patients with diabetes mellitus.

What Are the Clinical Implications?
- Determining a patient’s haptoglobin genotype may be a strategy to identify patients most likely to benefit from interventions targeting reverse cholesterol transport in those with diabetes mellitus.
- In addition, interventions targeting high-density lipoprotein function may have differing effects based on demographics including sex.

**Nonstandard Abbreviations and Acronyms**

| Abbreviation | Definition
|--------------|------------------------------------------------|
| ACCORD       | Action to Control Cardiovascular Risk in Diabetes |
| BODIPY       | boron dipyrromethene difluoride |
| CEC          | cholesterol efflux capacity |
| CETP         | cholesteryl ester transfer protein |
| CODAM        | Cohort on Diabetes and Atherosclerosis Maastricht |
| DEFINE       | Determining the Efficacy and Tolerability of CETP Inhibition With Anacetrapib |
| EPIC         | European Prospective Investigation into Cancer |
| JUPITER      | Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin |
| Lp(a)        | lipoprotein (a) |
| MESA         | Multi-Ethnic Study of Atherosclerosis |
| PREDMED      | Prevention With Mediterranean Diet |
| PREVEND      | Prevention of Renal and Vascular End-Stage Disease |
| RCT          | reverse cholesterol transport |
| REVEAL       | Randomized Evaluation of the Effects of Anacetrapib Through Lipid-Modification |
for 18 months. The details about the trial design along with inclusion/exclusion criteria have been previously reported.\textsuperscript{25} For this current study, the main inclusion criteria were history of CHD, clinical risk factors, lipid measurements, and available plasma at baseline and at 24 weeks. Exclusions included anyone without data at both time points and anyone developing an incident cardiovascular event before the follow-up visit. The inclusion of only participants with CHD restricted the number of participants to 888. Given that the DEFINE trial enrolled mostly White men with a small number of women and non-White participants, we selected a random sampling of white men from each treatment group matched for age, sex, DM status, and type of statin based on prior work regarding the variability of the assay. All women and all non-White participants were included. This selection process ensured adequate information in important subgroups. A total of 600 participants were selected a priori. Of these, 574 participants had complete data and were assessed at baseline and 24-week follow-up (Figure S1).

Clinical and Anthropometric Measurements
Demographic characteristics, presence of DM and CHD, and statin use were obtained at enrollment during the trial. Fasting lipid and lipoprotein measurements (HDL-C, low-density lipoprotein cholesterol [LDL-C], ApoA-I, ApoB, Lp(a), and triglycerides) were collected at baseline and 24-week follow-up and performed by the study core laboratory.\textsuperscript{25} LDL-C was calculated using the Friedewald equation; if triglycerides were >400 mg/dL, LDL-C was directly measured. The remainder of lipid measurement methods can be found as previously reported in the DEFINE trial.

Measurement of CEC
CEC was assessed from fasting EDTA plasma samples obtained at baseline and at 24-week follow-up stored at \textdegree{}80°C. CEC measured the efflux of boron dipyrromethene difluoride (BODIPY) fluorescently labeled cholesterol from J774 macrophages to ApoB-depleted plasma from study participants using a previously described method\textsuperscript{26} (Table S1). Baseline and follow-up specimens were measured on the same plate. CEC was normalized to efflux elicited by pooled human plasma run on every plate. Study personnel were blinded to treatment group and demographic and clinical information.

Measurement of Haptoglobin
Haptoglobin copy number variant status was ascertained in all participants using a commercially available ELISA assay (Savyon Diagnostics, Ltd.) from the same baseline frozen EDTA plasma used for CEC. This copy number variant (termed \textit{allele} 2) results in polymerization of haptoglobin and a structurally abnormal protein, which can be reliably detected by ELISA. Using the assay, each participant was identified as having the 1-1 (normal), 2-1 (heterozygous for abnormal “2” allele), or 2-2 (homozygous for abnormal “2” allele) copy number variant genotypes.\textsuperscript{27} Prior studies have established that allele 2 confers impaired HDL function only among patients with DM and not among those without DM. Thus, the focus of this analysis in our study was on participants with DM. Study personnel were blinded to treatment group, CEC measurements, demographics, and clinical information.

Statistical Analysis
Study participants were stratified by treatment status (placebo versus anacetrapib). Clinical and demographic characteristics along with CEC were compared among groups using Wilcoxon rank sum test for unpaired variables and Wilcoxon matched-pairs signed rank test for paired variables comparing patient data between time points. Unadjusted changes in CEC were reported as percent change in each treatment group. In multivariate models, the main outcome was follow-up CEC adjusted for baseline CEC. Multivariate adjusted linear regression analyses were performed to evaluate the independent associations between lipoproteins and follow-up CEC in the placebo and anacetrapib groups separately, adjusted for baseline CEC, covariates of sex, ethnicity, DM status, and type of statin, along with baseline and change in HDL-C, ApoA-I, ApoB, and triglycerides. Each lipoprotein, for example HDL-C (baseline and change) was tested in separate models and then all lipoproteins were combined into one model. The association between anacetrapib 100 mg versus placebo and follow-up CEC was tested in linear regression models adjusted for baseline CEC, sex, ethnicity, DM, and type of statin, along with HDL-C, ApoA-I, ApoB, and triglycerides. Each lipoprotein, for example HDL-C (baseline and change) was tested in separate models and then all lipoproteins were combined into one model. The association between anacetrapib 100 mg versus placebo and follow-up CEC was tested in linear regression models adjusted for baseline CEC, sex, ethnicity, DM, and type of statin, along with HDL-C, ApoA-I, ApoB, and triglycerides. Each lipoprotein, for example HDL-C (baseline and change) was tested in separate models and then all lipoproteins were combined into one model. The association between anacetrapib 100 mg versus placebo and follow-up CEC was tested in linear regression models adjusted for baseline CEC, sex, ethnicity, DM, and type of statin, along with HDL-C, ApoA-I, ApoB, and triglycerides. Each lipoprotein, for example HDL-C (baseline and change) was tested in separate models and then all lipoproteins were combined into one model. The association between anacetrapib 100 mg versus placebo and follow-up CEC was tested in linear regression models adjusted for baseline CEC, sex, ethnicity, DM, and type of statin, along with HDL-C, ApoA-I, ApoB, and triglycerides. Each lipoprotein, for example HDL-C (baseline and change) was tested in separate models and then all lipoproteins were combined into one model. The association between anacetrapib 100 mg versus placebo and follow-up CEC was tested in linear regression models adjusted for baseline CEC, sex, ethnicity, DM, and type of statin, along with HDL-C, ApoA-I, ApoB, and triglycerides. Each lipoprotein, for example HDL-C (baseline and change) was tested in separate models and then all lipoproteins were combined into one model. The association between anacetrapib 100 mg versus placebo and follow-up CEC was tested in linear regression models adjusted for baseline CEC, sex, ethnicity, DM, and type of statin, along with HDL-C, ApoA-I, ApoB, and triglycerides. Each lipoprotein, for example HDL-C (baseline and change) was tested in separate models and then all lipoproteins were combined into one model.
statistical analyses were performed in SAS version 9.4 (SAS Institute Inc.).

RESULTS

Baseline Characteristics

Baseline characteristics of the study participants in the placebo and anacetrapib groups are shown in Table 1. There were no significant differences at baseline between the placebo and anacetrapib groups regarding age, race, BMI, or presence of DM. However, there was a greater percentage of women in the placebo group compared with the anacetrapib group (46% versus 35%, respectively; *P*=0.008). The type of statin significantly differed between groups such that in the placebo group more participants took simvastatin while fewer participants took atorvastatin, rosuvastatin, pravastatin, or lovastatin (overall *P*=0.007). Due to the selection criteria for this substudy resulting in inclusion of all women and non-White participants with CHD, baseline characteristics differ from the overall DEFINE trial participants (Table S2). The percentage of women in this substudy is greater than in the overall DEFINE trial (35%–46% versus 22%–24%, respectively). The percentage of White participants is lower than in the overall DEFINE trial (77%–79% versus 82%–85%, respectively). The percentage of participants with DM was greater than in the overall DEFINE trial (60%–65% versus 53%, respectively). Finally, the percentage of those on each statin differed from the overall DEFINE trial (higher percentage of rosuvastatin and lower percentage of simvastatin in the anacetrapib group, respectively).

Effect of Anacetrapib on Lipids and Apolipoproteins

Baseline and follow-up lipids, apolipoprotein levels, and CEC are shown in Table 2. At baseline, there

Table 1. Baseline Characteristics Comparing the Placebo Group Versus the Anacetrapib Group

|                          | Placebo (n=289) | Anacetrapib (n=285) | *P* Value* |
|--------------------------|-----------------|---------------------|------------|
| Age, median (IQR), y     | 63.1 (56.9–69.6) | 62.1 (55.2–68.1)   | 0.24       |
| Women                    | 132 (46)        | 99 (35)             | 0.008      |
| White race               | 223 (77)        | 226 (79)            | 0.55       |
| BMI, median (IQR), kg/m² | 29.6 (27.0–33.4) | 29.4 (26.3–33.2)   | 0.56       |
| DM                       | 188 (65)        | 171 (60)            | 0.23       |
| Atorvastatin             | 97 (33.6)       | 101 (35.4)          | 0.007      |
| Simvastatin              | 132 (45.7)      | 95 (33.3)           |            |
| Rosuvastatin             | 49 (17.0)       | 62 (21.8)           |            |
| Pravastatin              | 4 (1.4)         | 13 (4.6)            |            |
| Lovastatin               | 6 (2.1)         | 13 (4.6)            |            |

Values are presented as number (percentage) unless otherwise indicated. BMI indicates body mass index; DM, diabetes mellitus; and IQR, interquartile range.

* *P* values are obtained using Wilcoxon rank sum test assuming nonparametric distributions for continuous variables and chi-square test for categorical variables.

†The *P* value for statins is shown as an overall *P* value.

Table 2. Baseline, Follow-Up, and Percent Change in Lipids, Apolipoproteins, and CEC

|                          | Placebo (n=289) | Anacetrapib (n=285) | *P* Value* |
|--------------------------|-----------------|---------------------|------------|
| Week 0                   | Week 24         | Median Percent Change | Week 0 | Week 24 | Median Percent Change |            |
| LDL-C, mg/dL             | 81 (68–94)      | 75 (65–87)          | −7.0 (−18.8 to 5.0) | 82 (69–94) | 42 (32–54) | −48.9 (−59.0 to −33.2) | <0.0001    |
| HDL-C, mg/dL             | 40 (34–47)      | 45 (38–51)          | 11.5 (2.1 to 26.3) | 40 (34–47) | 100 (82–117) | 145.2 (118.8 to 188.1) | <0.0001    |
| Triglycerides, mg/dL     | 131 (101–194)   | 130 (94–177)        | −3.3 (−21.2 to 16.4) | 125 (93–172) | 114 (90–146) | −8.7 (−25.1 to 13.7) | 0.09       |
| ApoA-I, mg/dL            | 145 (130–159)   | 145 (132–159)       | 0 (−7.8 to 9.2)    | 142 (127–157) | 207 (183–233) | 45.3 (31.6 to 59.6) | <0.0001    |
| ApoB, mg/dL              | 88 (77–101)     | 89 (77–101)         | 0 (−8.1 to 11.7)   | 86 (76–98) | 69 (60–78) | −20.9 (−30.7 to −8.7) | <0.0001    |
| Lipoprotein (a), nmol/L  | 27 (12–66)      | 31 (13–98)          | 6.7 (−3.4 to 42.5) | 27 (10–59) | 16 (5–55) | −15.1 (−49.7 to 0) | <0.0001    |
| CEC normalized           | 0.87 (0.77–1.01)| 0.84 (0.72–0.95)    | −3.9 (−20.4 to 12.8) | 0.86 (0.76–0.97) | 0.89 (0.79–1.01) | 4.6 (−13.6 to 28.4) | 0.0001     |

Values are presented as median (interquartile range). ApoA-I indicates apolipoprotein A-I; ApoB, apolipoprotein B; CEC, cholesterol efflux capacity; HDL-C, high-density lipoprotein cholesterol; and LDL-C, low-density lipoprotein cholesterol.

* *P* values for median percent change are obtained using Wilcoxon rank sum test assuming nonparametric distributions.
was a trend toward higher levels of triglycerides in the placebo group compared with the anacetrapib group (131 mg/dL versus 125 mg/dL, respectively; \( P \) value 0.06). There was no significant difference between the remainder of lipoproteins/apolipoproteins at baseline. After 24 weeks, in participants treated with anacetrapib, HDL-C increased by 60 mg/dL (145%) and ApoA-I increased by 65 mg/dL (45%); LDL-C decreased by 40 mg/dL (49%) and ApoB decreased by 17 mg/dL (21%); triglycerides decreased by 11 mg/dL (9%); and Lp(a) decreased by 11 nmol/L (15%). In the placebo group, HDL-C increased by 5 mg/dL (12%), ApoA-I and ApoB remained unchanged; LDL-C decreased by 6 mg/dL (7%); triglycerides decreased by 1 mg/dL (3%); and Lp(a) increased by 4 nmol/L (7%).

**Effect of Anacetrapib on CEC**

Compared with placebo, anacetrapib was associated with an 8.6% median increase in unadjusted CEC (\( P=0.0001 \)). When stratified based on sex and DM status in univariate analysis, a 12.5% and 15.5% median increase was seen in men and those without DM, respectively (\( P<0.0001 \) for both men versus women and DM versus no DM) (Figure 1). In multivariable models, anacetrapib was associated with increased CEC (standard \( \beta \), 0.23; 95% CI, 0.05–0.41) (Figure 2). When stratified based on sex, anacetrapib was associated with increased CEC in men (standard \( \beta \), 0.36; 95% CI, 0.13–0.58) but not in women (standard \( \beta \), −0.04; 95% CI, −0.35 to 0.26) (\( P \) for interaction=0.002) (Figure 2). However, when stratified based on the presence of DM in multivariable models, there was no significant interaction between anacetrapib and DM status (\( P \) for interaction=0.23) with similar standard \( \beta \) coefficients (Figure 2).

**Associations Between Changes in Apolipoproteins and Lipids With Change in CEC**

In the placebo group, change in CEC was positively associated with changes in ApoA-I (standard \( \beta \), 0.21; \( P=0.003 \)), ApoB (standard \( \beta \), 0.26; \( P=0.0002 \)), and triglycerides (standard \( \beta \), 0.24; \( P=0.0006 \)) (Table 3). In the fully adjusted model including all lipids/lipoproteins (HDL-C, ApoA-I, ApoB, and triglycerides), change in CEC remained positively associated with ApoA-I (standard \( \beta \), 0.21, \( P=0.02 \)) and ApoB (standard \( \beta \), 0.16; \( P=0.04 \)). Of note, in the placebo group there was no significant association between change in HDL-C and change in CEC in either the separate (standard \( \beta \), 0.05; \( P=0.39 \)) or fully adjusted (standard \( \beta \), −0.01; \( P=0.89 \)) models.
In the anacetrapib group, there remained a positive association between change in ApoB and change in CEC (standard $\beta$, 0.17; $P=0.02$) in the fully adjusted model (Table 3). There were no significant associations between change in HDL-C, ApoA-I, or triglycerides and change in CEC.

### Association Between Haptoglobin Copy Number and CEC

In 332 participants with DM, 51 (15%) had the 1-1 genotype, 162 (49%) had the 2-1 genotype, and 119 (36%) had the 2-2 haptoglobin genotype. Anacetrapib was positively associated with a significant increase in CEC in those with the 1-1 genotype (standard $\beta$, 0.42; $P=0.003$) but not in those with either 2-1, 2-2, or the combined 2-1/2-2 genotypes ($P$ for interaction=0.02) after adjustment for baseline CEC (Figure 3). After adjusting for baseline risk factors and change in lipoproteins, the positive association between anacetrapib and CEC in those with the “1” allele remained borderline significant (standard $\beta$, 0.55; $P=0.05$) ($P$ for interaction=0.08). In contrast, among those without DM, there was no significant interaction between anacetrapib and haptoglobin genotype for CEC ($P$ for interaction=0.36) (data not shown).

### DISCUSSION

In this post hoc substudy of patients with CHD enrolled in the DEFINE trial, anacetrapib 100 mg was associated with increased CEC independent of changes in lipids and apolipoproteins. The effect of anacetrapib was blunted in women and those with DM. Increases in CEC were linked more to changes in apolipoprotein levels than cholesterol levels. Among patients with DM, the effect of anacetrapib on CEC was blunted in those with the haptoglobin “2” allele. These findings suggest that anacetrapib improves a key HDL function, CEC, with heterogeneous effects by sex, DM status, and haptoglobin polymorphism.

CETP inhibitors cause marked increases in HDL-C levels and have also been shown to increase CEC, with varying effects by transporter-specific efflux. Transporter-specific CEC has typically been described for ABCA1-, ABCG1-, and SR-B1–mediated CEC; however, the consistency and accuracy of assay methodology has varied among studies. In patients with dyslipidemia, evacetrapib, when combined with statin therapy, increased both ABCA1-specific (27%) and non-ABCA1–specific CEC (15%) compared with statin monotherapy. When compared against...
placebo, evacetrapib demonstrated a larger increase in both ABCA1-specific (26%) and non-ABCA1–specific CEC (47%). In small studies involving patients with dyslipidemia, torcetrapib increased non-ABCA1–specific CEC. In a small number of patients with low to average HDL-C levels on pravastatin treated for 12 weeks, dalcetrapib 600 mg and 900 mg significantly increased SRB1 efflux 19% and 21%, respectively, with no effect on ABCA1-mediated CEC. A Study of Dalcetrapib in Patients Hospitalized for an Acute Coronary Syndrome (Dal-ACUTE), a multicentered trial of patients post–acute coronary syndrome, total and non-ABCA1–specific CEC was increased with 600 mg of dalcetrapib at 4, 12, and 20 weeks; however, ABCA1-specific CEC was not significantly increased. In 187 patients with dyslipidemia, increased ABCA1–specific CEC (67%) and total CEC (31%) compared with placebo; ABCA1-specific CEC increased by 16%, although nonsignificantly. These studies varied in type of CETP inhibitor, intensity of CETP inhibition, baseline risk and demographics of study populations, and duration of treatments, making comparisons of results related to CEC challenging.

In a small sample of 20 patients, treatment with anacetrapib 300 mg increased radiolabeled CEC by 2.4-fold. In our substudy involving 574 participants with CHD enrolled within a larger randomized placebo-controlled trial, anacetrapib 100 mg, a dose shown to improve cardiovascular outcomes, increased ApoA-I levels 45% while decreasing LDL-C levels 42%, ApoB levels 21%, Lp(a) levels 22%, and triglyceride levels 6%, results similar to the complete DEFINE trial. Since CETP inhibitors have varied effects on lipids including HDL-C, ApoA-I, and ApoB, it is not surprising that there may be varied effects on HDL function. We showed that anacetrapib at a dose proven to improve ASCVD outcomes (100 mg) increased CEC 8.6% compared with placebo on a background of statin therapy. We used fluorescently labeled cholesterol (BODIPY) in our assay system, which has been shown by others to be mostly driven by lipid-poor particles via ABCA1. However, defining ABCA1–specific CEC remains challenging and varies by assay methodology; thus, direct comparisons with prior studies may be limited.

Overall, changes in CEC were associated with changes in apolipoprotein levels but not in HDL-C. Prior studies have reported variations in these associations, likely related to differences in study populations and assay methodology. Studies using radiolabeled cholesterol to assess CEC typically report modest correlations with HDL-C (baseline and change), reflecting movement of cholesterol via ABCG1 and SR-BI transporters to larger, mature HDL particles. In the assay system used in this study, BODIPY cholesterol was used, showing primarily transport via ABCA1 to lipid-poor acceptor particles, hence the poor or negligible correlation with HDL-C. With respect to apolipoproteins, increased CEC in our study among patients with CHD was associated with increased ApoA-I and ApoB in the placebo group and only an increase in ApoB in the anacetrapib group. These findings are somewhat consistent with prior studies, most of which used radiolabeled cholesterol. In a case-control substudy of the JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial assessing the efficacy of rosuvastatin in patients free of CHD, baseline CEC was positively associated with ApoA-I and ApoB in addition to HDL-C and LDL-C. After 12 months, positive associations were seen between change in CEC and change in HDL-C and ApoA-I. In a nested case-control substudy from the EPIC-Norfolk study (patients free of CHD), CEC was positively associated with baseline HDL-C and ApoA-I but not ApoB. In the dal-ACUTE study in patients post–acute coronary syndrome, baseline ApoA-I but not HDL-C was positively associated with ABCA1–specific CEC, similar to our results. After 4 weeks, there was no association between ApoA-I or HDL-C levels and ABCA1–specific CEC in the placebo group. However, in the dalcetrapib group, there was a positive association between week 4 ApoA-I levels in addition to HDL-C levels and ABCA1–specific CEC. Associations between CEC and ApoB levels were not tested. The dal-ACUTE study population differed from our study in that they were within 1-week post–acute coronary syndrome. In addition, they found that dalcetrapib increased CEC primarily through the non-ABCA1–mediated pathway. Thus, apolipoproteins are clearly linked to change in CEC and either directly mediate CEC or reflect a lipid-protein milieu associated with CEC functionality. Comparison of the effects of CETP inhibitors on CEC is likely to reflect differences in effects on apolipoprotein levels, study population, and background statin therapy.

Intriguingly, we found that anacetrapib increased CEC in men but not in women, reflecting a statistically significant interaction by sex. To our knowledge, the impact of sex on CETP inhibition and CEC has not been thoroughly studied thus far. Baseline associations between sex and CEC have not been consistent among studies. Large studies including the JUPITER trial and EPIC (European Prospective Investigation into Cancer) Norfolk population-based cohort studies have shown that women have higher CEC than men using radiolabeled cholesterol. On the other hand, in ~3000 participants from the Dallas Heart Study, there was no association between sex and CEC using BODIPY cholesterol assay. Similar null associations were seen in controls from the PREVEND (Prevention of Renal and Vascular End-Stage Disease) and MESA (Multi-Ethnic Study of Atherosclerosis) trials using differentiated THP
human macrophages. However, in patients who developed incident ASCVD in the PREVEND cohort, CEC was significantly decreased in men compared with women. There was a significant interaction with sex such that CEC was inversely associated with ASCVD in men while it was directly associated in women. In a prospective case-control study from EPIC-Norfolk, an interaction between CEC and sex was seen with regard to incident cardiovascular disease such that there was an inverse association with men, while no association was seen with women. Addressing transporter-specific CEC, one study of healthy volunteers found that women had greater CEC via the SR-BI pathway but lower CEC compared with men via the ABCA1 pathway; there was no significant difference with regards to the ABCG1 pathway. The differences seen between CEC and sex in the literature may be attributable to the study population including the presence or absence of CHD in addition to the method used to obtain CEC. Our study examined the difference in anacetrapib-mediated CEC by sex among patients with CHD, adding to the current literature on sex and CEC. In our study involving patients with CHD, BODIPY cholesterol was employed, primarily reflecting the ABCA1 pathway and resulting in increased CEC with anacetrapib only among men, consistent with the transporter-specific investigation of baseline CEC. Thus, future investigation of therapies affecting CEC may need to account for possible sex-based effect modifications.

In addition to sex, we also explored the impact of DM on anacetrapib-mediated changes in CEC. Several prior studies except for one have shown little to no correlation between DM status and CEC. In almost 300 participants from the PREDIMED (Prevention With Mediterranean Diet) trial, the presence of DM at baseline visit was not associated with CEC adjusted for HDL-C. In the MESA cohort, Shea et al. also found no significant correlation between DM and CEC. Similar null associations were seen in the CODAM (Cohort on Diabetes and Atherosclerosis Maastricht) study. In addition, in vitro and mouse studies have shown that nonenzymatic glycation of HDL does not diminish CEC. Similar to sex, differences seen between CEC and DM in the literature may be attributable to the study population including the presence or absence of CHD in addition to the method used to obtain CEC. This relationship may also be influenced by the duration of DM, something not uniformly captured in studies. Our study examined the difference in anacetrapib-mediated CEC by DM status adding information concerning the relationship between DM and CEC. In our study, although there appeared to be a blunted effect of anacetrapib on CEC among patients with DM, in adjusted analyses there was no significant interaction, consistent with prior reports. Although this lack of effect modification by DM status is consistent with prior reports, it remains possible that the study was underpowered to detect an interaction in this analysis. Thus, DM by itself may not signify differential effects on CEC.

Despite this lack of effect modification by DM status, we sought to explore the potential for a pharmacogenomic interaction among patients with DM. Haptoglobin is an HDL-associated protein and binds to HDL and hemoglobin in a complex, primarily scavenging free hemoglobin to protect against oxidative damage. In the human genome, haptoglobin genes have been shown to exhibit copy number variance, with duplication events of exons 3 and 4 of the haptoglobin 1 allele, leading to the formation of 2 major common classes of alleles of haptoglobin (referred to as Hp1 and Hp2). The Hp2 category is further split into Hp 2-1 and Hp 2-2, depending on the number of Hp1 or Hp2 alleles that are expressed. Of importance to our study, the Hp2 allele (both 2-1 and 2-2 variants) can lead to altered HDL protein structure and function, specifically impaired antioxidative function and impaired cholesterol efflux. The abnormal “2” allele has been linked to impaired coronary endothelial function and increased cardiovascular risk in patients with DM. It has been hypothesized that the complex between glycosylated haptoglobin 2-2 protein and hemoglobin binds and oxidizes HDL along with the related lectin-cholesterol acyltransferase, decreasing the formation of mature HDL in the process of RCT. It is plausible that the decreased formation of mature HDL from lipid-poor HDL via the ABCA1 pathway may result in decreased CEC. In non-Hispanic Whites from the ACCORD (Action to Control Cardiovascular Risk in Diabetes) trial, it was shown that haptoglobin genotype could be helpful in guiding glycemic targets in DM to lower CHD risk. In this analysis, intensive glycemic control (glycated hemoglobin target <6.0%) decreased risk of CHD in the haptoglobin 2-2 genotype but not in those with the other genotypes. Some investigations have suggested that this haptoglobin-dependent HDL dysfunction can be improved with vitamin E supplementation, suggesting that haptoglobin genotype may signal potential pharmacogenomic interactions, but only in patients with DM.

Thus, we sought to investigate whether such an interaction exists for anacetrapib. Indeed, we found that the effect of anacetrapib on CEC was significantly blunted in individuals with DM and the haptoglobin “2” allele, whereas it was preserved among patients homozygous for the normal “1” allele. No such effect modification was noted in patients without DM. These findings support the notion that in the milieu of genetically impaired HDL structure/function, the beneficial effects of anacetrapib on CEC are mitigated, which may lead to differential protection against cardiovascular events.
Pharmacogenomic interactions have been identified for another CETP inhibitor, dalcetrapib, albeit a different genetic polymorphism. In the dal-OUTCOMES and dal-PLAQUE-2 trials, polymorphisms in the ADCY9 gene, an isoform of adenylate cyclase, were associated with impaired CEC and cardiovascular events.48,49 Different genotypes of the ADCY9 gene were associated with varying outcomes with dalcetrapib treatment showing either increased or decreased cardiovascular events. This same polymorphism did not translate into a pharmacogenomic interaction with evacetrapib or anacetrapib on lipid levels or outcomes.50,51 Thus, just as CETP inhibitors vary in their effects on lipids, apolipoproteins, and CEC, they may also vary with respect to pharmacogenomic interactions. Whether such an interaction between anacetrapib and haptoglobin “2” allele is linked to differential cardiovascular outcomes remains unknown, as does whether this pharmacogenomic interaction exists for other CETP inhibitors or alternative lipid-modifying drugs. Identifying a patient’s haptoglobin genotype suggests a strategy to identify patients most likely to benefit from interventions targeting RCT, specifically in patients with DM.

Several limitations in our study merit remark. First, this study was a substudy of a randomized trial and may not be generalizable to other populations. Second, whether anacetrapib-mediated increase in CEC is linked to the cardiovascular reductions seen in the REVEAL (Randomized Evaluation of the Effects of Anacetrapib Through Lipid Modification) trial is unknown. Third, this subset of patients was predominantly White, limiting generalizability. Fourth, we used an assay system that highlights the role of lipid-poor cholesterol acceptors primarily via ABCA1, thus direct comparisons with studies employing other assay systems may be limited. However, ABCA1-mediated CEC is thought to be the predominant pathway linked to CHD risk. Fifth, given the limited size of this study population, we are unable to draw firm conclusions on the interaction between DM status and haptoglobin genotype on CEC. Sixth, the restriction on the sample size to include only patients with complete data introduces the possibility of selection bias. In addition, excluding patients with incident cardiovascular events restricts the population to those with stable CHD, limiting generalizability.

CONCLUSIONS
Among patients with CHD, anacetrapib at a dose linked to improved CHD outcomes increased CEC. The effect of anacetrapib on CEC appears to be blunted in women. This CEC-raising effect was also blunted among patients with DM and concurrent polymorphism in the haptoglobin gene linked to dysfunctional HDL and increased cardiovascular risk.

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Supplemental Material
### Table S1. Major Resources.

#### Cultured Cells

| Name      | Vendor or Source | Sex (F, M, or unknown) | Persistent ID / URL               |
|-----------|------------------|------------------------|-----------------------------------|
| J774A.1   | ATCC® TIB-67™    | F                      | https://www.atcc.org/products/all/TIB-67.aspx |
Table S2. Comparison of Baseline Characteristics between overall DEFINE study and current substudy comparing placebo group vs. anacetrapib group.

|                          | DEFINE study Placebo (N = 812) | Substudy Placebo (N = 289) | DEFINE study Anacetrapib (N = 811) | Substudy Anacetrapib (N = 285) |
|--------------------------|--------------------------------|-----------------------------|-----------------------------------|-------------------------------|
| **Age, years, mean (SD)*** | 62.9 (9.0)                     | 62.4 (9.1)                  | 62.5 (8.7)                        | 61.5 (9.1)                    |
| **Females**              | 194 (23.9)                     | 132 (46)                    | 182 (22.4)                        | 99 (35)                       |
| **White race**           | 669 (82.4)                     | 223 (77)                    | 686 (84.6)                        | 226 (79)                      |
| **Body Mass Index**      | 30.1 (5.2)                     | 30.5 (5.4)                  | 30.4 (5.5)                        | 30.4 (5.6)                    |
| **Diabetes Mellitus**    | 432 (53.2)                     | 188 (65)                    | 430 (53.0)                        | 171 (60)                      |
| **Atorvastatin**         | 275 (33.9)                     | 97 (33.6)                   | 276 (34.0)                        | 101 (35.4)                    |
| **Simvastatin**          | 372 (45.8)                     | 132 (45.7)                  | 367 (45.3)                        | 95 (33.3)                     |
| **Rosuvastatin**         | 86 (10.6)                      | 49 (17.0)                   | 104 (12.8)                        | 62 (21.8)                     |
| **Other statin†**        | 72 (8.9)                       | 10 (3.5)                    | 60 (7.4)                          | 26 (9.1)                      |

*Values are presented as N (%) unless otherwise stated. †Other statin includes pravastatin and lovastatin.
Randomized in the DEFINE Trial (n = 1,623)

Participants with CHD (n = 888)

Participants selected for this substudy (n = 600)

Participants with complete data for both baseline and follow-up (n = 574)

Selection of all women and non-white participants plus random selection of white males matched for age, sex, diabetes status, type of statin

Diagram of participants included in substudy.