PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Serum perfluoroalkyl acids concentrations and memory impairment in a large cross-sectional study
AUTHORS	Gallo, Valentina; Leonardi, Giovanni; Brayne, Carol; Armstrong, Ben; Fletcher, Tony

VERSION 1 - REVIEW

REVIEWER	Alan Ducatman, MD, MS Professor of Public Health Professor of Medicine West Virginia University (USA) I have performed externally funded projects which employ summary data from the same population reported in this study. I do not think this represents a conflict of interest.
REVIEW RETURNED	24-Jan-2013

THE STUDY	In my detailed response to the authors, I do raise the issue of whether they should include a more direct discussion of the differences of their findings to the predecessor paper. It is about making the paper better, not about a problem with the paper.
GENERAL COMMENTS	The review below should also be attached and cleaned up a bit if I did this right. This is a valuable and interesting addition to the literature. There is no reason to list the many good things about it. Here are topics for the authors' consideration for minor edits. Abstract and Population: The abstract could be improved by clarifying that the study population is aged 50 and above. (this is clear, but not in the abstract). Style: The paper has some misspellings of key terms (perfluoroctanoate - not octanate - if the purpose is to refer to the anion) and inconsistencies of expression between anions and acids. Your may prefer to use the more conventional terms for the acids, but it does not matter so long as the form is consistent. The discussion even says "perflourochlorinated" at one point, that is incorrect. One format should be used consistently (choose one and stick with it), and spellings corrected. I understand what the authors are trying to get at with their discussion of "impossible to estimate" participation rates. However, this statement is followed by a citation of a well supported estimation (that is also in a paper by Steenland). What the authors mean is that the estimation applies only to those who were living in the affected water districts at the time of the survey. It would be easy to reword this so that a casual reader will not be misled into thinking there is

	an apparent contradiction. The second paragraph of the discussion contains the word "effect," which may be read to imply causation. Stick with association. Context: The key predecessor paper, by Power et al, had slightly different findings than this paper, including the finding that the association was concentrated in diabetics who were not on medication. There are physiologic reasons, related to the dementing effects of diabetes and the PPAR effects of PFAAs, why the finding by Power and colleagues is satisfying. (That does not mean it is right, it is simply intellectually satisfying.) The differences in findings between the two papers could be better delineated, and a report of diabetics not taking drugs could be included in the main body of the work instead of the appendix. Further, the discussion is somewhat dismissive about survey listings of diabetic medications. However, in this survey, patients also had the opportunity to actually list their medications. Where that occurred, the data are very likely to be good. Animal toxicity testing suggests concerns not raised in the paper. Articles by Viberg et al and Johansson et al concerning animal neuorbevioral and neurodevelopmental studies are worth considering. The references concerning associations between PFAAs and diabetes are generally correct. However, there are a small number of papers with different findings (for example Lin et al and Lundin et al). It is a question whether these should be mentioned. Sensitivity analysis: The choice of sensitivity analyses is always open to question. Here are my questions about this paper and its approach to sensitivity analysis. 1. The actual research question can be stated in the affirmative. Are PFAAs neuroprotective? In that case, it seems to me that the best sensitivity analysis is a simple look at those who answered their memory loss surveys with "never." (The ordinal regression gets at this, but is not so clearly visible as simply reporting on it). 2. The logic behind the inclusion of those who moved residence is unclear. Moving may connote upward or downward economic mobility, or neither, and it is hard to understand why there should be an association independent of the clear water district association with ingested PFOA . The absence of an independent association is what one expects, and also what is found. The value of proving that absence of effect is not well explained. Unless there is a compelling reason, consider leaving this out (or, at least not calling it a sensitivity analysis). Confounders: The sugar sweetened drink discussion is not up to the standards of the rest of the paper. Further, it is not clear that it is relevant. The discussion of adolescent consumption is particularly perplexing in light of the population age for this study (the serum half life is long, but not so long as three decades). The hypothesis that there might be an interaction (independent of diabetes and BMI, already part of the methods) addresses a topic that is already controversial in the literature. There is no room to discuss the controversy in this paper, nor reason to do so. Addressing it interrupts rather than adds to the discussion. Consider leaving this

	topic out, or reducing it to a line which says something like: consumption of sugar sweetened beverages was considered and found to not affect the measured outcome. Further, the discussion of who drinks less water appears in some papers by this group. It is a question whether it deserves inclusion as well. First, the authors start with a biomarker - one of several PFAAs. Water consumption may be one of the variables that goes into that biomarker (especially PFOA in this population) but is not known to be related to the outcomes (demented patients may stop drinking, a different topic). Further, the authors are right to point out that hypotheses about other drinks replacing water is not well supported by available literature. It is unclear why there is a need to raise and dismiss the topic. It is reasonable to consider leaving out the topic (unless there is concern that critics will raise it). Methods issues: Papers by this group frequently make the distinction of findings within water districts compared to the whole population. The reasons to do this are unclear, unless the context is a sensitivity analysis. The attendant problem is that water district is a great predictor of the biomarker, and.....The biomarker is the biomarker. The author's concept that comparisons of adjacent water districts adds meaning to consideratino of socio-economic or environmental confounders is charactristic of this group's work but more idiosnycratic than supported by available literature. (Nearby populations are used as control groups for a reason). Mostly, it is way to find out if the same analysis is robust to a much smaller "n." Is there a good reason to do that? I know the authors think the comparison has merit, as they keep doing it. I leave it to them and editors to consider if sufficient value is added. Discussion: The water consumption discussion applies to PFOA only but is attributed to all PFAAs at one point in the discussion (6th paragraph I think). Again, the easiest and scientifically best, and most parsimonious approach is to leave out the water consumption discussion. The biomarkers are the biomarkers. So far we know, Water consumption relates to and is already factored into the dependent variable, and known to be important for PFOA only. The discussion of nuclear receptors and antiinflammation is nicely done, including acknowledgement that the issue is more complex than PPAR alpha (and, of course, PPAR gamma, an emerging literature for these toxicants) only. However, should the authors strengthen the discusion about the difference between statistical significance (present) and the size of the negative association (present yet quite small, and for a self-attributed survey characteristic)? That is a difficult topic. With a clinical outcome (disease presence), or a clnical marker with gradations (a laborotory test with cut-offs for normal), this group has been right to show that relatively small differences are associated with clinically relevant outcomes. However, those differences were greater than these in all instances, and there was a measureable clinical outcome. I leave it to the authors to consider whether a stronger caution is warrented concerning the potential difference between statistical and clincial significance in the setting of a survey opinion about one's memory.

REVIEWER	Anders Glynn

	Senior Risk and Benefit Assessor, toxicologist Swedish National Food Agency Sweden I have no competing interest.
REVIEW RETURNED	10-Feb-2013

THE STUDY	There is a lengthy discusiion about the involvement of PPARgamma in a possible mechanism behind the key findings in the study. This is not supported by the references given.
RESULTS & CONCLUSIONS	There is also confusion in the Discussion about the possibility of reverse causation. The Discussion is confusing but I get the feeling that the authors are leaning towards reverse causation as an explanation of the finding. If this is the case, this should be mentioned in the Abstract and in the conclusions.
GENERAL COMMENTS	This cross-sectional study addresses the interesing hypothesis that increased exposure to perfluoroalkyl acids is associated with a better cognitive function. The study is well conducted and the results are in most cases clear-cut. However, the discussion about causality is confusing and the speculations about possible mechanism behind the observed associations unclear. **Comments** Abstract, line 18: "prefluoroclorinated"? Introduction, page 4, lines 17-18: Environmentally contaminated fish is a major source of exposure for PFOS and long-chain PFCA. Page 4, lines 21-48: The section correctly starts with some lines about PFAA and PPARalpha. It then goes into a more speculative part dealing with PPARgamma. The authors have not given any references from the literature dealing with PFAA and PPARgamma activation. There are some studies looking into interactions between PFAA and PPAR. None of these have suggested an interaction between PFAA and the gamma version, for instance Takacs and Abbot (2007). How does this fit with the hypothesis proposed by the authors? Page 5, line 18: "3.65-4.27))". On parenthesis too much? Methods, page 6, lines 21-29: There is no information about timing of memory loss, i.e. when did the study participants suffer from this? Now, 5 years ago, 10 years ago. Would be important information to associate with the onset of exposure. In the description of the study population, it is stated that "individuals were eligible to

	participate…….if they had consumed water for at least one year between 1950 and Dec 2004". Were the study subjects blinded to the exposure situation? Page 5, lines 42-57: Details are given about analyses of PFOA and PFOS, but nothing about PFHxS and PFNA. Please include this info in the text. Results, page 9, lines 38-39: Something missing in this sentence? Discussion, the first 3 sections: It is hypothesized that PPAR receptors are involved in a causal relationship between PFAAA and short-term memory. However, it is not clear which receptor(s) that the authors are refereeing to. PPAR-gamma is mentioned, but is there any data in the literature showing that PFAA interacts with this receptor? Page 10, lines 30-51: The association with PFOA was entirely within water districts. The authors further states that the between-district estimate is not vulnerable to reverse causation in this study, and conclude that "this (reverse causation?) is a more plausible explanation. This is an important conclusion (if I have interpreted the discussion correctly), and should be included in the abstract. Page 11, last section: The hypothesis that the inverse association is explained by an PPAR agonist effect is highly speculative, and not clearly supported in the discussion. Reverse causation a more plausible explanation? The Introduction and Discussion have to be thoroughly revised in order to convince the reader about the plausibility of the hypotheses.

VERSION 1 – AUTHOR RESPONSE

Reviewer: Alan Ducatman, MD, MS
Professor of Public Health
Professor of Medicine
West Virginia University (USA)

I have performed externally funded projects which employ summary data from the same population reported in this study. I do not think this represents a conflict of interest.

This is a valuable and interesting addition to the literature. There is no reason to list the many good things about it. Here are topics for the authors' consideration for minor edits.

Abstract and Population: The abstract could be improved by clarifying that the study population is aged 50 and above. (this is clear, but not in the abstract).
Thank you for noticing it, good point.

Style: The paper has some misspellings of key terms (perfluoroctanoate - not octanate - if the purpose is to refer to the anion) and inconsistencies of expression between anions and acids. Your may prefer to use the more conventional terms for the acids, but it does not matter so long as the form is consistent. The discussion even says "perflourochlorinated" at one point, that is incorrect. One format should be used consistently (choose one and stick with it), and spellings corrected.

- Thanks for noticing this. Inconsistencies have been corrected.

I understand what the authors are trying to get at with their discussion of "impossible to estimate" participation rates. However, this statement is followed by a citation of a well supported estimation (that is also in a paper by Steenland). What the authors mean is that the estimation applies only to those who were living in the affected water districts at the time of the survey. It would be easy to reword this so that a casual reader will not be misled into thinking there is an apparent contradiction.

- The sentence was in fact not very clear. It has been changed as follows: “The participation rate for the C8 Health Project based on US census counts of residents in the affected water districts during Project enrolment, have been estimated at around 80%”

The second paragraph of the discussion contains the word "effect," which may be read to imply causation. Stick with association.

- Thank you, good point.

Context: The key predecessor paper, by Power et al, had slightly different findings than this paper, including the finding that the association was concentrated in diabetics who were not on medication. There are physiologic reasons, related to the dementing effects of diabetes and the PPAR effects of PFAAs, why the finding by Power and colleagues is satisfying. (That does not mean it is right, it is simply intellectually satisfying.) The differences in findings between the two papers could be better delineated, and a report of diabetics not taking drugs could be included in the main body of the work instead of the appendix. Further, the discussion is somewhat dismissive about survey listings of diabetic medications. However, in this survey, patients also had the opportunity to actually list their medications. Where that occurred, the data are very likely to be good.

- We have now underlined more explicitly the difference between this and the previous study, having in the discussion: “In a previous published study an inverse association between PFAAs and memory impairment was observed specifically among non-medicated diabetics19. In the present study, this

pattern was not repeated, with the inverse association between PFAAs and cognitive impairment being more evident in those without diabetes; among diabetics, the association was not present, irrespective of treatment status."
Regarding medication use, we accept that uncertainties in classifying self-reported medication should not be overstated, and haved added to the discussion: "However we consider it very unlikely that any mis-reporting would be confounded with serum PFAA. "

Animal toxicity testing suggests concerns not raised in the paper. Articles by Viberg et al and Johansson et al concerning animal neuorbevioral and neurodevelopmental studies are worth considering.

- The sentence "There is some evidence of detrimental effects of PFAAs in neurodevelopment of mice affecting the cholinergic system and cognitive function38-40, thus timing of exposure may also be relevant in order for the PFAAs to exert this hypothesised anti-dementing role. " has been added to the discussion, to reflect this concern.

The references concerning associations between PFAAs and diabetes are generally correct. However, there are a small number of papers with different findings (for example Lin et al and Lundin et al). It is a question whether these should be mentioned.

- Thanks, we agree that these should be cited too and they have now been added.

Sensitivity analysis: The choice of sensitivity analyses is always open to question. Here are my questions about this paper and its approach to sensitivity analysis.

1. The actual research question can be stated in the affirmative. Are PFAAs neuroprotective? In that case, it seems to me that the best sensitivity analysis is a simple look at those who answered their memory loss surveys with "never." (The ordinal regression gets at this, but is not so clearly visible as simply reporting on it).

- The magnitude of the results are a little sensitive to the case definition (whether "rarely" is included in the baseline or case category), or whether ordinal odds ratios are presented, so we present them to allow comparison.
However, the sensitivity analysis has been changed according to suggestions. In order to maintain comparability of data, we compared those with any memory impairment (frequent, sometimes, and rare) with those reporting no memory problems. Results are shown in table 4, and described in the relevant result section.

2. The logic behind the inclusion of those who moved residence is unclear. Moving may connote upward or downward economic mobility, or neither, and it is hard to understand why there should be an association independent of the clear water district association with ingested PFOA . The absence of an independent association is what one expects, and also what is found. The value of proving that absence of effect is not well explained. Unless there is a compelling reason, consider leaving this out (or, at least not calling it a sensitivity analysis).

- We agree that the analysis of mobility is somewhat redundant for the scope of this paper, and it has been deleted.

Confounders: The sugar sweetened drink discussion is not up to the standards of the rest of the paper. Further, it is not clear that it is relevant. The discussion of adolescent consumption is particularly perplexing in light of the population age for this study (the serum half life is long, but not so long as three decades). The hypothesis that there might be an interaction (independent of diabetes

and BMI, already part of the methods) addresses a topic that is already controversial in the literature. There is no room to discuss the controversy in this paper, nor reason to do so. Addressing it interrupts rather than adds to the discussion. Consider leaving this topic out, or reducing it to a line which says something like: consumption of sugar sweetened beverages was considered and found to not affect the measured outcome.

- We do not have direct measure of sweetened beverage consumption in this population, so cannot consider it in a formal sense. Further given that only one of the PFAAs considered (PFOA) is clearly associated with the drinking water consumption and given the old age of the study population, we agree that confounding with soft drinks is at speculative as an explanation and have dropped this part of the discussion as suggested.

Further, the discussion of who drinks less water appears in some papers by this group. It is a question whether it deserves inclusion as well. First, the authors start with a biomarker - one of several PFAAs. Water consumption may be one of the variables that goes into that biomarker (especially PFOA in this population) but is not known to be related to the outcomes (demented patients may stop drinking, a different topic). Further, the authors are right to point out that hypotheses about other drinks replacing water is not well supported by available literature. It is unclear why there is a need to raise and dismiss the topic. It is reasonable to consider leaving out the topic (unless there is concern that critics will raise it).

- While we agree that there is no compelling argument that water consumption is associated with memory impairment other than through PFOA, we believe that sound scientific practice requires that investigators discuss biases that are possible, not just biases for which there is evidence. There is also value, as the reviewer notes, in addressing concerns readers might have even where investigators may not share those concerns. We have however acknowledged more prominently that the water consumption issue is not relevant for PFAAs other than PFOA.

Methods issues: Papers by this group frequently make the distinction of findings within water districts compared to the whole population. The reasons to do this are unclear, unless the context is a sensitivity analysis. The attendant problem is that water district is a great predictor of the biomarker, and.....The biomarker is the biomarker. The author's concept that comparisons of adjacent water districts adds meaning to consideratino of socio-economic or environmental confounders is charactristic of this group's work but more idiosnycratic than supported by available literature. (Nearby populations are used as control groups for a reason). Mostly, it is way to find out if the same analysis is robust to a much smaller "n." Is there a good reason to do that? I know the authors think the comparison has merit, as they keep doing it. I leave it to them and editors to consider if sufficient value is added.

- We respectfully disagree with the reviewer on this point. An association of serum PFOA within and between water districts are at risk of different potential biases. As noted in the text, a between water district association could not for example be subject to a bias due to a genetic factor increasing risk of memory impairment in linkage disequilibrium with one affecting excretion of the biomarker but could be subject to an “ecological” bias. For within district analyses these bias potentialities reverse. Overall analysis would include the combination of all these biases, if present. Where an association is present both within and between water districts, this would be more convincing as it seems less likely that independent biases would both lead to the same artefactual association. These bias vulnerabilities rather than a “small n” issue is what is addressed by the within/between water district analysis. We have attempted to more clearly explain this reasoning in the text and have explicitly referred to this analysis as a sensitivity analysis.

Discussion: The water consumption discussion applies to PFOA only but is attributed to all PFAAs at one point in the discussion (6th paragraph I think). Again, the easiest and scientifically best, and most parsimonious approach is to leave out the water consumption discussion. The biomarkers are the biomarkers. So far we know, Water consumption relates to and is already factored into the dependent variable, and known to be important for PFOA only.

- We agree that the water consumption discussion is little relevant for PFAAs other than PFOA, and simplified the discussion.

The discussion of nuclear receptors and antiinflammation is nicely done, including acknowledgement that the issue is more complex than PPAR alpha (and, of course, PPAR gamma, an emerging literature for these toxicants) only.

- Thank you.

However, should the authors strengthen the discusion about the difference between statistical significance (present) and the size of the negative association (present yet quite small, and for a self-attributed survey characteristic)? That is a difficult topic. With a clinical outcome (disease presence), or a clnical marker with gradations (a laborotory test with cut-offs for normal), this group has been right to show that relatively small differences are associated with clinically relevant outcomes. However, those differences were greater than these in all instances, and there was a measureable clinical outcome. I leave it to the authors to consider whether a stronger caution is warrented concerning the potential difference between statistical and clincial significance in the setting of a survey opinion about one's memory.

- We have reviewed the text and attempted to clarify our position on what we agree is a difficult issue.

Reviewer: Anders Glynn
Senior Risk and Benefit Assessor, toxicologist
Swedish National Food Agency
Sweden

I have no competing interest.

There is a lengthy discusiion about the involvement of PPARgamma in a possible mechanism behind the key findings in the study. This is not supported by the references given.

- Additional references have now been inserted to support the discussion on PPAR.

There is also confusion in the Discussion about the possibility of reverse causation. The Discussion is confusing but I get the feeling that the authors are leaning towards reverse causation as an explanation of the finding. If this is the case, this should be mentioned in the Abstract and in the conclusions.

- Thank you, we agree that this was not clear enough. We have now more clearly set forward the argument. As these are cross sectional data they remain uncertain as to whether the PFAA exposure is driving the association or not and the conclusion reflects that uncertainty.

This cross-sectional study addresses the interesing hypothesis that increased exposure to perfluoroalkyl acids is associated with a better cognitive function. The study is well conducted and the results are in most cases clear-cut. However, the discussion about causality is confusing and the

speculations about possible mechanism behind the observed associations unclear.

- In response this and reviewer 1's detailed comments on the discussion we trust that the discussion of the strength of evidence and mechanisms is now clearer.

Comments
Abstract, line 18: "prefluoroclorinated"?

- Thank you, this spelling error is now corrected.

Introduction, page 4, lines 17-18: Environmentally contaminated fish is a major source of exposure for PFOS and long-chain PFCA.

- We agree and this has been added to the exposure sources.

Page 4, lines 21-48: The section correctly starts with some lines about PFAA and PPARalpha. It then goes into a more speculative part dealing with PPARgamma. The authors have not given any references from the literature dealing with PFAA and PPARgamma activation. There are some studies looking into interactions between PFAA and PPAR. None of these have suggested an interaction between PFAA and the gamma version, for instance Takacs and Abbot (2007). How does this fit with the hypothesis proposed by the authors?

- Thanks for highlighting this very good point. The paper has been acknowledged "However, a study in vitro showed that PFOA and PFOS activate differentially PPARα and PPARγ receptors, but it is not possible to directly extrapolate these results to toxicity studies in vivo" in the introduction.

Page 5, line 18: "3.65-4.27))". On parenthesis too much?

- Thanks

Methods, page 6, lines 21-29: There is no information about timing of memory loss, i.e. when did the study participants suffer from this? Now, 5 years ago, 10 years ago. Would be important information to associate with the onset of exposure. In the description of the study population, it is stated that "individuals were eligible to participate…….if they had consumed water for at least one year between 1950 and Dec 2004". Were the study subjects blinded to the exposure situation?

- This is a cross-sectional study: in the original questionnaire, there is no mention of the time frame within which the subjects had experienced memory loss. Both measures (self-reported memory impairment and PFAAs measurements were taken at the same point tin time).

Page 5, lines 42-57: Details are given about analyses of PFOA and PFOS, but nothing about PFHxS and PFNA. Please include this info in the text.

- Thanks for pointing this out. Figures have been added and corrected.

Results, page 9, lines 38-39: Something missing in this sentence?

Discussion, the first 3 sections: It is hypothesized that PPAR receptors are involved in a causal relationship between PFAAA and short-term memory. However, it is not clear which receptor(s) that the authors are refereeing to. PPAR-gamma is mentioned, but is there any data in the literature showing that PFAA interacts with this receptor?

- Thanks, this was a concern raised also by the other reviewer, the issue had been discussed with greater clarity both in the introduction and in the discussion.

Page 10, lines 30-51: The association with PFOA was entirely within water districts. The authors further states that the between-district estimate is not vulnerable to reverse causation in this study, and conclude that “this (reverse causation?) is a more plausible explanation. This is an important conclusion (if I have interpreted the discussion correctly), and should be included in the abstract.

- We agree that the abstract does not adequately reflect the balance of discussion, and have corrected this by adding the sentence “This can be potentially explained by preventive anti-inflammatory effect exerted by a PPAR agonist effect of these PFAAs, but confounding or even reverse causation cannot be excluded as an alternative explanation. “

Page 11, last section: The hypothesis that the inverse association is explained by an PPAR agonist effect is highly speculative, and not clearly supported in the discussion. Reverse causation a more plausible explanation? The Introduction and Discussion have to be thoroughly revised in order to convince the reader about the plausibility of the hypotheses.

- We believe the improved discussion is more consistent now. The consistency of findings across the different PFAAs could reflect a common pathway and there is some evidence that PPAR agonism could be a plausible mechanism, conversely there could be some confounding which leads to both the memory loss outcome and is associated with reduced bioaccumulation, affecting all four PFAAs. As this is cross sectional study the data we have cannot conclusively distinguish these conclusions, and so further research will be needed for addressing these issues.

VERSION 2 – REVIEW

REVIEWER	Ducatman, Alan West Virginia university, Department of Occupational & Environmental Health Sciences
REVIEW RETURNED	21-Mar-2013

GENERAL COMMENTS	This topic is interesting, useful to the affected population, and well done in my view. The authors have responded reasonably to critiques and have a lot of reasons to feel good about this work, for both science content and the message it sends to the studied population. Two topics follow, one may be important. Consider omitting the concept of important genetic variants in the context of between-water district comparisons. No data support this speculation, and, importantly, it raises the potential for a sharp-eyed critic to read old prejudices concerning Applachia and consanguinity into the manuscript. The authors are likely unaware of this US regional bias, or the regional sensitivity about it. Since the speculation does not add and carries some small risk of offense, it is in the authors interest to omit it. The authors are likely aware that recent reviews suggest that the anti-inflammatory activites (or could also be unrelated to PPARs. (What is going on in humans is still pretty mysterious.) Whether to add a more specific sentence about this is up to the authors.

REVIEWER	Glynn, Anders National Food Agency, Sweden
REVIEW RETURNED	25-Mar-2013

- **The reviewer completed the checklist but made no further comments.**

VERSION 2 – AUTHOR RESPONSE

Reviewer: Alan Ducatman

This topic is interesting, useful to the affected population, and well done in my view. The authors have responded reasonably to critiques and have a lot of reasons to feel good about this work, for both science content and the message it sends to the studied population. Two topics follow, one may be important.

Consider omitting the concept of important genetic variants in the context of between-water district comparisons. No data support this speculation, and, importantly, it raises the potential for a sharp-eyed critic to read old prejudices concerning Applachia and consanguinity into the manuscript. The authors are likely unaware of this US regional bias, or the regional sensitivity about it. Since the speculation does not add and carries some small risk of offense, it is in the authors interest to omit it.

We agree that a genetic explanation is a hypothesis with no data to support it and have edited the text to leave the possible nature of individual confounding more generic ("some unmeasured individual characteristic").

The authors are likely aware that recent reviews suggest that the anti-inflammatory activites (or could also be unrelated to PPARs. (What is going on in humans is still pretty mysterious.) Whether to add a more specific sentence about this is up to the authors.

Assuming that this comment is suggesting we comment on human anti-inflammatory effects with or without PPAR agonism, there is very little evidence for pro- or anti-inflammatory mechanisms for these compounds in humans, so we prefer to refer to the published work and we have not expanded this part of the discussion to speculate further.

Whilie I have stated that I would be willing to do a review, it my view it is not needed. Any further changes should be at authors discretions.