

Cardiovascular, endothelial function, and immune markers in response to treatment with a polysaccharide in HIV+ adults in a randomized, double-blind placebo-control trial

John E. Lewis, Steven E. Atlas, Muhammad H. Abbas, Ammar Rasul, Ashar Farooqi, Laura A. Lantigua, Frederick Michaud, Sharon Goldberg, Lucas C. Lages, Jinrun Gao, Oscar L. Higuera, Andrea Fiallo, Philip D. Harvey, Eduard Tiozzo, Judi M. Woolger, Stephanie Ciraula, Armando Mendez, Allan Rodriguez, Janet Konefal

Corresponding author:

John E. Lewis

Department of Psychiatry & Behavioral Sciences University of Miami Miller School of Medicine 1120 NW 14th Street, Suite #1482A (D28), Miami, FL 33136, United States

Handling editor:

Michal Heger

Department of Pharmaceutics, Utrecht University, the Netherlands

Department of Pharmaceutics, Jiaying University Medical College, Zhejiang, China

Review timeline:

Received: May 23, 2019

Editorial decision: July 3, 2019

Revision received: November 26, 2019

Editorial decision: November 27, 2019

Published online: April 23, 2020

1st editorial decision

3-Jul-2019

Ref.: Ms. No. JCTRes-D-19-00007

Cardiometabolic, endothelial function, and immune markers in response to treatment with a polysaccharide in HIV+ adults in a randomized, double-blind placebo-control trial
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 submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Aug 02, 2019.

To submit a revision, go to <https://www.editorialmanager.com/jctres/> 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: The paper describes the effects of RBAC versus placebo on cardio-metabolic, endothelial and immune function in people living with HIV. I find of high relevance the fact that the authors acknowledged the modest effect of the treatment on the variables of study. This has an important value to the scientific community. However, the conclusion in the discussions section "In the current study, systolic blood pressure, skin blood flow in response to nitric oxide, and CD4+ count differentially changed overall between the RBAC and placebo groups over time" (lines 9 to 12, page 14) is too strong in favor of the RBASC group. This statement could mislead the reader or could be wrongly interpreted if considered out of context. It is clarified in the paragraphs below that the effect is not statistically significant for two of the variables, but I'd suggest to state that fact as the main conclusion of the study given the potential benefit to the study population.

The authors recognize the effect of the small samples size, and describe the relevance of obtaining positive effects even with the small group of participants enrolled. However, the lack of control/report of additional variables (confounders) on the study outcomes, could be limiting the results of the study. Additional description of adjustment by other control variables like exercise and diet could strength the positive effect of RBAC on systolic blood pressure and CD4.

The discussion could elaborate in the results of interaction between treatment and time and provide interpretation to the reader. It is presented as an important section in the statistical analyses, but it is not mentioned in the discussion.

Editorial Reviewer #2:

Dear authors,

Thank you for submitting your work to JCTR.

The editorial board underscores the importance of your study for HIV+ patients who are on ART.

In addition to the comments of Reviewer 1, please consider the following remarks in preparing your resubmission.

- 1) In the Introduction you summarize the effects of RBAC on NK cells. However, the relationship between NK cells and HIV+ is unclear. Please establish this link as you have done for endothelial function and CD4+ cells. The same applies to blood pressure (the prevalence of cardiovascular disease, as alluded to in the last sentence of the first paragraph, is too broad). It should be clear to readers why the authors have chosen to measure these parameters in the context of HIV+ from the Introduction.
 - 2) The Results section is rather difficult to read for several reasons. Firstly, rather complex statistical tests are used to measure therapeutic efficacy. The reader is forced to fully understand the respective methods section in order to comprehend the results. It would improve the legibility and understandability of the Results section if the authors specified the outcome parameters rather than using generic terms such as "overall multivariate interaction," "dependent variables," and "contrasts." It is therefore encouraged that the text is reformulated in a manner that the results can be fully understood without having to revert to the Methods section. Secondly, it would greatly benefit the Results section if the authors briefly explained what the multivariate analysis implies about the results and what the univariate analysis implies about the results. The most important issue here is the interpretation and contextualization difficulty that arises when the multivariate analysis yields significant differences, but the univariate analysis chiefly does not. We believe that especially the clinical readership needs a helping hand in results interpretation, and we kindly request you to provide that accordingly. The sentence in the Discussion "Overall, we found a significant interaction in the simultaneous changes in cardiometabolic, endothelial function, and immune markers comparing the RBAC group to placebo in the absence of significant individual univariate effects" is quite a quagmire in terms of what to actually make of the results.
 - 3) It would further benefit the data interpretation if the authors could indicate in the Results section the direction in which the variables should change to be considered beneficial for patients. This holds especially true for the blood flow measurements.
 - 4) It seems to me that the overall effect of RBAC is that supplementation results in a steady systolic blood pressure during 6 months, a less expeditious decline in skin blood flow in response to NO during 6 months, and a short-term (3 months) increase in CD4+ cell count relative to the control group. These factors altogether cause the multivariate analysis in terms of treatment effect to be significant. If the interpretation is correct, then it should be better stated in the manuscript, preferably in the first paragraph of the Discussion.
 - 5) Table 2 should denote the units in the Measure column.
 - 6) Statistically speaking, if a difference between 2 ordinal variables is not significant, one cannot speak of a change.
 - 7) Individual results do not have to be restated in the Discussion section, as is done in the second paragraph.
 - 8) How was compliance measured during 6 months? If the compliance related to supplement/placebo intake was not assessed or monitored, the authors should state the reason and explain why they have confidence that each subject abided by the imposed dosing schedule.
-

Author's response

UNIVERSITY
OF MIAMI



Department of Psychiatry & Behavioral Sciences
Clinical Research Building
1120 NW 14th Street, Suite 1482A (D28)
Miami, FL 33136

Office: 305-243-6227
Cell: 305-962-5286
Fax: 305-243-1619
E-mail: jelewis@miami.edu

November 25, 2019

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

The paper describes the effects of RBAC versus placebo on cardio-metabolic, endothelial and immune function in people living with HIV. I find of high relevance the fact that the authors acknowledged the modest effect of the treatment on the variables of study. This has an important value to the scientific community. However, the conclusion in the discussions section "In the current study, systolic blood pressure, skin blood flow in response to nitric oxide, and CD4+ count differentially changed overall between the RBAC and placebo groups over time" (lines 9 to 12, page 14) is too strong in favor of the RBASC group. This statement could mislead the reader or could be wrongly interpreted if considered out of context. It is clarified in the paragraphs below that the effect is not statistically significant for two of the variables, but I'd suggest to state that fact as the main conclusion of the study given the potential benefit to the study population.

We agree with the Reviewer and understand that the statement could be considered too strong and interpreted incorrectly by the reader, so we have changed the statement to: "In the current study, systolic blood pressure, skin blood flow in response to nitric oxide, and CD4+ count were slightly different overall between the RBAC and placebo groups over time."

The authors recognize the effect of the small samples size, and describe the relevance of obtaining positive effects even with the small group of participants enrolled. However, the lack of control/report of additional variables (confounders) on the study outcomes, could be limiting the results of the study. Additional description of adjustment by other control variables like exercise and diet could strength the positive effect of RBAC on systolic blood pressure and CD4.

We agree with the Reviewer that many other behaviors could have influenced the results of our study. We were limited in how much attention we could devote to these other behaviors and factors, and thus they were not collected or accounted for. Nonetheless, we have added

the following sentence to the Limitations sub-section: “In addition to co-morbidities, other behaviors, such as diet, exercise, stress management, and sleep, may have at least partially influenced the results of the study.”

The discussion could elaborate in the results of interaction between treatment and time and provide interpretation to the reader. It is presented as an important section in the statistical analyses, but it is not mentioned in the discussion.

We encourage the Reviewer to understand that the use of the term “time” is much more about statistical parlance than it is about a specific reference to time’s importance in a clinical sense. You cannot discuss the results of interactions of between and within subjects effects without acknowledging the importance of the “time” factor. Thus, in any intervention study where you are comparing two or more groups over time with two or more assessment points, “time” is going to be a key statistical component to the discussion of those results. Nonetheless, we have made sure to highlight the fact that this study is limited to short-term results, given that our intervention was only for 6 months and that a suggestion for future research would be to look at the effect of RBAC over at least a 12-month period.

Editorial Reviewer #2

The editorial board underscores the importance of your study for HIV+ patients who are on ART.

We thank the Reviewer for recognizing the importance of our work.

In addition to the comments of Reviewer 1, please consider the following remarks in preparing your resubmission.

1) In the Introduction you summarize the effects of RBAC on NK cells. However, the relationship between NK cells and HIV+ is unclear. Please establish this link as you have done for endothelial function and CD4+ cells. The same applies to blood pressure (the prevalence of cardiovascular disease, as alluded to in the last sentence of the first paragraph, is too broad). It should be clear to readers why the authors have chosen to measure these parameters in the context of HIV+ from the Introduction.

We thank the Reviewer for pointing out the problems with our background justification and information. To correct our mistake, we have deleted almost all of the text that referred to the prior research on RBAC and NK cell cytotoxicity. While we only intended that point to be informational about RBAC’s general effect on NK cell cytotoxicity, it turned out to be distracting. Thus, we only now mention the finding on NK cell cytotoxicity in our first study on RBAC to point out its immunomodulatory effect and our extensive experience with testing RBAC. The rest of the text has been deleted, so that the focus for immune function remains on CD4+ cells for this paper. In addition, we have deleted some of the other findings about cardiometabolic effects in the HIV population that drive CVD risk and instead now focus primarily on the link between hypertension and risk of CVD to be more clear for why systolic blood pressure was chosen as one of the outcome variables for this analysis. Also, it now seems better to change “cardiometabolic” to “cardiovascular” throughout the paper, including the title, given that some of the other background information has now been deleted.

2) The Results section is rather difficult to read for several reasons. Firstly, rather complex statistical tests are used to measure therapeutic efficacy. The reader is forced to fully understand the respective methods section in order to comprehend the results. It would improve the legibility and understandability of the Results section if the authors specified the outcome parameters rather than using generic terms such as "overall multivariate interaction," "dependent variables," and "contrasts." It is therefore encouraged that the text is reformulated in a manner that the results can be fully understood without having to revert to the Methods section. Secondly, it would greatly benefit the Results section if the authors briefly explained what the multivariate analysis implies about the results and what the univariate analysis implies about the results. The most important issue here is the interpretation and contextualization difficulty that arises when the multivariate analysis yields significant differences, but the univariate analysis chiefly does not. We believe that especially the clinical readership needs a helping hand in results interpretation, and we kindly request you to provide that accordingly. The sentence in the Discussion "Overall, we found a significant interaction in the simultaneous changes in cardiometabolic, endothelial function, and immune markers comparing the RBAC group to placebo in the absence of significant individual univariate effects" is quite a quagmire in terms of what to actually make of the results.

We appreciate the Reviewer's comments that the results may not be easily understandable for the reader who does not have a background in multivariate statistics that includes repeated measure ANOVAs. The nomenclature we used is very standard, but we understand that it is still difficult for many people to grasp. Nonetheless, we have changed the text to make it as readable as possible, given the very technical nature of the analyses. We have also highlighted in the results what the significant findings mean. Also, the text in the Methods and Results sections is very consistent, which should make the interpretation of the findings easier. Regarding the "quagmire" sentence previously in the Discussion section, it has been modified to be clearer.

3) It would further benefit the data interpretation if the authors could indicate in the Results section the direction in which the variables should change to be considered beneficial for patients. This holds especially true for the blood flow measurements.

We have added an additional footnote to Table 2: "For systolic blood pressure and skin blood flow in response to nitric oxide, a decrease in value from one time point to another would typically be clinically beneficial, whereas for CD4+ cell count an increase in value from one time point to another would typically be clinically beneficial." We used the word "typically" to imply that changes in one direction or another are not necessarily always absolutely beneficial.

4) It seems to me that the overall effect of RBAC is that supplementation results in a steady systolic blood pressure during 6 months, a less expeditious decline in skin blood flow in response to NO during 6 months, and a short-term (3 months) increase in CD4+ cell count relative to the control group. These factors altogether cause the multivariate analysis in terms of treatment effect to be significant. If the interpretation is correct, then it should be better stated in the manuscript, preferably in the first paragraph of the Discussion.

The Reviewer's interpretation of the primary finding from the study is correct, and we have changed the text in the Discussion section to better state our results. The first and second paragraphs of the Discussion have been revised to address the Reviewer's suggestions.

5) *Table 2 should denote the units in the Measure column.*

The units for each dependent variable have been added to Table 2 and to the first sentence of the second paragraph on page 12.

6) *Statistically speaking, if a difference between 2 ordinal variables is not significant, one cannot speak of a change.*

Our dependent variables are not on the ordinal level, as they are continuous, not categorical. In addition, the dependent variables are evaluated simultaneously in the multivariate model, which leads to the significant interaction and difference between the two treatment groups. Thus, changes in the dependent variables are significantly occurring together as a group, not individually.

7) *Individual results do not have to be restated in the Discussion section, as is done in the second paragraph.*

The individual data were deleted from the text of the Discussion section.

8) *How was compliance measured during 6 months? If the compliance related to supplement/placebo intake was not assessed or monitored, the authors should state the reason and explain why they have confidence that each subject abided by the imposed dosing schedule.*

A compliance measure was not utilized for this analysis, but weekly check-in calls were conducted with participants to discuss any problems and to encourage adherence to the protocol. Also, the HIV patients who participated in our study all have experience as subjects in other studies, as our University and medical center are widely known, and they are one of the oldest sites in the US for ongoing HIV/AIDS research. Thus, our participants were knowledgeable about the importance of adherence to the study's procedure, and they were reminded of such with our weekly phone calls.

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

Best regards,



John E. Lewis, Ph.D.
Associate Professor

2nd editorial decision
27-nov-2019

Ref.: Ms. No. JCTRes-D-19-00007R1

Cardiovascular, endothelial function, and immune markers in response to treatment with a polysaccharide in HIV+ adults in a randomized, double-blind placebo-control trial

Journal of Clinical and Translational Research

Dear authors,

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

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

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