PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Contributions of Body Mass Index and Exercise Habits on Inflammatory Markers: A Cohort Study of Middle Aged Adults Living in the United States
AUTHORS	Strohacker, Kelley; McCaffery, Jeanne; wing, rena

VERSION 1 - REVIEW

REVIEWER	Caroline Schmidt, CLS, Associate Professor. Wallenberg Laboratory for Cardiovascular Research Institution for Medicin, Department for Molecular and Clinical Medicine Sahlgrenska Academy, Gothenburg University
REVIEW RETURNED	29-Jan-2013

THE STUDY	Description of inclusion and exclusion criteria of the subjects is missing. Further, the decription of the sample is not thorough, therefore it is hard to tell if the material isrepresentative. The statistical methods are not clearly described and therefore it is not possible to state if they are appropriate.
GENERAL COMMENTS	The authors should present the number of subjects in each of the weight groups. The table may be supplemented with this information and the demographic variables could be presented for each weight group. The authors should also rewrite the Statistical part so it will be presented more clearly. Figure 1, panel B, concerning sICAM-1: in the statistic section of the manuscript, the authors describes that "race was excluded as a covariate to increase sample size in all analyses excluding sICAM-1", the figure legend describes that "these analyses were adjusted for age, sex, smoking and relevant medication", for panel B this is not true, therefore this should be amended.

REVIEWER	Tore Christiansen Department of Endocrinology, Odense University Hospital
REVIEW RETURNED	17-Feb-2013

THE STUDY	Contribution of BMI and exercise habits on inflammatory markers: A Cohort study of middle aged adults living in the United States General Comments: As both obesity and physical inactivity in epidemiological studies has been shown associated with a low grade of inflammation, and this low grade of inflammation is a known risk factor for CVD, the authors have used a large cohort to disentangle the relative contributions of

	BMI and physical (in) activity to circulating levels of inflammatory markers. Before the paper is accepted for publications the authors the following points should be addressed. Page 4 Line 83; regarding IL-6 as a establish marker for CVD, there seems to be a paradox!. I accept that several papers have shown that elevated levels of circulating IL-6 are associated with CVD and mortality in the elderly population (papers from Harris TB). On the other hand, the group of Pedersen BK has in several publications pinpointed out that IL-6 has potential anti-inflammatory properties as the skeletal muscle during exercise is producing and releasing IL-6 into circulation. The authors should raise this complexity about IL-6 in the introduction. This complexity leads to Line 87 at the same page. Here, it is stated that physical activity appears to have anti-inflammatory effects. This could be true when reading epidemiological studies; however in randomized clinical trials the data regarding the anti-inflammatory effect of exercise is not so consistent (papers from Christiansen T,. And furthermore, it is resistance training, aerobic training or the combination the authors refers to? The authors should introduce these inconsistent data in their introduction. To strengthen the paper (introduction and method) the author should use some space describing the importance of body fat contribution in relation to systemic low grade inflammation. For instance, accumulation of fat in the abdominal region is known to have a stronger association with low grade inflammation as compared to accumulation of fat in the hip region. Thus, instead of a crude estimate of obesity (BMI), the authors should consider to implement some data on waist/hip ratio in relation to low grade inflammation and if possible show data on this ratio in the result section. Page 4, line 95; In my opinion the introduction is not strong enough to lead to this hypothesis. At least the authors must introduce the reader for some data regarding the dose response of physical activity (and be more precise – shat they do mean with physical activity) in relation to status of low grade inflammation! Page 5; line 115: I am not sure what the sample size calculation refers to! Page 5; Line 117; Do the authors have data on body fat distribution e.g. waist and hip circumference. This will strengthen the method section? Page 6; line 125; Do the authors have some data on information bias in relation to the self-reported data of levels of physical activity? Page 7; line 147 “reported >500 mmw” should be “reported < 500 mmw” Page 7; Line 157 – Was IL-6 (and the other inflammatory markers) measured in single or double batch Page 7; line 157 – In relation to collection the blood samples – what about the female participant’s menstrual cycle! Page 9 – general: As IL-6 initiates the acute response, marked by the release of hepatic CRP (stated at page 12,line 261), what was the association between IL-6 and CRP and why did CRP but not IL-6 show a significant interaction between BMI and MMW ? Page 9;Line 205 and 209. Perhaps I am a bit old fashioned, but it is my opinion that the authors should use the result section to present results and leave the discussion section for comments, speculations and discussion of the observed results! Page 9; line 200-209. Did the data show any dose response

	relationship? For instance did obese subjects with “MMW” >1000 had lower CRP (or any of the other measures inflammatory markers) as compared to obese subjects with MMW 500-1000?. Furthermore, looking at Figure 1 (a), how will the authors explain that subjects with MMW 500-100 have higher levels of CRP as compared to subjects with MMW<500 and , but lower than subjects with “MMW” >1000 ? Page 9-10: How are the different inflammatory markers inter correlated ?

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

1. Description of inclusion and exclusion criteria of the subjects is missing. Page 4/5, Lines 96-101: We have added the statements to clarify participant enrollment – “Participants were eligible for the Biomarker Study if they were previously enrolled in MIDUS 1, which recruited non-institutionalized, English-speaking adults residing in the contiguous United States aged 25-74. The random digit dialing sample for the parent study was selected from working telephone banks and a list of all individuals between the ages of 25 and 74 years within each household was generated in order to select a random respondent.”

2. Further, the description of the sample is not thorough, therefore it is hard to tell if the material is representative. The authors should present the number of subjects in each of the weight groups. The table may be supplemented with this information and the demographic variables could be presented for each weight group. Table 1 has now been amended to show descriptive variables for all BMI groups, as well as provide data on the number of subjects in each BMI group.

3. The authors should also rewrite the Statistical part so it will be presented more clearly. Page 7, Lines 169-187: We have added additional wording the statistical analysis section to clarify our statistical methods. In reviewing prior studies published in BMJ Open that have used general linear models, we believe we provide a similar level of detail. If there are aspects that remain unclear, we are happy to respond to comments that more specifically address perceived issues with the statistical section.

4. Figure 1, panel B, concerning sICAM-1: in the statistic section of the manuscript, the authors describes that "race was excluded as a covariate to increase sample size in all analyses excluding sICAM-1", the figure legend describes that "these analyses were adjusted for age, sex, smoking and relevant medication", for panel B this is not true, therefore this should be amended. Page 21, Line 514: The legend for figure 1 has been amended to state that “the analysis for sICAM-1 was further adjusted for race”.

Reviewer: 2

1. Page 4 Line 83; regarding IL-6 as a establish marker for CVD, there seems to be a paradox!. I accept that several papers have shown that elevated levels of circulating IL-6 are associated with

CVD and mortality in the elderly population (papers from Harris TB). On the other hand, the group of Pedersen BK has in several publications pinpointed out that IL-6 has potential anti-inflammatory properties as the skeletal muscle during exercise is producing and releasing IL-6 into circulation.

The authors should raise this complexity about IL-6 in the introduction.
This complexity leads to Line 87 at the same page. Here, it is stated that physical activity appears to have anti-inflammatory effects. This could be true when reading epidemiological studies; however in randomized clinical trials the data regarding the anti-inflammatory effect of exercise is not so consistent (papers from Christiansen T,. And furthermore, it is resistance training, aerobic training or the combination the authors refers to?
The authors should introduce these inconsistent data in their introduction. We have added substantial information to the introduction to incorporate this literature (Page 3, Lines 68-77).

2. To strengthen the paper (introduction and method) the author should use some space describing the importance of body fat contribution in relation to systemic low grade inflammation. For instance, accumulation of fat in the abdominal region is known to have a stronger association with low grade inflammation as compared to accumulation of fat in the hip region. Page 3, Lines 64-67: We have added the following statements – “Further, body fat distribution is also an important factor relating to inflammatory status. Accumulation of fat in visceral depots is more strongly associated with low-grade inflammation compared to accumulation of fat in subcutaneous or hip-region depots”

3. Thus, instead of a crude estimate of obesity (BMI), the authors should consider to implement some data on waist/hip ratio in relation to low grade inflammation and if possible show data on this ratio in the result section. We have analyzed the impact of waist circumference and physical activity on inflammatory biomarkers. We have added these results in the supplemental material as results are generally similar to those for BMI.

Additional Changes:

Page 5, Lines 112-114: we have added a statement regarding the methods of waist circumference measurement – “A single measure of WC was taken directly on the skin or over a single layer of light, close-fitting clothing at the narrowest point between ribs and the iliac crest in centimeters to the nearest millimeter”

Page 11, Lines 241-250: We have added a section titled “Waist Circumference (WC) and Inflammatory Markers” in order to briefly summarize WC results within the main document.

4. Page 4, line 95; In my opinion the introduction is not strong enough to lead to this hypothesis. At least the authors must introduce the reader for some data regarding the dose response of physical activity (and be more precise – what they do mean with physical activity) in relation to status of low grade inflammation! We believe that in addressing the concerns highlighted by the reviewer’s first comment, the introduction has been strengthened in order to introduce our primary hypothesis.

5. Page 4; line 92: I am not sure what the sample size calculation refers to! The statement “Based on the sample of 1254 participants, 80% power was achieved to detect small effects of 0.08 or greater with alpha level at 0.05 for a two-tailed test” refers to the statistical power to detect effects based upon the previously collected sample.

6. Page 5; Line 117; Do the authors have data on body fat distribution e.g. waist and hip circumference. This will strengthen the method section? Waist circumference data has been added in a supplement

7. Page 6; line 125; Do the authors have some data on information bias in relation to the self-reported data of levels of physical activity? Page 14, Lines 312-314: We have added this statement regarding information of physical activity bias – “No objective measures of physical activity were available in the MIDUS sample. Therefore, the use of self-report physical activity data may have diminished our ability to detect effects”

8. Page 7; line 147 “reported >500 mmw” should be “reported < 500 mmw” This error has been corrected.

9. Page 7; Line 157 – Was IL-6 (and the other inflammatory markers) measured in single or double batch. This aspect of blood processing was not specified in the Blood, Urine, and Saliva Documentation data provided by the Inter-University Consortium for Political and Social Research regarding the MIDUS Biomarker Project.

10. Page 7; line 157 – In relation to collection the blood samples – what about the female participant’s menstrual cycle! Page 7, Lines 146-147: We have noted that “blood samples were not collected at any specific point during the menstrual cycle in female participants”.

11. Page 9 – general: As IL-6 initiates the acute response, marked by the release of hepatic CRP (stated at page 12,line 261), what was the association between IL-6 and CRP and why did CRP but not IL-6 show a significant interaction between BMI and MMW? We have added a table showing the associations between all inflammatory markers in the supplement.

Page 13, Lines 295-300: we have added the following statements to address the findings that CRP, but not IL-6, showed a significant interaction between BMI and MMW – Although IL-6 produced in hypertrophied adipose tissue(33, 34) initiates the acute phase response, marked by the release of hepatic CRP (35, 36), an interaction between BMI and physical activity was only detected for CRP. While IL-6 and CRP were significantly correlated (r=0.514), this correlation suggests that IL-6 levels do not fully explain CRP levels at any given moment. Further, CRP is a more stable biomarker, owing to its substantially longer plasma half-life (37), which may improve our ability to detect interaction effects in CRP compared to IL-6.

12. Page 9;Line 205 and 209. Perhaps I am a bit old fashioned, but it is my opinion that the authors should use the result section to present results and leave the discussion section for comments, speculations and discussion of the observed results! Any remarks relating to the discussion have been removed from the results section.

13. Page 9; line 200-209. Did the data show any dose response relationship? For instance did obese subjects with “MMW” >1000 had lower CRP (or any of the other measures inflammatory markers) as compared to obese subjects with MMW 500-1000? Page 12, Lines 264-269 and Page 13, Lines – 292-294: We address this question. Our results suggest that any level of regular physical activity was associated with lower CRP and IL-6. We did not observe effects that may be considered dose-dependent, such that inflammatory levels in those reporting >1000 MMW were greater than 500-1000 MMW.

14. Furthermore, looking at Figure 1 (a), how will the authors explain that subjects with MMW 500-100

have higher levels of CRP as compared to subjects with MMW<500 and , but lower than subjects with "MMW" >1000?
Overall, while these levels appear to be numerically different, they were not statistically significant, perhaps owing to potential biases of self-reported physical activity levels.

15. Page 9-10: How are the different inflammatory markers inter correlated? We have added a table showing the associations between all inflammatory markers in the supplement.

VERSION 2 – REVIEW

REVIEWER	Caroline Schmidt, Associate Professor, Institution of Medicine, Sahlgrenska University Hospital, Sweden
REVIEW RETURNED	02-Apr-2013

THE STUDY	The authors have not described any exclusion criteria.
GENERAL COMMENTS	Page 5, line 109 - 110: This scentence needs to be clarified.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1

1. The authors have not described any exclusion criteria.

Page 5, lines 99-100: We have added the following sentence: "Exclusion criteria included non-participation in MIDUS and MIDUS II and unwillingness to travel to specified sites for biomarker assessment."

2. Page 5, line 109 - 110: This sentence needs to be clarified.

Page 5, lines 110-112: We apologize for not including the effect size metric. We have clarified this sentence to read "Based on the MIDUS Biomarker Project sample of 1254 participants, 80% power was estimated to detect small effect sizes (delta=0.08 and higher) with an alpha level at 0.05 for a two-tailed test".