Author’s response to reviews

Title: Associations of Perfluoroalkyl Substances with Blood Lipids and Apolipoproteins in Lipoprotein Subspecies: the POUNDS-Lost Study

Authors:
Gang Liu (liugang026@hust.edu.cn)
Bo Zhang (bzhang@hsph.harvard.edu)
Yang Hu (huyang1989@gmail.com)
Jennifer Rood (jennifer.rood@pbrc.edu)
Liming Liang (lliang@hsph.harvard.edu)
Lu Qi (lqi1@tulane.edu)
George Bray (brayga@pbrc.edu)
Lilian DeJonge (edejonge@gmu.edu)
Brent Coull (bcoull@hsph.harvard.edu)
Philippe Grandjean (pgrandjean@health.sdu.dk)
Jeremy Furtado (jfurtado@hsph.harvard.edu)
Qi Sun (qisun@hsph.harvard.edu)

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Reviewer #1: - Missing some important articles in the references and text: Nelson; Shankar; Geiger; and possibly others.

Response: We thank the Reviewer for pointing it out. We have cited the following articles:

- Please say how you determined which potential confounders to include in the models.

Response: We firstly considered some traditional covariates, including demographic, socio-economic and lifestyle factors. Given that our study was based on a dietary intervention trial, we further included dietary intervention groups in the models. In addition, considering that lipid-lowering medication use might confounder the association of interest, we also took this variable into account in the models, although the result did not significantly change when we further restricted the analysis among participants without lipid-lowering medication use. We have added more information to the text:

“In terms of multivariate adjustment, we considered traditional covariates, including demographic, socio-economic, and lifestyle factors. In addition, given the clinical trial study design, we further included dietary intervention groups in the model. Lastly, considering that lipid-lowering medication use might confound the association of interest, we also took this variable into account in multivariate analyses.” (Line 147-151, Page 7)

- Table 1: even though the sample is mostly white, can you further break out race categories?

Response: We have further categorized race into 4 groups: white, black, Hispanic, and other. Please see updated Table 1.

- Table 1: showing weight in pounds would be more intuitive for many readers (could even do so in addition to kg)

Response: We have added weight in pounds to the table. Please see updated Table 1.

- Please comment on the correlation among PFASs and also on the outcome side. What issues, if any, could this present, and how have you dealt with it (if at all)?

Response: We observed moderate-to-high correlations among PFOS, PFOA, PFHxS, PFNA, and PFDA (rs ranged from 0.32 to 0.84), probably reflecting some common exposure sources of these chemicals. Additionally, we have also tried factor analysis and only one factor was identified and strongly associated with all individual PFASs. This phenomenon can be observed when the number of variables is small. The inter-correlations between lipid species were shown in Supplementary Figure 1. There were moderate-to-high correlations among most of the lipoprotein and apolipoprotein subspecies (rs ranged from -0.29 to 0.97, P<0.001). In light of these correlations, we are inclined to not adjust for other PFASs to avoid inflation of standard errors and confidence intervals. In fact, after mutual adjustment of other PFASs, we observed little changes in the LS means: the LS means for apoC-III according to tertile of PFOA were 13.7±1.0, 14.7±0.9, 15.3±1.0 (P trend=0.14), although the P for trend was not significant because of inflated standard errors resulted from collinearity between PFASs. For the same reason, we are inclined to not mutually adjust for other lipids.

- Why did you choose to focus on LS Means?
Response: We showed least square means of lipids and apolipoproteins according to tertiles of PFASs after accounting for covariates, and further tested the linear trend by assigning a median value to each tertile and treating it as a continuous variable. LS means are more intuitive to understand than a single beta coefficient.

- In the methods you stated that those on lipid-lowering medication were excluded, but in Table 2 the variable is shown in the covariate list

Response: We apologize for the confusion. The main analysis did not exclude the participants with lipid-lowering medication use, and the variable was adjusted for in the model (as shown in Table 2). As a sensitivity analysis to test the robustness of the findings, we further restricted the analysis to participants without lipid-lowering medication use, and the results did not significantly change. “Several sensitivity analyses were performed…Second, analyses were further restricted to participants without lipid-lowering medication use or to non-current smokers.” (Line 159-165, Page 8)

- It would be helpful if you would annotate Table 2 more overall. Define what follows the +/- indicate significance in some way, etc. so it's easier for the reader to identify key findings. Also somewhere indicate the range or mean value of each tertile as appropriate.

Response: We have revised Table 2 accordingly. Standard error follows the +/- . We now indicate at footnote of Table 2 that data are presented as least squares (LS) means +/- standard error (SE).

- The setup of table 2 is counterintuitive (to me, perhaps not to others). I recognize the challenge of presenting this type of results in an intuitive manner, but I would actually flip the column and row headings. When presented the current way, it seems as if the PFASs are the outcome.

Response: We thank the Reviewer for this comment. We have flipped the column and row headings accordingly. Please see updated Table 2.

- Very nice figures 1 and 2

Response: Thank you for the comment.

- I would like to see a more thoughtful comparison of your findings with the existing literature in the discussion section.

Response: We have added more discussions to the text. “Evidence from human studies regarding PFAS exposure and lipids is mixed [11, 27, 28]. Positive associations between PFASs and total cholesterol, LDL cholesterol, or triglycerides were observed in some cross-sectional and prospective studies [4, 8, 9, 29-31], whereas other studies reported null associations or even inverse associations [11, 28, 32]. We are among the first to examine apolipoprotein species within the broad categories of lipoproteins that were not considered in previous studies [13, 14]. Indeed, the present study showed no clear association between PFAS and total cholesterol, triglycerides, or lipoproteins in plasma, but PFAS exposures were associated with apoC-III levels and
also the lipid contents in IDL, LDL, and HDL particles that contain apoC-III. The diverging functions of apolipoproteins in the same class of blood lipids may also explain the previous inconsistent findings regarding PFAS exposures and total blood lipid levels.” (Line 255-265, Page 12)

- Very nice paper overall!

Response: We thank the Reviewer for the positive comment.

Reviewer #2: In this study entitled "Associations of Perfluoroalkyl Substances with Blood Lipids and Apolipoproteins in Lipoprotein Subspecies: the POUNDS-Lost Study", Liu et al. investigates the associations between plasma PFAS concentrations and lipoprotein and apolipoprotein subspecies in samples from the POUNDS-LOST study conducted in a subset of 326 American women and men. The study is important since it includes 5 PFAS and investigates different lipoproteins apolipoprotein subfractions separately, which is the first time. The authors find that PFAS are associated with LDL and HDL that carry ApoC-III, which are associated with increased CVD risk in observational studies. Overall, the study is excellently conducted and reported and I have only minor suggestions for improvements.

Response: We thank the Reviewer for the positive comment.

- From the current paper it appears the PFAS is associated with lipid metabolism and it is hinted that they may cause altered lipid metabolism (through provision of reference examples in animals where this has been shown). However, could it be that the PFAS are transported in lipoproteins fractions and thus are determined by the concentrations of such fractions? Could the authors elaborate on this please and mention what implication it may have. I know that several authors have looked into this with regards to POPs.

Response: We thank the Reviewer for this comment. To our knowledge, whether PFASs could be transported in lipoproteins fractions remains unclear. Some study suggested that a transfer route of persistent organic pollutants via LDL-receptor mediated endocytosis might be possible (Ljunggren et al, Environ Int. 2014), although an in vitro study showed that LDL-receptors play a minor role in the cellular uptake of DDT-lipoprotein complexes (Hjelmborg et al., Environ Res, 2008). In addition, unlike many other persistent organic pollutants, PFASs are not lipophilic and mainly bind to serum albumin rather than lipoprotein (Zhang et al, BMC Mol Biol, 2009; Bischel et al, Environ Toxicol Chem, 2011).

To further address this concern, we also examined PFASs in relation to the ratio of IDL, LDL and HDL that carry apoC-III to total cholesterol as a surrogate marker of apoC-III composition rather than absolute concentrations. We found similar results: i.e., the LS means (±SE) for apoC-III/total cholesterol according to tertile of PFOA were 0.068±0.005, 0.071±0.004, 0.075±0.004 (Ptrend=0.048). Nonetheless, more studies are warranted to clarify the association between PFASs exposure and lipoprotein subspecies. We have added more discussions to the text:

“Of note, we cannot exclude the possibility that our observed associations may not bear any causal interpretation if PFASs are incorporated in the same lipoprotein species that contain apoC-III, although we believe that such possibility is small because the current evidence suggests that the majority of
PFASs in circulation are carried by albumin rather than lipoproteins [39]. In addition, in a sensitivity analysis, we observed similar results when we examined the ratio of IDL, LDL, and HDL particles that contain apoC-III to total cholesterol levels as a surrogate measure of lipoprotein compositions. More studies are warranted to elucidate the distribution of PFASs in blood compartments and other tissues in human body.” (Line 291-299, Page 14)

Reference:

- The authors report significant associations in particular for PFOS and APOC-III in LDL. However, the difference across tertile, how can that be translated into altered CVD risk? Could the authors provide a rough estimate?

Response: In our study, the least square means (±SE, mg/dl) of ApoC-III on IDL+LDL according to tertile of PFOA were 1.66±0.2, 1.94±0.2, 2.03±0.2, respectively (Ptrend<0.05). Based on a recent meta-analysis (Wyler et al, J Clin Lipidol 2015), a pooled risk estimate was 2.48 (1.48–4.32) for a cardiovascular event with a 5-mg/dl increase in apoC-III on non-HDL. Therefore, we estimated a 18% increased risk of CVD according to difference in apoC-III levels between the extreme tertiles of PFOA in the present study population.

“We estimated that the difference in apoC-III levels between the extreme tertiles of PFOA would lead to an 18% increased risk of cardiovascular disease (CVD), based on a pooled estimate of 148% increased CVD risk for each 5-mg/dl increase in apoC-III levels [20]” (Line 281-283, Page 13)

Reference:

Line 109: Were both base line and 2 year samples for the 326 used?

Response: Yes. Those 326 participants had data of lipoprotein subspecies both at baseline and 2 years.

Line 141: "Assessment of other covariates" -> "Assessment of covariates"

Response: Revised.

Line 181: Since many of the PFAS are highly correlated, why did the authors not consider to derive latent variables reflecting orthogonal variation in PFAS and use such variables in studies in relation to lipids (latent variables derived by PCA for example).
Response: We thank the Reviewer for this comment. We have tried factor analysis, although only one factor was identified and strongly associated with all individual PFASs. This phenomenon can be observed when the number of variables is small.

Table 2: As now stated with PFAS in the columns and lipids in the rows, it gives the impression that PFAS is expressed per tertile of lipid and not the other way around. I suggest the authors move PFAS to the rows and lipids to the columns. Moreover, please provide concentrations ranges of PFAS for the tertiles.

Response: We have revised Table 2 according to the Reviewer’s suggestion. Please see updated Table 2.