Author’s response to reviews

Title: Malnutrition impairs mitochondrial function and leukocyte activation

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Author’s response to reviews:

Dear Editor,

Thank you very much for considering our previously submitted manuscript “Malnutrition impairs mitochondrial function and leukocyte activation” (NUTJ-D-18-00192) for publication in Nutrition Journal. We have read with great interest and examined thoroughly all the comments and criticisms raised by the reviewers and have modified the manuscript accordingly. Therefore, we are sending a new version of the present manuscript and respond to the reviewers below. Changes are marked in red throughout the manuscript.

We sincerely hope that this new revised version of the manuscript is now suitable for publication in Nutrition Journal.

Sincerely,

Prof Antonio Hernandez-Mijares
A point-by-point letter follows.

Reviewer #1: The paper confirms an interesting topic that is related to the malnutrition inflammation syndrome. A major review in formal English is required. Many informal sentences are included in the text like: our results, we, in short. That should not be used in papers. And still I am confused if you should use the terms DRM + I and DRM-I, because either the DRM-I have biomarkers of inflammation high, so they are not inflamed? For me it seems the difference is the presence or not of a disease.
Response: We appreciate your comments and we thank you for your contribution, which we feel has greatly improved our manuscript.
We have revised the manuscript and made the corresponding changes in accordance with the comments of the reviewer.
We understand the reviewer’s point regarding the terminology used in the groups (DRM +I or –I) being confusing, and the presence or not of a disease probably does play an important role in the inflammatory system, but we believe that categorization of these study groups allows us to establish the importance of inflammatory-based disease in malnutrition. In addition, according to the latest guidelines of Nutritional Societies, DRM has been classified by different groups based on the presence or not of inflammatory disease. In this context, we have found two types of DRM: DRM with inflammation (DRM+I), when malnutrition is associated with a chronic or acute inflammatory-based disease; and DRM without inflammation (DRM-I), which occurs in starvation-related malnourished patients.

Abstract:
Line 60: a total of 83 subjects, suggest to include the word were included, just after the sentence.
Response: Done
Line 63: Do not need to include the word mitochondrial parameters.
Response: Done
Line 67: "protein levels with respect to the NN group, differences that were less noticeable in the DRM-I 68 group. DRM+I was associated with a significant increase in inflammatory parameters (hsCRP, 69 and TNFα, IL6) ", explain which differences.
Response: We have explained differences in inflammatory parameters
Line 71: Suggest to change the phrase construction for "... decrease in mitochondrial oxygen consumption and glutathione".
Response: Done

Background:
Line 96: Explain better the sentence "it has been demonstrated that disorders that lead to inadequate nutrition"
Response: We have changed the sentence to explain how malnutrition per se or DRM could lead to inadequate nutrition.
Line 112: "In addition, reduced dietary intake has been associated with an increase in leukocyte-endothelium interaction and, therefore, enhanced leukocyte migration ", would be interesting if you explain the association between the dietary intake and leukocyte-endothelium interaction.
Response: We appreciate the reviewer’s comment and have discussed this issue in the Background section (lines 105 to 115). In brief, malnutrition enhances ROS production, which activates inflammation pathways, and stimulates the production of adhesion molecules in endothelial cells. Hence, activated immune cells can attach to the endothelium monolayer and infiltrate into the inner organs. Several studies have confirmed this relationship. We have previously demonstrated that anorexia nervosa characterized by a reduced dietary intake is associated with an increase in leukocyte-endothelium interaction and, therefore, enhanced leukocyte migration [13]. This relationship between...
malnutrition and leukocyte activation has also been described by Stevinkel et al, who reported a strong relationship between undernourishment, inflammation and soluble adhesion molecule levels in pre-dialysis patients [14], a situation that could lead to increased leukocyte-endothelium interaction. Many times, you use the word malnourished instead malnutrition. For example in line 120. Suggest to look all over the text and correct. Malnourished is different from malnutrition. Response: We apologize for this mistake. We have changed this incorrect terminology throughout the text, and have highlighted the changes in red.

Line 122-123 : Explain better. Can't understand what does this sentence want it to mean. Response: We completely agree with the reviewer. In fact, we have rewritten the sentence in the Background as follows “Multiple definitions of malnutrition are used in the literature, which creates certain confusion. Recent evidence suggests that varying degrees of acute or chronic inflammation is a key contributing factor in the pathophysiology of DRM. An etiology-based approach that incorporates current understanding of the inflammatory response would seem to be appropriate.”

Line 128: Let it clear which consensus criteria is used. Response: We have added more information about the ASPEN consensus criteria used (reference 18).

Line 129 : Suggest to change the word causing to leading Response: Done

Line 141: Explain why Chronic Kidney Disease (CKD) patients and those with hepatic disease, were not included if they are an inflammatory group. And also, you say it was not included CKD patients but in your results they appear. How? Response: In the new version, when referring to the exclusion criteria, we have explained that only “severe renal diseases (stage ≥4 chronic kidney disease and glomerular filtration rate<30mL/min) and severe hepatic disease (Child-pugh B or C) were excluded from the study. According to the literature, both of these severe diseases alter biochemical parameters such as albumin and make it confusing to assess malnutrition. In addition, these patients usually develop edema and ascites that alter their weight, one of the criteria for assessing malnutrition. Finally, these alterations of biochemical and anthropometrical parameters could affect the correlations between clinical and oxidative stress studied. In nutritional assessment: Explain with more details how loss of muscle mass and subcutaneous fat was evaluated? Response: To assess nutritional status we performed a physical examination and anthropometric determinations. These clinical characteristics were obtained according to the recommendations of the Academy of Nutrition and Dietetics (Academy)/ American Society for Parental and Enteral Nutrition (ASPEN). Specifically, loss of muscle mass was evaluated according to wasting of the temples; clavicles; shoulders; interosseous muscles; scapula; thigh and calf. Loss of subcutaneous fat was evaluated by orbital, triceps, and fat overlying the ribs.

Line 169: It is mid-upper arm muscle circumference (MUAMC) and not Arm muscle perimeter (AMP), the most used in the literature. Response: As we have described in the methodology, we measured MUAC (line 167-168) and AMP (line 170-171). We described the results of these parameters in table 1.

Biochemical determinations: You explain to much. I think it is necessary you say which method was used and thresholds, if you used to classify malnutrition. Response: Although not all variables were used to classify malnutrition, we have detailed all the methodology applied in the study. We think that is of interest to readers to know how the study was performed.

Results:

Line 255: Don’t need to say preliminary, it is not you already published 2 papers! Response: Done

Line 258: Is numberS, Response: Done
chronic renal disease (2.0%), check exclusion criteria.
Response: According to the exclusion criteria, severe renal disease patients (stage ≥4 chronic kidney
disease and glomerular filtration rate<30mL/min) were not included, but patients with chronic renal
disease in the early stages (1-3) were included in the study.
"Surprisingly, DRM-I group showed increased levels of TNFα with respect to NN group."
And How about DRM+I group?
Response: We have explained that DRM+I showed significant differences in TNFα compared to
controls, as can be seen in Figure 1.

Discussion:
Need major improvement are necessary, many times it is used personal subjects and also you present
again your results. You need to discuss it.
Response: We have now checked and corrected this issue in the discussion section.
You need to discuss it, not show results.
Response: We have tried to discuss the results with more depth, but sometimes we need to introduce
the result in question to follow up and link with other studies.
Avoid words: we have found, diagnosis, said, as we have confirmed.
Response: Done.
Did not understand what you mean with: "22% anorexia subjects" and where did you found
this number.
Response: In the results section we describe the disease-related malnutrition of each group, with
anorexia being present in 22.4% of the DRM-I group.
it is malnutrition not malnourishment
Response: We apologise for this mistake, which we have now corrected.
Limitations: It is necessary to be more formal, avoid to use very little, you may use a few instead. And
also personal names.
Response: A native english editor has thoroughly checked the manuscript and we made the
corresponding changes where applicable.
Conclusion: It is not necessary the word overall.
Response: We have removed “Overall” from the Conclusion paragraph

Table 1.
#explain what is it ? Use a label
## explain what is it ?Use a label
###explain what is it ?Use a label
Response: These symbols are explained in the legend of Table 1.
It is not necessary to explain the statisc test.
Response: We have removed the explanation of the statistical test in the legend.

Reviewer #2: I appreciate the authors for writing the manuscript in a very good way, I also suggested
some few corrections in your manuscript.
Concentrate on your spell checking and punctuation as well.
Response: We are grateful to the reviewer for his/her evaluation of the MS and thank him/her for
considering that our study has been written in a very good way.
We have revised the manuscript and made the corresponding changes in accordance with the comments
of the reviewer.
Line 89-91. Remodify the statement, which has shown repeated (And) between the statements. 
Response: Done

Line 132-134 - Hypotheses and the prevalence. Clarify that the background of your study says that the prevalence is reported to be lower in out-patients, then why your study samples were chosen from outpatients? Do the DRM related research with outpatients is less? Clarify it.
Response: We previously carried out a research project to assess the prevalence of nutritional risk and malnutrition in outpatient, institutionalized and hospitalized populations in a health department (Nutr Hosp. 2017;34(4):889-898). The overall prevalence of DRM was 26.4% (outpatients 2.8%, institutionalized patients 30.13%; hospitalized patients 30.2%). However, as part of a second approach, we evaluated inflammatory and oxidative stress parameters in an outpatient population because few studies had assessed this type of population. In addition, we selected an outpatient population because, due to logistic reasons, it is easier to obtain blood samples and perform follow up at our hospital’s outpatient Endocrinology than to study hospitalized patients.

Line 143 - modify the word categorized instead of Distributed according to the classification of your study with NN, DRM-1, DRM+1.
Response: Done

Line 171 - Concentrate on the Punctuation error.
Response: We have done this.

Line 172 - Clarify the subtitle
Response: Done

Line 190 - Word correction Intercellular
Response: Changed.

Line 213 - check for the proper mentioning of the serum concentration level \{5x106\}
Response: Done

Line 272- Clarify the statement about differences remained after adjustment for age and gender.
Response: Done

Line 329 - As mentioned Corroborates it is presented in a good way but I suggest you to remodify the vocabulary for the purpose of clear understanding for the journal readers'
Response: Done

Line 329 - remodify the statement inflammation induces increased lipolysis or inflammation induced increases lipolysis.
Response: Changed

Line 336 - Correct spelling mistake of haemodialysis
Response: Done

Line 352-355 - concentrate on the punctuation error found in the line
Response: Done

Line 355 - I suggest that you can avoid or change the words as y in this way, in this sense. You can start
Response: We agree.

Line 357 - You can also avoid words like in said patients, you can mention them as among samples, among population studied etc., which sounds better.
Response: Done

Line 362 - Avoid said researchers. You can change your express of words in some other forms.
Response: Done

Line 371-372 - remodify the sentence as it is not written properly.
Response: Done