I heard the Global Principal Investigator for the REDUCE-IT study comment in his late-breaker presentation at AHA that there was no change in hsCRP in the placebo-arm of the study, please explain why that was referenced?

What is C-reactive protein (CRP)?

C-reactive protein (CRP) is a substance produced by the liver in response to inflammation. Other names for CRP are high-sensitivity C-reactive protein (hsCRP) and ultra-sensitive C-reactive protein (usCRP). A high level of CRP in the blood is a marker of systemic inflammation. It can be caused by a wide variety of conditions, from infections due to temporary illness to chronic inflammatory states associated with chronic disease conditions.

High hsCRP levels can indicate inflammation in the cardiovascular system, which can mean a higher degree of events associated with cardiovascular disease, such as heart attacks. However, as a marker of broad systemic inflammation, hsCRP is recognized as an inherently nonspecific marker, subject to unreliable outcomes that may not accurately reflect a drug's effect on a chronic disease condition. This is due in part to the fact that hsCRP levels can be elevated from any inflammatory condition, even those related to temporary infections. Nonetheless, it remains a marker of systemic inflammation commonly measured in clinical studies.

Vascepa has been shown to significantly lower hsCRP in prior studies and in REDUCE-IT. While the baseline levels of hsCRP studied in REDUCE-IT were not considered particularly high, the results support that hsCRP was significantly lowered and future analyses will help establish if inflammation was a contributing factor to the overall CV risk reduction observed in REDUCE-IT.

Standard method to handle hsCRP results: Log hsCRP[1]

Extreme outliers due to infections caused by temporary illness or other factors can heavily influence summary statistics of hsCRP, even beyond what is handled by using a non-transformed data approach (e.g., a conventional mean or median on a nominal scale). These individual outlier results can affect a mean or median population measurement in a way that can convey a misleadingly skewed result for the population studied. For this reason, a more reliable log transformation of hsCRP is used to incorporate outlier data appropriately within the context of the entire data set. Log transformation of hsCRP is a standard and generally recognized method employed to put the impact of outlier data into appropriate context within the entire data set.

hsCRP in REDUCE-IT

The REDUCE-IT statistical analysis plan (SAP) prespecified both direct hsCRP and log transformed hsCRP methods to measure changes in hsCRP. The comment from the Global Principal Investigator regarding hsCRP not changing from baseline in the placebo-arm of REDUCE-IT relied upon the log hsCRP method. Again, the log hsCRP method is a standard and generally recognized method to avoid a misleadingly skewed result due to the high variability of hsCRP for the population studied, as well as others.

Using the log hsCRP method, there was no increase in log hsCRP from baseline in the placebo arm and the between-group change was a 22.5% reduction at Year 2. This reflects that the reduction in hsCRP was driven by Vascepa therapy. These data are presented within the NEJM publication Supplemental Table 4 entitled "Lipid, Lipoprotein, and Inflammatory Marker Data Over Time – ITT Population". hsCRP data from that table is as follows:

Source Data for Bhatt NEJM 2018 Supplementary Table 4.
Lipid, Lipoprotein, and Inflammatory Marker Data Over Time – ITT Population.[2]

		Icosapent Ethyl (N=4089)					Placebo (N=4090)					Between Group Difference		
Biomarker	Visit	Median Observed Values	Median Absolute Change from Baseline	Median % Change from Baseline	Median % Change P value[1]		Median Observed Values	Median Absolute Change from Baseline	Median % Change from Baseline	Median % Change P value[1]		Median Absolute Change from Baseline[2]	Median % Change from Baseline[2]	Median % Change P value[3]
hsCRP (mg/L)	Baseline	2.2					2.1							
	Year 2	1.8	-0.2	-13.9	0.0365		2.8	0.5	32.3	<.0001		-0.9	-39.9	<.0001
	Last Visit	1.8	-0.2	-12.6	0.7509		2.8	0.4	29.9	<.0001		-0.8	-37.6	<.0001
Log hsCRP (mg/L)	Baseline	0.8					0.8							
	Year 2	0.6	-0.1	-21.8	<.0001		1.0	0.3	0.0	0.9203		-0.4	-22.5	<.0001
	Last Visit	0.6	-0.1	-23.1	<.0001		1.0	0.3	-4.0	0.0481		-0.4	-21.2	<.0001

[1] P-value from Wilcoxon Signed-Rank test

[2] Based on Hodges-Lemann Estimation

[3] P-Value from Wilcoxon Rank-Sum test

To summarize measurements from REDUCE-IT presented in the above table, the median percent change from baseline using the standard and generally recognized log hsCRP method, reflected the following at Year 2:

- **No increase from baseline in the mineral oil placebo arm (0.0%, p=0.9203)**
- **Vascepa change from baseline of -21.8% (p<0.0001)**
- **Between-group difference in change: 22.5% reduction of hsCRP in the Vascepa versus placebo arms (p<0.0001)**
    - The placebo-corrected median percent change was calculated by a Hodges-Lehmann method, whereby each possible comparison between each individual placebo and each individual Vascepa patient change is generated, and then a median of all possibilities is taken. This well-accepted statistical comparison improves the predictive accuracy of determining the most likely between group difference, which at Year 2 = -22.5%.
- **Last visit results, within and between arms, are consistent with Year 2 results**

A point of interest may be that the within group placebo absolute change from baseline suggested an increase of hsCRP (+0.3 log mg/L), but the median of the within group placebo percent changes showed no change from baseline (0.0%). Both baseline value and change from baseline value would impact individual percentage change from baseline (e.g., a patient with high baseline hsCRP value may see an absolute increase/decrease that seems numerically impactful, but is ultimately small on the percent scale [denominator effect]), which is why absolute values may change while observing little or no effect in percent change from baseline in the placebo group.

Further Details on Calculation Methods

<u>Log hsCRP data in REDUCE-IT, as published in *NEJM*</u>

To obtain the log hsCRP values presented in the NEJM supplement:

- Individual timepoint medians: Individual patient-level values were each log transformed and then the median values were calculated for the baseline and Year 2 timepoints:
    - Vascepa Baseline = 0.8 log mg/L and Year 2 = 0.6 log mg/L
    - Placebo Baseline = 0.8 log mg/L and Year 2 = 1.0 log mg/L
- Log-transformed change from baseline medians: To accurately represent median group changes from baseline, one cannot simply subtract median Baseline values from Year 2 median values (e.g. for Vascepa, one cannot say 0.6 log mg/L – 0.8 log mg/L = – 0.2 log mg/L change from baseline). To accurately represent group median change from baseline on log-transformed data, the median absolute and median percent changes from baseline within each treatment arm were calculated independently by first subtracting the log transformed hsCRP baseline value from the post-baseline value for each patient, and then the within-group median change (or percent change) was calculated across those individual differences (e.g. Year 2 – Baseline) for all patients in that group.
    - For the log-transformed median absolute change values, there is an increase in the placebo arm and a decrease in the Vascepa arm:
        - Vascepa change from baseline at Year 2 = -0.1 log mg/L
        - Placebo change from baseline at Year 2 = 0.3 log mg/L
    - While for the log-transformed percent change values, there is no change in the placebo arm and a decrease in the Vascepa arm:
        - Vascepa change from baseline at Year 2 = -21.8% (p<0.0001)
        - Placebo change from baseline at Year 2 = 0.0% (p=0.9203)
- Between-group comparisons with log-transformed data: The placebo-corrected median percent change with log-transformed data was calculated by the Hodges-Lehmann method, in which every possible difference is computed between each placebo patient and all Vascepa patients; then the median of all those differences is computed. This well-accepted statistical method improves the predictive accuracy of the between group difference, which at Year 2 = -22.5% (p<0.0001).

[1] Crawford SL. Correlation and Regression. Circulation. 2006;114:2083-2088.

[2] Bhatt DL, Steg PG, Miller M, et al. Cardiovascular Risk Reduction with Icosapent Ethyl in Hypertriglyceridemia. N Engl J Med. 2018. Epub ahead of print. *See* supplemental index.