# Low-dose obicetrapib significantly increases concentrations of lipophilic antioxidants, apoE, and S1P in HDL subfractions

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## Background

- Obicetrapib is a next generation CETP inhibitor in phase 3 clinical development for the treatment of patients at risk for cardiovascular disease with elevated LDL cholesterol (1, 2).
- Obicetrapib also dramatically increases HDL cholesterol, HDL particle concentration, and apoA1, the main apolipoprotein in HDL (1, 2).
- Broadly, HDL can be distinguished into two subfractions: lipid-enriched HDL2 particles with low-density (1.063-1.125 g/mL) and protein-enriched HDL3 particles with high-density (1.125-1.21 g/mL); S1P is a bioactive sphingolipid that can be bound to lipoproteins and albumin, which is necessary for many of the physiological functions of HDL (3).
- Non-lipidated apoA1 (also known as pre-beta-1 HDL) captures cholesterol and dietary lipophilic antioxidants (see Figure 1) via the ATP-binding cassette transporter A1 (4-7).
- The majority of lutein and zeaxanthin is transported by HDL; it has been speculated that the smallest HDL subfractions are the ones largely responsible for moving antioxidants from the intestine to the plasma pool to the retina, where they would be important for preventing diseases such as age-related macular degeneration, and to the cerebrospinal fluid/brain, where they would be important for preventing diseases such as Alzheimer's disease (8-10).
- Preliminary investigations demonstrated CETP inhibition with obicetrapib increased pre-beta-1 HDL and increased the uptake of lipophilic antioxidants (lutein, zeaxanthin, and α-tocopherol) in HDL particles (11).
- This investigation was undertaken to further examine the effect of low-dose obicetrapib on the distribution among lipoproteins of apolipoproteins, S1P, cholesterol, and lipophilic antioxidants.

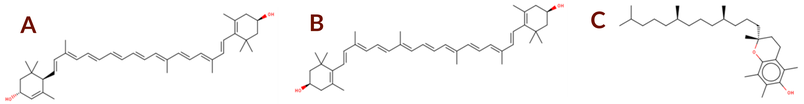


**Figure 1.** Chemical structures of (A) lutein, (B) zeaxanthin, and (C) α-tocopherol.

## Objective

To evaluate the effect of low-dose obicetrapib on the lipoprotein distribution of apoA1, apoE, S1P, cholesterol, lutein, zeaxanthin, and α-tocopherol in a trial of Japanese participants.

## Methods

- Phase II clinical trial (2) registered at ClinicalTrials.gov: NCT05421078.
- Participants: Japanese men and women (n=24 per dose group in the original trial; n=12 per dose group in this analysis) who had not achieved 2022 Japanese Atherosclerosis Society Guidelines for the Prevention of Atherosclerotic Cardiovascular Disease, with documented LDL cholesterol >70 mg/dL (or non-HDL cholesterol >100 mg/dL), while receiving stable statin therapy with either atorvastatin 10 or 20 mg/d or rosuvastatin 5 or 10 mg/d only for at least 8 weeks prior to screening.
- Participants received placebo or obicetrapib 2.5, 5, or 10 mg/d for 8 weeks. Groups included in this analysis were those that received placebo or 2.5 mg/d.
- Plasma samples for this analysis were collected at clinic visits occurring at baseline (V2) and after 2 weeks (V3) and 8 weeks (V5) of treatment.
- The separation of the two HDL subclasses (HDL2 and HDL3) from non-HDL was done with lipoprotein fractionation ultracentrifugation using a Beckman TLA-100.4 rotor.
- ApoA1 and apoE were measured by enzyme-linked immunosorbent assays (Diazyme Laboratories and abcam, respectively).
- S1P was determined by reversed phase ultra-performance liquid chromatography and tandem mass spectrometry (in multiple reaction monitoring mode). An external calibration curve was prepared by spiking different concentrations of S1P with isotopic internal standard.
- Cholesterol in the HDL and non-HDL fractions was measured by a colorimetric assay (Elabscience).
- Lipophilic antioxidants including lutein, zeaxanthin, and α-tocopherol were quantified by liquid chromatography-tandem mass spectrometry (Waters Corporation) in electrospray ionization mode.

## Results

- Placebo: apoA1, apoE, cholesterol, and antioxidants remained unchanged in the ultracentrifugation fractions, confirming stability of these parameters and reproducibility of the methods.
- Obicetrapib 2.5 mg:
  - Cholesterol
    - HDL cholesterol increased 135%; non-HDL cholesterol decreased 25.4%.
    - HDL2 fraction: cholesterol increased 172% and 302% at V3 and V5, respectively (both p < 0.0001)
    - HDL3 fraction: cholesterol increased 26.1% at V3 (p=0.062) and 49.7% at V5 (p = 0.0008).
  - ApoA1 and apoE
    - HDL2 fraction: apoA1 increased 265% and 355% at V3 and V5, respectively (both p < 0.0001)
    - ApoE shifted from the protein fraction (unbound apoE) to the HDL fractions, particularly the HDL2 fraction which increased 133% at V5 (p = 0.047).
  - Antioxidants
    - No significant and consistent changes in lutein, zeaxanthin, or α-tocopherol in the non-HDL, HDL3, and protein fractions at V3 and V5.
    - HDL2 fraction: obicetrapib increased lutein 202% at V3 (p = 0.0033) and 246% at V5 (p = 0.0002), zeaxanthin increased 203% and 302% at V3 and V5, respectively (both p < 0.0001), and α-tocopherol increased 250% and 258% at V3 and V5, respectively (both p < 0.0001)
  - S1P
    - Non-significant decrease in S1P in the non-HDL and protein fractions; decrease of S1P in the "protein-bound" fraction.
    - S1P in HDL2 increased 267% and 518% at V3 and V5, respectively (p < 0.0001), and 40.8% in HDL3 at V5 (p = 0.0378).

**Table 1.** Effects of obicetrapib 2.5 mg on the percentage changes from baseline (V2) to weeks 2 (V3) and 8 (V5) in the concentrations of apoA1, apoE, S1P, cholesterol, and lipophilic antioxidants in the HDL2 fraction.

| Timepoint | % change vs V2 | | | | | | |
|---|---|---|---|---|---|---|---|
| | ApoA1 | ApoE | S1P | Cholesterol | Lutein | Zeaxanthin | α-tocopherol |
| V3 | 265.0**** | 20.9 | 266.8**** | 172.4**** | 201.8** | 202.5**** | 250.3**** |
| V5 | 355.02**** | 133.02* | 517.90**** | 301.92**** | 245.79*** | 301.97**** | 258.22**** |

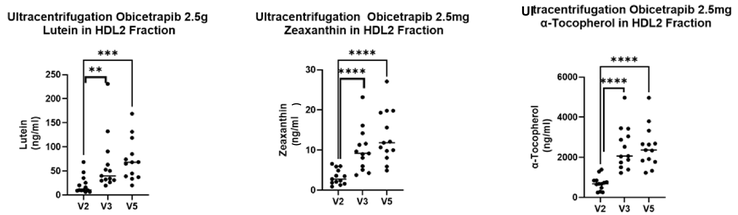


**Figure 2.** Lipophilic antioxidant levels in the HDL2 fraction at baseline (V2) and after 2 (V3) and 8 (V5) weeks of 2.5 mg obicetrapib treatment.

### p-values

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

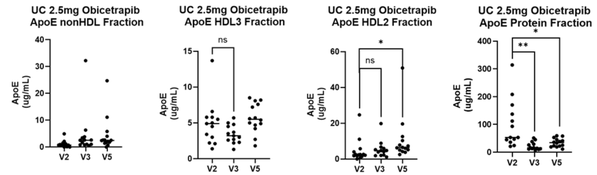


**Figure 3.** ApoE levels in the non-HDL, HDL2, HDL3, and protein fractions at baseline (V2) and after 2 (V3) and 8 (V5) weeks of 2.5 mg obicetrapib treatment.

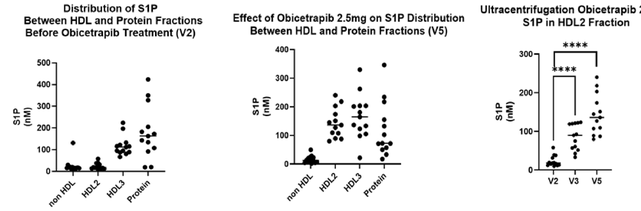


**Figure 4.** Distribution of S1P between non-HDL, HDL2, HDL3, and protein fractions at baseline (V2) and after 8 weeks (V5) of 2.5 mg obicetrapib treatment, and S1P concentration in the HDL2 fraction at baseline (V2) and after 2 (V3) and 8 (V5) weeks of 2.5 mg obicetrapib treatment.

## Conclusions

The results of this investigation demonstrated that treatment with obicetrapib not only raised HDL2 cholesterol and apoA1 but also shifted important lipophilic antioxidants, S1P, and apoE to the HDL subfractions. These effects might have important therapeutic implications for atherosclerotic cardiovascular disease, age-related macular degeneration, Alzheimer's disease, and diabetes.

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## Abbreviations

apo, apolipoprotein; CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ns, not significant; S1P, sphingosine-1-phosphate; UC, ultracentrifugation; V, visit; VLDL, very low-density lipoprotein

## Support

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