A double-blind, randomized trial on the effect of a broad-spectrum dietary supplement on key biomarkers of cellular aging including inflammation, oxidative stress, and DNA damage in healthy adults

Lucas C. Lages, Johanna Lopez, Ana Maria Lopez-Medrano1, Steven E. Atlas, Ana H. Martinez1, Judi M. Woolger, Eduard Tiozzo, Janet Konefal, Armando J. Mendez, Herbert G. Simoes, John E. Lewis

Corresponding author:
Lucas C. Lages
Department of Psychiatry and Behavioral Sciences, University of Miami Miller School of Medicine, Miami, Florida, United States

Handling editor:
Michal Heger
Department of Experimental Surgery, Academic Medical Center, University of Amsterdam, the Netherlands

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A double blind, randomized trial on the effect of a broad-spectrum dietary supplement on key biomarkers of cellular aging including inflammation, oxidative stress, and DNA damage in healthy adults

Journal of Clinical and Translational Research

Dear Dr. Lewis,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you resubmit your work.

Your revision is due by Oct 14, 2016.

To submit a revision, go to http://jctres.edmgr.com/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger

Editor-in-Chief

Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: This research is well-planned and well-described, however, the decision of using such multi-component product as the object of study is not convincingly motivated. Healthycell preparation is a mixture of extracts, which are already mixtures, and many single compounds. It should be explained why a less complex mixture has not been used. It is true that the supplementation with single components has largely failed in improving any health parameters, but in my opinion the more logical step would be studying the mixture with limited number of components (although still containing different types of nutrients), such as one extract, maybe enriched with vitamins and minerals. This way, the components responsible for obtained results would be possible to identify.

There are also some issues that should be discussed in more detail:

1. What is the cause of the increase of IL-1α and IL-2 in placebo group? In the latter case, this increase is probably the reason why after a supplementation period the difference between groups was observed, since in the Healthycell group no changes were observed (as ilustrated in Fig. 3).

2. The tendency of change of IL-5 is different for different age groups (decrease in <35 group and increase in >35). What is the possible

explanation of this observation? Could it be related to the difference in baseline value for these groups?

Reviewer #2: Thank you for the opportunity to review this interesting paper. Considering the widespread use of nutritional supplements within the community, in particular combination supplements, it is important that the effects of these products are assessed.

Overall I found the paper to be well written and complete in the information provided. I have some questions/concerns regarding the statistical aspects of the paper, and a few minor editing points.

Introduction.

The introduction is well written and clearly leads to the objectives of the study. The authors discuss aging but do not tie this to any hypothesis around the age split sub-analysis. More specific hypotheses could be added here, for example, if improvements are expected rather than worsening of the biomarkers.

Abstract.

Need to add that the findings were in the treatment group compared with placebo - especially for CRP it is a bit unclear whether it rose in just the treatment group - sounds like it was across the board.

Methods.

Power analysis - 0.70 is a fairly low power, usually it is at least 0.80. Was there a reason to choose this level? Do you think there may have been further changes observed if the sample size was larger?

The low sample size is a serious limitation considering the number of tests that were undertaken and should given more consideration in the manuscript. This is particularly true for the sub-analyses which made the groups very small.

Two-tailed tests were used however if the hypotheses were directional you could consider using one-tailed tests.

Results.

The paper reports post-hoc tests for ANOVA but does not report the ANOVA results. Please include the ANOVA results or provide

justification why these are not included in the report.

When reporting tests, please restate the tests that were used to obtain the p values.

Figures: I'm not sure if it is how my copy printed out because the figure legends are with the text, not under the figure. It looks as if the statistics are reported in the figure legend, however these should be within the main text.

Discussion and Limitations.

The discussion was well written and limitations were acknowledged. Again, mention of the small sample size is made with regard to generalizability but not low power - this should be added.

Editing.

Check spacing around the = sign when reporting results.

When a sentence starts with a number, use the word not the number, e.g. Forty not 40.

For numbers up to ten in text use the word, e.g. one tablet not 1 tablet.

Reviewer #3: Dear Authors,

The manuscript JCTRes-D-16-00028 examined the effects of the dietary supplementation with a broad-spectrum of supplement in the human biomarkers for cellular aging, oxidative stress and DNA damage. The authors suggested that the dietary supplementation could be an important tool to fight against age-related chronic diseases and contribute to decrease health care cost. This work is interested in this regard, however there major points that must be explained and/or corrected.

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

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General to the author

The manuscript JCTRes-D-16-00028 examined the effects of the dietary supplementation with a broad-spectrum of supplement in the human biomarkers for cellular aging, oxidative stress and DNA damage.

The authors suggested that the dietary supplementation could be an important tool to fight against age-related chronic diseases and contribute to decrease healthcare cost. This work is interesting in this regard, however there major points that must be explained and/or corrected:

Title:

- Authors says “Oxidative stress”, however do not present results....

Abstract:

- The author’s declare that their supplement counteract against aging and age-related chronic diseases. However, according to WHO aging is considered at 60 years or older. With this, why the authors conducted the study with ages that is not considered as aged people?
- The results showed at abstract don’t include anything related to oxidative stress.
- The conclusion need be re-think because of the missing results and concepts.

Introduction

- The authors need determine what is the theory that they will focus latter in the results, discussion and conclusion, I say this because in the title and abstract the authors focus on inflammatory and oxidative stress, however in this section the authors did not show concept or mechanisms related to inflammatory and oxidative stress isolated or associated with ageing.
- Rethink about the approach that the authors want follow through the manuscript.

Methods

- There is a lack of information for all assays, for example how was the kit used for all interleukin?
- Same thing for 8-hydroxydeoxyguanosine, isoprostanes, homocysteine, CRP and cortisol

Results

- Authors showed in table 2 a significant increase in TNFR I and II (important mediator in apoptotic process and normally increased with ageing) in 4 weeks and after 6 weeks in healthycell group. With this how the authors can sustain that the supplement counteract ageing, since in the placebo group was lower than healthycell group????
- Huge relevance to inflammatory data, with very small relevance to others data present such as CRP, 8-OHdG and cortisol. For these 3 the effective results (i.e values) are missing.
- Missing isoprostanes, and homocysteine results.

Discussion

- As mention above the authors need determine whether this manuscript is only about the supplements and inflammation, or others concepts are also relevant. Because the way that the authors conducted the manuscript and the discussion it is not in sync.

******Author response******

Michal Heger, Ph.D.
Editor-in-Chief
Journal of Clinical and Translational Research

Dear Dr. Heger:

We thank the reviewers for their thorough evaluation of our manuscript. As per the requests of the reviewers, we have endeavored to modify our paper to improve its quality and suitability for publication. We have addressed the following reviewers' comments:

Reviewer #1

This research is well-planned and well-described, however, the decision of using such multi-component product as the object of study is not convincingly motivated. Healthycell preparation is a mixture of extracts, which are already mixtures, and many single compounds. It should be explained why a less complex mixture has not been used. It is true that the supplementation with single components has largely failed in improving any health parameters, but in my opinion the more logical step would be studying the mixture with limited number of components (although still containing different types of nutrients), such as one extract, maybe enriched with vitamins and minerals. This way, the components responsible for obtained results would be possible to identify.

We attempted to investigate the effects of a multi-nutrient supplement containing ingredients used in other multi- and single-component supplements available in the marketplace. Delineating the mechanisms by which the results were obtained was not within the scope of this study. However, we agree that investigating the individual components or a less complex mixture is warranted. Nonetheless, given poor outcomes with single-component supplements, it is perhaps best to establish efficacy with a broad-spectrum supplement before investigating its individual ingredients.

There are also some issues that should be discussed in more detail:

1. What is the cause of the increase of IL-1α and IL-2 in placebo group? In the latter case, this increase is probably the reason why after a supplementation period the difference between groups was observed, since in the Healthycell group no changes were observed (as ilustrated in Fig. 3).

After reviewing the data again, the IL-2 results were apparently due to random chance and the latest version of the manuscript has those findings removed. We attribute the IL-1a placebo increases to small sample size.

2. The tendency of change of IL-5 is different for different age groups (decrease in <35 group and increase in >35). What is the possible explanation of this observation? Could it be related to the difference in baseline value for these groups?

Actually both group's (young and old) IL-5 levels increase at certain times. For the older group, this occurs from baseline to the four-week time point, while in the younger group it occurs between the four- and six-week time points. We do not know why the younger group initially experienced a decrease between baseline and four weeks.

Reviewer #2

Introduction. The introduction is well written and clearly leads to the objectives of the study. The authors discuss aging but do not tie this to any hypothesis around the age split sub-analysis. More specific hypotheses could be added here, for example, if improvements are expected rather than worsening of the biomarkers.

We have modified the study hypotheses and theories and have been modified the Introduction and Discussion sections accordingly.

Abstract. Need to add that the findings were in the treatment group compared with placebo - especially for CRP it is a bit unclear whether it rose in just the treatment group - sounds like it was across the board.

We have made these edits accordingly.

Methods. Power analysis - 0.70 is a fairly low power, usually it is at least 0.80. Was there a reason to choose this level? Do you think there may have been further changes observed if the sample size was larger?

The level of power was chosen considering the results of previous work with this supplement and our financial limitations to run the study. Nonetheless, even with a fairly low power level, we still found several significant findings. We have noted the concern on lines 300-301 and based on the findings we believe that additional changes may have been shown with a larger sample size.

The low sample size is a serious limitation considering the number of tests that were undertaken and should given more consideration in the manuscript. This is particularly true for the sub-analyses which made the groups very small.

This is true. The increase in placebo's IL-1a levels have been attributed to small sample size, and small sample size is mentioned as one of the limitations in the study.

Two-tailed tests were used however if the hypotheses were directional you could consider using one-tailed tests.

To maintain impartiality when analyzing the results, we used two-tailed tests.
Some markers like cortisol were found to change in the opposite direction than what would be expected, but we argued that those results were in fact, positive (see lines 306-314).

Results. The paper reports post-hoc tests for ANOVA but does not report the ANOVA results. Please include the ANOVA results or provide justification why these are not included in the report.

Please see lines 178-182 for the justification for why the ANOVA results were not presented.

When reporting tests, please restate the tests that were used to obtain the p values.

We have modified the text to state the "t" values for the multiple t-tests performed.

Figures: I'm not sure if it is how my copy printed out because the figure legends are with the text, not under the figure. It looks as if the statistics are reported in the figure legend, however these should be within the main text.

Legends and figures have been adjusted accordingly.

Discussion and Limitations. The discussion was well written and limitations were acknowledged. Again, mention of the small sample size is made with regard to generalizability but not low power - this should be added.

We have added a statement in the Discussion section in lines 331-332 regarding the power of the study.

Editing. Check spacing around the = sign when reporting results.

Spaces before and after the "=" have been eliminated. Thus, all uses of the equality sign have no space behind or after it, e.g., p=0.01.

When a sentence starts with a number, use the word not the number, e.g. Forty not 40.

We have changed any use of a number starting a sentence to be spelled out.

For numbers up to ten in text use the word, e.g. one tablet not 1 tablet.

We have changed any use of a number up to ten to be spelled out.

Reviewer #3

Title: Authors says "Oxidative stress", however do not present results.

We apologize this was unclear in the original version of the manuscript. In the new version, the results of 8-hydroxydeoxyguanosine are discussed on line 270 in the context of oxidative stress, as 8-OHdG is a marker for oxidative damage.

Abstract: The author's declare that their supplement counteract against aging and age-related chronic diseases. However, according to WHO aging is considered at 60 years or older. With this, why the authors conducted the study with ages that is not considered as aged people?

The Reviewer is correct in that the average age in the older group was 44 years of age and the average age in the younger group was 30. We respectfully suggest that the definition of "older" is far from absolute, as even within the WHO controversy exists about what age is older, as it is multidimensional (http://www.who.int/healthinfo/survey/ageingdefnolder/en/). Please see the following websites for additional discussion on the difficulty in defining elderly or older: https://www.sciencedaily.com/releases/2013/12/131212100144.htm and http://www.npr.org/2013/03/12/174124992/an-age-old-problem-who-is-elderly. Thus, given the inconsistent definitions associated with aging or older, we are comfortable with the ranges for age we chose in the current study. Additionally, we have changed the Introduction (lines 58-70), Discussion (lines 289-295), and Conclusion (line 316) to emphasize the possible preventative aspects of using Healthycell in an approach to counteract age-related chronic diseases, which would be appropriate for anyone regardless of his/her age.

The results showed at abstract don't include anything related to oxidative stress.

The results have been clarified to report that 8-OHdG is a marker for oxidative damage.

The conclusion need be re-think because of the missing results and concepts.

Our Conclusion focuses on Healthycell's ability to counteract age-related chronic diseases before they begin. Given that we have modified the paper to address the Reviewer's prior concerns, the Conclusion should follow logically.

Introduction: The authors need determine what is the theory that they will focus latter in the results, discussion and conclusion, I say this because in the title and abstract the authors focus on inflammatory and oxidative stress, however in this section the authors did not show concept or mechanisms related to inflammatory and oxidative stress isolated or associated with ageing.

The Introduction has been modified to include the theory that the gradual deterioration of the immune system with age (i.e., immunosenescence) is linked to age-related diseases. In our study, we analyzed key biomarkers of oxidative stress and inflammation that allowed us to observe changes in our sample's immune functioning. We found positive results for those who took Healthycell. In our Conclusion, we suggest that Healthycell may be useful in counteracting age-related chronic disease, as it decreased markers of inflammation and oxidative stress that are implicated in senescence and age-related diseases.

Rethink about the approach that the authors want follow through the manuscript.

The Introduction, Discussion, and Conclusion sections have been modified to be consistent with the Reviewer's suggested changes.

Methods: There is a lack of information for all assays, for example how was the kit used for all interleukin?

We have added a subsection in the Methods section that lists sufficient information about the laboratory techniques performed for the assays (lines 143-155).

Same thing for 8-hydroxydeoxyguanosine, isoprostanes, homocysteine, CRP and cortisol.

This information has been added to the same section as well.

Results: Authors showed in table 2 a significant increase in TNFR I and II (important mediator in apoptotic process and normally increased with ageing) in 4 weeks and after 6 weeks in healthycell group. With this how the authors can sustain that the supplement counteract ageing, since in the placebo group was lower than healthycell group????

We measured soluble tumor necrosis factor receptor (sTNFR), not receptor bound TNFR. sTNFRs work as decoys and inhibit TNF-a. References 42-44 describe soluble tumor necrosis factor receptor I/II and lines 261-264 of our paper discuss this result.

Huge relevance to inflammatory data, with very small relevance to others data present such as CRP, 8-OHdG and cortisol. For these 3 the effective results (i.e values) are missing.

The results for CRP, 8-OHdG, and cortisol can be found in the Results section in lines 233-239. On lines 300-305, we deem the OHdG results meaningful because of its utility as a marker for oxidative stress and cancer (an age-related disease). We make the argument on lines 306-314 that lowering cortisol levels could indicate decreased levels of stress. We discussed on lines 315-319 how an increase in CRP levels would only be worrisome if IL-6 and TNF-α increased as well and that the increase in CRP was lost when participants who reported being sick during the intervention were excluded from the analysis. It may seem that more relevance was placed on the inflammatory data, but that is because we collected much more immune/inflammatory data compared to these three markers. Nonetheless, OHdG, cortisol, and CRP all represent important markers and their results help to form the conclusion of this paper.

Missing isoprostanes, and homocysteine results.

We have modified line 239 to reflect their non-significant results.

Discussion: As mention above the authors need determine whether this manuscript is only about the supplements and inflammation, or others concepts are also relevant. Because the way that the authors conducted the manuscript and the discussion it is not in sync.

The Introduction and Discussion sections have been edited, so that they are in sync with the Conclusion section. These edits are consistent with our response to the Reviewer's aforementioned prior criticisms.

Please let us know if you have any additional questions or clarifications, and we look forward to the next review of our paper.

2nd editorial decision
Date: 21-dec-2016
Ref.: Ms. No. JCTRes-D-16-00028R1
A double blind, randomized trial on the effect of a broad-spectrum dietary supplement on key biomarkers of cellular aging including inflammation, oxidative stress, and DNA damage in healthy adults
Journal of Clinical and Translational Research

Dear Dr. Lewis,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

Comments from the editor and reviewers can be found below.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers:

Reviewer #1: I'm recommending this work in the revised version for the publication in the Journal of Clinical and Transactional Research. Although this is rather a preliminary study (due to the small sample size and a very complicated composition of studied supplement), as the Authors themselves admit in the "Limitations" section, it reports some interesting findings that could be helpful in the further studies of dietary supplements trials.

Reviewer #2: Thank you again for the opportunity to review this paper. I have noted the changes made in response to my previous comments and am satisfied with the authors' response.

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