We thank both reviewers for their well-considered and reasonable critiques to our work. We have revised the manuscript accordingly and detail these changes here as well. Our point-by-point responses are included below in **bold**, and verbatim text added to the manuscript is noted in **red bold** font. We have also included line numbers for the changes for your convenience.

# Point-by-Point Response

The manuscript applies AI to investigate which are the factors that are independently associated with plasma cholesterol levels in the diversity outbread mice dataset.  
As expected, gender and diet are the major factors correlating with changes in cholesterol levels. Intriguingly calcium levels appear to the third.  
The manuscript will benefit from the following analysis if feasible:  
-Correlation of plasma cholesterol levels with the extension of the atherosclerotic plaque

**We agree that this is an important pathophysiology that was not measured in this study, so we are unable to perform this analysis. We do look forward to using these data in future studies using mouse models with atherogenic susceptibility, such as *Apoe* or *Ldlr* knockout mice. This is now noted in the limitations section on lines 226-228.**

**Finally, as cardiovascular disease is extremely rare in mice of this age we did not assess cardiovascular disease, or atherogenic lesions as an endpoint in this study.**

-Correlation of cholesterol content in lipoprotein subsets with calcium levels.

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**Response Figure 1**: Associations between HDL-C and non-HDL cholesterol with calcium levels at endpoint. These data are presented as Supplementary Figures 2A and 2B in the revised manuscript. Both associations are significant after diet and sex adjustment (p<1x10-7).

**This is an excellent point, given the differing directional associations of LDL vs HDL cholesterol with respect to cardiovascular disease. To answer this question have repeated this analysis for HDL-C which was directly measured and non-HDL cholesterol which was calculated by subtracting HDL-C from the total cholesterol. We are unable to detect other apolipoproteins such as VLDL or chylomicrons or separate those from non-HDL-C, nor are we able to evaluate correlations with subpopulations of lipoprotein particles. The correlations between serum calcium and these values are now presented in Supplementary Figures 2A-B and Response Figure 1. In both cases there was a significant association after adjusting for diet and sex. For HDL-C vs calcium the effect was 10.4 +/- 0.6 mg/dL per mg/dL of calcium (p=3 x 10-47). For non-HDL cholesterol the effect was 2.0 +/- 0.4 (p=4.5 x 10-8). From these data one could reasonably conclude the effect is stronger for the HDL than non-HDL cholesterol association, however we believe these effects are similar in scale since HDL levels are 3.5-7.1x higher than non-HDL cholesterol levels depending on the diet/sex group. Our interpretation is therefore that both subgroups of apolipoprotein fractions are associated with calcium, thus our focus on total cholesterol. It is fascinating that there is only a diet effect (after adjusting for changes in calcium) in the HDL-C but not the non-HDL-C group (comparing the black to the grey lines). While we don’t want to speculate here what that may mean, but we would have expected a stronger diet effect in the non-HDL-C fraction which contains among other things the LDL. This is something we look forward to examining further, and we thank the reviewer for encouraging this analysis. These data are now described in the results section on lines XXX:**

**Given the differences in the physiology and cardiovascular disease associations between HDL versus LDL cholesterol concentrations, we repeated this analysis assessing the diet- and sex-adjusted associations between HDL cholesterol and non-HDL cholesterol and calcium (Supplementary Figures 2A-B). Both of these fractions have positive associations with calcium levels, indicating that the positive associations with calcium are found in both apoplipoprotein fractions.**

-Correlation of cholesterol with the other parameters when a cholesterol rich diet is used.

We performed the requested analysis of cholesterol associations with all available clinical parameters. These data are presented below in order of significance.

-Evaluation of the translational relevance of the findings by for instance investigating the correlation between plasma chol levels, atherosclerosis and calcium in human datasets.  
  
  
Additional analysis.  
-How do the authors explain the weaker association between plasma chol and plasma TG in figure 2, panel A, male mice on HFHS diet compared to normal diet?  
  
  
Reviewer: 2  
  
Comments to the Author  
Comment:  
The authors analyzed in two distinct mouse datasets, Diversity Outbred and BXD, using a machine learning approach. This is an attractive study; however, some concerns are needed to address.  
  
Major concerns:  
1.      How long was the HSFS diet fed to the mice?  
2.      Are all of these mouse datasets from healthy mice fed NCD or HFHS? Are these phenotypes, including the relationship between cholesterol and calcium, for healthy cases and are different in disease conditions?  
3.      Aging is also thought to be an important factor in elevated calcium and/or cholesterol levels. Do the authors’ findings apply to both young and old?  
4.      Authors described that these data reflect associations between calcium and the HDL pool because of the absence of CETP in mice. Is there possibility that calcium levels affect LDL-C or both?  
5.      Are patients with hypercalcemia more likely to have dyslipidemia or vice versa? Any reports?  
  
Minor points:  
1.      There is no citation in texts and figure legend in Figure 1C. Please add.  
2.      The lines of HFHS group in Figures 1 and 2 are hard to see due to the light color. Please change it.