

Relationships between inflammatory and metabolic markers, exercise and body composition in young individuals

Sarah L. Dunn, Desiree L. Vera, Kathleen F. Weaver, and Jerome V. Garcia

Corresponding author

Sarah L. Dunn

California State University, San Bernardino, 5500 University Parkway, San Bernardino, CA
92407

Handling editor:

Michal Heger

Department of Pharmaceutics, Utrecht University, the Netherlands

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

Review timeline:

Received: 2 November, 2017
Editorial decision: 8 April, 2018
Revision received: 10 October, 2020
Editorial decision: 25 December, 2020
Revision received: 11 January, 2021
Editorial decision: 5 March, 2021
Revision received: 31 March, 2021
Editorial decision: 4 April, 2021
Published online: 14 May, 2021

1st Editorial decision
8-Apr-2018

Dear authors,

Your submission entitled "Young Trained Individuals Have Lower Plasma Concentrations of Inflammatory Markers Compared to Young Untrained Individuals" has been reviewed by the Editorial Board and by 5 experts in the field. As you can see from the reviewers' comments below and attached, there were major concerns with your study. Initially, we invited 2 reviewers who rendered a reject and major revision verdict. Because the editorial board wanted to give your manuscript another chance, 3 more reviewers were invited to critically appraise the manuscript. The reviewer report of the last reviewer came in today, altogether yielding a 2x major revision and 3x reject.

The editorial board has therefore decided to reject your manuscript in its current form, but with the opportunity for you to resubmit an improved version of the manuscript granted that all the reviewers' comments have been properly addressed and the methodological flaws completely eliminated.

Please address the above issue(s) prior to resubmitting your work, if you decide to do so, and contact the Editorial Office at info@jctres.com if you have any questions regarding your submission. We are more than willing to help you improve the study as long as you are

amenable to do the same. Please inform us if you intend to work together on a resubmission to JCTR.

Lastly, we apologize for the delay in handling your manuscript, but we wanted to give your study a fair chance. In order to do that, we had to invite additional reviewers and await their appraisal of your work.

Kindest regards,

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

Reviewer #1: The authors report that chronic physical exercise protects young, healthy adults from low grade systemic inflammation. The manuscript is well written; however, I find the interpretation of the results slightly misleading. For one, the groups are not comparable. To truly evaluate the role of exercise in your question then the comparative group should only differ in exercise behavior. The fact that your "untrained" group were overweight with high BF% and TRGs demonstrates that the groups were markedly different. Second, I think you have the data available to determine which of these factors mediates low grade inflammation. Is it BMI, BF%, TRG, exercise, and so on? From your analyses you cannot simply say that it is exercise. Please see comments below.

Line 68. I do not see the purpose of this first paragraph. Most of this information is re-stated in the subsequent intro paragraphs

Line 71. I believe you could begin the manuscript with this paragraph.

Line 75. I believe you could begin the manuscript with this paragraph.

Line 107. confusing sentence

Line 108. Mainly in disease states? Why would a normal healthy human need to have a decrease in inflammatory cytokines?

Line 136. What is meant by the word "response"? Are you applying a stimulus/intervention?

Line 193. Why did you culture the cells? Seems like you would've frozen immediately after collection.

Line 196-202. Please state the sensitivity range of each assay.

Line 209. Significance level? Power analysis?

Table 1. Just state n values for male and females. Percentage is confusing

Table 1. Looks like it was undetectable? How many samples were undetectable? Were these counted in the analysis?

Line 231. Correlations performed across all subjects?

Line 245. Is this in all subjects? I think a figure would be nice to see for these correlations.

Line 258. Your subjects were overweight, so this is not a true comparison to a healthy counterpart

Line 263. To truly evaluate whether or not exercise mediates the inflammatory state, you must compare subjects of similar characteristics except exercise. You may want to consider performing some regression analyses to determine which of these variables accounts for the inflammation. Is it BMI, BF, exercise behavior, FFM, TRG? This would tell an interesting story.

Line 281. This statement is conflicting and doesn't agree with your previous statements. Based on your results, this population is not an equal "healthy" group.

Line 299. The comparative group in the Borges et al study was of similar characteristics.

Line 316. Again, the Adams et al study was among a homogeneous group

Line 333. You do not know if exercise is the reason for this. It is apparent that the level of TRG or BF% or BMI play a role in inflammation. Your data even supports this with the positive relationships. This should be highlighted.

Reviewer #2: The authors, by a merely descriptive approach, compared plasma levels of some cytokines in two small group of individuals, relating them to the degree of physical activity.

The paper suffers from major weaknesses, including:

1) a very low number of participants, that makes a non-sense even performing a statistical analysis. The Authors did not consider at all the issue of the sample size, that should had been calculated "a priori".

2) these study participants are badly matched for a number of variables: gender, BMI, waist, triglycerides and so on. When you decide to test the effect of physical activity on something, even more in the presence of such small cohorts, you have to be sure that the two groups just differ for the degree of training. These individuals show significant differences in a series of phenotypic characteristics (BMI, waist, triglycerides and so on) that "per se" may induce different degrees of subclinical inflammation.

3) NF-kB is involved in many intracellular pathways; a quite rational approach to the link between such intracellular factor and cytokines would have been at least to measure IL-1beta, whose secretion can be primarily regulated by NF-kB via an alternative final pathway of the NLRP3-inflammasome platform.

4) How the Authors built the manuscript is pretty strange, indeed: a large introduction, with even a Figure reproduced by Author's permission (all better fitting to a review article, rather than an original article) and then a very elementary methodological approach and poor results.

5) Some unusual and unclear definition, like "blood plasma"; some typo and grammar errors.

Reviewer #3: This study examined differences in inflammatory cytokines and NF-Kb between trained and untrained males and females. No exercise intervention was utilized in this study. The authors attribute differences in CRP to differences in the training status between the groups, and even goes as far to suggest CRP to be a clinically relevant biomarker based on these results. This is a bold statement and is not adequately justified by the methodology. To answer the question as to whether CRP is lowered in response to training, one should train untrained individuals and examine the CRP response to training. There are also major issues with the sample used that I believe prevent real interpretations being drawn from the data. The untrained group is heavily unbalanced towards being mostly females which is likely a major contributor to differences in body composition between groups, as groups were not separated by gender, nor was an attempt made to have the same relative distribution of males to females in the trained/untrained groups. I recommend recruiting more participants to attempt to balance out the groups to see if some of these differences and correlations in particular are still observed. Ultimately, while the study poses an interesting question, I cannot accept it for publication.

Introduction

The introduction is too long. I believe paragraph 1 and section 1.1 can be removed. Section 1.2 is more appropriate considering that this study did not utilize obese individuals. I recommend rewriting the introduction to accurately reflect what was studied and justify the rationale for the study.

Line 135: Your hypotheses statement states that you expect to see a greater anti-inflammatory response. This is quite general. Perhaps hypotheses specific to each outcome measure is appropriate. Additionally, you offer no rationalization for examining the relationship between for example: p65 NF-Kb and glucose. Even though this study is descriptive in nature, the physiological meaningfulness of such relationships should be considered a priori.

Methods

Line 144: please either include all relevant descriptives (age, height, weight) here or refer the reader to Table 1.

Line 154: Explain in greater detail when data collection was performed relative to the end of the competitive season.

Line 159: If you have body composition, why do you need to include BMI?

Line 171: Please report the validity and reliability of the device used to measure your lipid panel.

Line 181: Define Fib

Line 195: Please provide CV's for all immunoassays.

Line 207: Why were non-parametric statistics utilized? No mention is made regarding a non-normal distribution of the data.

Line 208: Please report how interpretations for correlations were made.

Line 209: Please report the α -level used in this study.

Results

Table 1: Body mass should be reported within this table as well, particularly if you collected the data.

Table 1: I would recommend either collecting more data to allow for separation into males and females with similar relative distributions between groups or only analyzing data from males or females. Because of the known gender effects for anthropometric measures such as

body composition, fat mass, and fat free mass, and the fact that the untrained group only contained one male, it is possible that these results are the result of a sampling bias. This makes the results of these measures difficult to accurately interpret because groups were likely heterogeneous.

Table 1: Please report actual count distributions of males and females in each group, not just the percentages.

Table 1: Please change the comma after p65 subunit to a semicolon, as is consistent with the rest of your abbreviations.

Line 228: Please report effect size measurements as well as confidence intervals for each variable to provide further context for interpretation.

Line 234: Previous research has indicated that IL-6 is correlated with adiposity (See Voarova et al, 2001), and this is also acknowledged in the introduction. Therefore, there are likely gender effects on resting concentrations of IL-6 and other cytokines related to adiposity due to greater average levels of adiposity in females. Again, this brings the results of your study into question, as lack of differences in circulating cytokine concentrations may be because the untrained group had over twice the relative percentage of females that the trained group had.

Discussion

Line 294: in the discussion you talk about exercise induced increases in IL-6 and NF- κ B, but this study didn't use an exercise intervention and you had subjects refrain from exercise specifically to minimize the effect of exercise on inflammatory markers. This seems to make the effect of exercise on inflammatory function not relevant to your results. I recommend removal.

Reviewer #5: Introduction and discussion

The introduction was disjointed. Was figure 1 modified from reference 31? Some parts of the manuscript were difficult to follow as some sentences did not link to the next sentence (ended abruptly). Was it necessary to spell out all authors (more than 6 authors) in text citation (destructive to the flow of the manuscript); what citation style was used? Some information should not have been included in the manuscript as it was irrelevant (e.g., citation 9).

Methods

Participants:

* The criteria of subject recruitment were not clear. How were trained and untrained individuals screened? Was VO₂max or resting heart rate assessed? Would the results be different in trained individuals who exercised regularly for \leq 5 years?

* The number of subjects was small: 7 trained and 9 untrained. Why were males and females combined? What was the power calculation for this study?

Protocol:

* Blood collection procedures were not described clearly. Was blood collected using EDTA or heparin vacutainer tubes? On which site was venipuncture performed? What was the coefficient variation of ELISA analysis for each inflammation marker?

* What was (for the blood pressure average) the time interval between the first and the second blood pressure measurements? Was blood pressure assessed during the upright or supine position?

* It would have been advantages to control for menstrual cycle on inflammatory markers in female subjects.

Data analysis/Results:

- * The rationale for the use of non-parametric analysis was unclear. Was it due to the small number of subjects that may have led to an abnormal data distribution?
 - * In table 1, the number of males and females in each group was not stated. For example, in the trained group: 3 females and 4 males; whereas in the untrained group: 8 females and 1 male. Why were percentages used?
-

2nd Editorial decision
25-Dec-2020

Ref.: Ms. No. JCTRes-D-17-00021R1
Plasma Concentrations of Inflammatory Markers in Young Trained Compared to Young Untrained Individuals
Journal of Clinical and Translational Research

Dear Dr Dunn,

Two editorial board members who are experts in the field have appraised your paper. You will see that they are advising that you revise your manuscript, citing fundamental flaws in methodology. 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. Please note that the most important concern is the statistically significant difference between the female fraction in the study groups, which has been discounted in the text yet can have considerable implications on the parameters studied. Both reviewers propose a distinct solution to this issue, altogether indicating that the manuscript should not be published without addressing the matter. Accordingly, one editor suggested a major revision while the other recommended a rejection of the paper. I am willing to give the authors a shot at improving the paper fundamentally, reiterating that the reviewers' comments should not be taken lightly.

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 Jan 24, 2021.

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 #6: Please see comments that are attached.

Reviewer #7: The study aims to compare inflammatory markers of trained and untrained healthy young people. The introduction is well written and demonstrates the literature gap. The methods are adequate and well described, guaranteeing the possibility to answer the study question. The results and discussion are well developed. However, the sample is small, as well as, there is no similar proportion of men and women between the groups, with only three women in one group while there are 8 in the other group, which may contribute to a higher difference in fat mass between groups already that there is a greater concentration of women in the untrained group. Comparisons between groups are fragile to the detriment of the small number of participants and the lack of proportionality of sex between groups. The sampling power was not presented, which would be clarified. For the study to reduce its fragility, I suggest excluding comparisons between groups, maintaining the other analyzes, adjusting results, and the discussion.

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.

Authors' response

January 10, 2021

Dear Dr. Michal Heger,

On behalf of my coauthors and I, we are pleased to resubmit an original research article entitled, *Relationships between Inflammatory and Metabolic Markers, Exercise and Body Composition in Young Individuals* for reconsideration in the Journal of Clinical and Translational Research. We appreciate the opportunity to resubmit and are grateful for the reviewers recommendations and suggested revisions.

In this version of the manuscript, we did our best to accommodate all reviewer comments and feedback which is further explained below (with author follow up comments highlighted green) and within the manuscript are brief track changes. We look forward to your review again and appreciate this second opportunity.

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

Thank you for your consideration!

Sincerely,

Sarah L. Dunn, Ph.D.

Coauthors:

Desiree L. Vera
Kathleen Weaver,
Ph.D., and Jerome
Garcia, Ph.D.

REVIEWER COMMENTS

Reviewer #6:

Abstract

Lines 13-17: I suggest the authors to alter the objective of the study without the methodological information, as follow as: “The objective of this study was to analyze the differences between the inflammatory cytokines C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF α), and a transcription factor Nuclear Factor- κ B (NF κ B) in trained and untrained young people”.

We have updated our objectives to assess the relationships and identify the variables significantly associated and have included regression analysis. Due to methodological flaws with a low sample size and no power calculations we removed the group comparisons as suggested and included new language about the methodological flaws.

Introduction

As this study is original research provided by a cross-sectional design, I strongly recommend the authors to rewrite a new shorter one version of the Introduction. Long introductions are recommended for literature review studies.

Addressed, we have shortened the introduction.

Lines 53- 62: Less attention should be given to the contextualization dedicated to obesity in the Introduction of the manuscript, as this study addresses apparently healthy young people trained and untrained, so I would like to suggest to the authors alter this part of the manuscript in a shorter one.

Less attention is given to obesity.

Line 65: After [...] “other disease states”, could you please insert a citation?

Updated!

Lines 65-66: After “Little has been done with respect to populations that are predisposed and have not developed the disease”. Could you please insert a citation?

Updated!

Lines 74-75: After “Cytokines not only play a vital role in the immune response, but are also involved in several metabolic behaviors”. Could you please add a citation?

This was removed with the reduction in text within the introduction.

Lines 92-93: There is no need to explain again that CRP is a “chronic inflammatory marker”, as the authors already said in the line 80. Could you please delete this sentence “a chronic inflammatory marker”.

Updated!

Lines 124-125: Could you please after the sentence “Overall, multiple studies have concluded that life long physical exercise carries greater health benefits in its promotion of anti-inflammatory myokines and increasing insulin sensitivity” add a citation?

Updated.

Results

Figure 2. Please provide footnote for the abbreviations in figure 2. For example: CRP.

Updated.

Discussion

Lines 308-310: Instead of this sentence below, please add “by previous reports” or “previous studies”. [...] “Ross, Janssen, Dawson, Kungl, Kuk, Wong, Nguyen-Duy, Lee, Kilpatrick and Hudson (32) and Christ-Roberts, Pratipanawatr, Pratipanawatr, Berria Belfort, Kashyap and Mandarino (8)”.

Updated.

The authors have to insert this following limitation in the manuscript: (i) cross-sectional design of the study, that is not possible to establish a causal relationship between independent variable and dependent outcomes; (ii) absence of sample size calculation.

Updated.

Conclusion

Lines 366-367: Please, delete this sentence “(as shown in cross country and track athletes)”

Updated.

REVIEWER COMMENTS

Reviewer #7:

The study aims to compare inflammatory markers of trained and untrained healthy young people. The introduction is well written and demonstrates the literature gap. The methods are adequate and well described, guaranteeing the possibility to answer the study question. The results and discussion are well developed. However, the sample is small, as well as, there is no similar proportion of men and women between the groups, with only three women in one group while there are 8 in the other group, which may contribute to a higher difference in fat mass between groups already that there is a greater concentration of women in the untrained group. Comparisons between groups are fragile to the detriment of the small number of participants and the lack of proportionality of sex between groups. The sampling power was not presented, which would be clarified. For the study to reduce its fragility, I suggest excluding comparisons between groups, maintaining the other analyzes, adjusting results, and the discussion. Addressed!

3rd Editorial decision
05-Mar-2021

Ref.: Ms. No. JCTRes-D-17-00021R2
Relationships between Inflammatory and Metabolic Markers, Exercise and Body
Composition in Young Individuals
Journal of Clinical and Translational Research

Dear Dr Dunn,

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 Apr 04, 2021.

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 # 6: The authors did not respond to any of my comments, particularly recognizing the significant gender difference in their sample, which likely accounts for at least part of their results. Without even acknowledging the statistically significant gender difference in their study, at the very least, all I can do is reject this paper again.

Reviewer #7: The requested changes were made as suggested. I am in favor of accepting the manuscript for publication. Congratulations!

Authors' response

March 30, 2021

Dear Dr. Michal Heger,

On behalf of my coauthors and I, we are resubmitting a revised original research article entitled, *Relationships between Inflammatory and Metabolic Markers, Exercise and Body Composition in Young Individuals* for reconsideration in the Journal of Clinical and Translational Research. We appreciate the opportunity to resubmit and hope the reviewers are satisfied with the updates made based on their feedback.

We re-reviewed each point made by reviewer 6 (who mentioned we did not respond to any comments). In our first revision, we did not specifically mention the differences in gender. In this version, we added a correlation analysis on female participants (page 12) as well as discussion of the potential impact of gender on the study (page 13). We did address the other comments provided by reviewer 6 and included them below in green highlight for your reference.

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

Thank you for your consideration!

Sincerely,

Sarah L. Dunn, Ph.D.

Coauthors:
Desiree L. Vera

Kathleen Weaver,
Ph.D., and Jerome
Garcia, Ph.D.

CURRENT REVIEWER COMMENTS

Reviewer # 6: The authors did not respond to any of my comments, particularly recognizing the significant gender difference in their sample, which likely accounts for at least part of their results. Without even acknowledging the statistically significant gender difference in their study, at the very least, all I can do is reject this paper again.

Thank you for raising the importance of looking at gender as part of our analysis. In this revision, we have made the following changes: 1) mentioned gender differences with inflammation early on in the paper; 2) provided correlation data for female participants in the results section; and 3) expanded the discussion to include previous work on differences found in inflammation based on gender, which may be due to the hypothalamic-pituitary-adrenal axis and sex hormones or secretion of more inflammatory or less anti-inflammatory cytokines by males or females.

We included the previous comments made by Reviewer 6 (below) and how we addressed each one.

Reviewer #7: The requested changes were made as suggested. I am in favor of accepting the manuscript for publication. Congratulations!

Thank you. We are grateful to Reviewer 7 for their comments.

PREVIOUS REVIEWER COMMENTS

Reviewer #6:

Abstract

Lines 13-17: I suggest the authors to alter the objective of the study without the methodological information, as follow as: “The objective of this study was to analyze the differences between the inflammatory cytokines C-reactive protei (CRP), interluekin-6 (IL-6), and tumor necrosis factor- α (TNF α), and a transcription factor Nuclear Factor- κ B (NF κ B) in trained and untrained young people”.

We have updated our objectives to assess the relationships and identify the variables significantly associated and have included regression analysis. Due to methodological flaws with a low sample size and no power calculations we removed the group comparisons as suggested and included new language about the methodological flaws.

Introduction

As this study is original research provided by a cross-sectional design, I strongly recommend the authors to rewrite a new shorter one version of the Introduction. Long introductions are recommended for literature review studies.

Addressed, we have shortened the introduction.

Lines 53-62: Less attention should be given to the contextualization dedicated to obesity in the Introduction of the manuscript, as this study addresses apparently healthy young people trained and untrained, so I would like to suggest to the authors alter this part of the manuscript in a shorter one.

Less attention is given to obesity.

Line 65: After [...] “other disease states”, could you please insert a citation?

Updated.

Lines 65-66: After “Little has been done with respect to populations that are predisposed and have not developed the disease”. Could you please insert a citation?

Updated.

Lines 74-75: After “Cytokines not only play a vital role in the immune response, but are also involved in several metabolic behaviors”. Could you please add a citation?

This was removed with the reduction in text within the introduction.

Lines 92-93: There is no need to explain again that CRP is a “chronic inflammatory marker”, as the authors already said in the line 80. Could you please delete this sentence “a chronic inflammatory marker”.

Updated.

Lines 124-125: Could you please after the sentence “Overall, multiple studies have concluded that lifelong physical exercise carries greater health benefits in its promotion of anti-inflammatory myokines and increasing insulin sensitivity” add a citation?

Updated.

Results

Figure 2. Please provide footnote for the abbreviations in figure 2. For example: CRP.

Updated.

Discussion

Lines 308-310: Instead of this sentence below, please add “by previous reports” or “previous studies”. [...] “Ross, Janssen, 309 Dawson, Kungl, Kuk, Wong, Nguyen-Duy, Lee, Kilpatrick and Hudson (32) and Christ-Roberts, Pratipanawatr, Pratipanawatr, Berria, Belfort, Kashyap and Mandarino (8)”.

Updated.

The authors have to insert this following limitation in the manuscript: (i) cross-sectional design of the study, that is not possible to establish a causal relationship between independent variable and dependent outcomes; (ii) absence of sample size calculation.

Updated.

Conclusion

Lines 366-367: Please, delete this sentence “(as shown in cross country and track athletes)”

Updated.

4th Editorial decision

04-Apr-2021

Ref.: Ms. No. JCTRes-D-17-00021R3

Relationships between Inflammatory and Metabolic Markers, Exercise and Body Composition in Young Individuals

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

Comments from the editors and reviewers:
Authors' response