Understanding the responsiveness of nitric oxide to acute eccentric resistance exercise in elderly obese women

Tatiane Gomes Teixeira, Dahan da Cunha Nascimento, Ramires Alsamir Tibana, Nuno Manuel Frade de Sousa, Vinicius Carolino de Souza, Jeeser Alves de Almeida, Amilton Vieira, Octavio Luiz Franco, Guilherme Borges Pereira, Jonato Prestes

Corresponding author:
Jonato Prestes, Graduation Program on Physical Education, Catholic University of Brasilia - Q.S. 07, Lote 01, EPTC – Bloco G. Zip code: 71966-700 – Taguatinga – Federal District, Brasilia, Brazil.

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

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Date: 7-May-2016

Ref.: Ms. No. JCTRes-D-16-00013
Responsiveness of nitric oxide to acute eccentric resistance exercise in elderly obese women
Journal of Clinical and Translational Research

Dear Dr. Prestes,

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 Jun 06, 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

******Editor's comments******

As you will see below, the reviewer, who is an expert in this field, has thoroughly appraised your work and there is certainly enthusiasm regarding your study. I kindly ask you to implement the reviewer's suggested changes and provide a point-by-point response, particularly for the critiques that you feel should be rebutted.

Also, please let me know whether you need help with the language editing. Try to do this as well as possible and, if requested, I will help with proofreading of the resubmitted version.

******Reviewers' comments******

Reviewer #1: # JCTRes-D-16-00013
Title: Responsiveness of nitric oxide to acute eccentric resistance exercise in elderly obese women
Firstly, thanks for giving me the chance to review the interesting study. Manuscript reports result of study aimed to investigate the responsiveness of nitric oxide levels (existence of high (HR) and low (LR) responders) following eccentric resistance exercise (ERE) in elderly obese women. The conclusion that HR displayed higher nitric oxide (NO) responses to ERE is obvious (the possible novelty is the existence of this subgroup) and requires changes. The same is applied to the aim of the study. However, other questions pointed below should be clarified and/or modified. However, in sum, the manuscript is well conducted and brings new findings to the area of investigation.

Status: Major revision.

TITLE:
1. The title is clear and represents the manuscript.

ABSTRACT:

1. Please, consider changing purpose, results and conclusion items (considering the more detailed commentaries below).

2. Please, present other important results at "results", as the equal levels o NO pre-exercise, and that LR group showed higher body fat, cholesterol concentrations, LDL levels (estimated) and lower upper limb fat-free mass, since this factors might affect the acute parameters along and after ERE. These concerns should be also included in the conclusions of abstract

3. Here and along the whole text: remember the using of "." rather than "," when reference is given to number (p value, numberS in tables and results section of manuscript) in English.

INTRODUCTION:
1. Lines 66-68. As previously cited, it is known the existence of higher inter-subject variability (references #4 and #12) for creatine kinase (CK). So, this should be omitted of the aim, except if this is specifically for elderly obese women and/or this model of eccentric resistance exercise. If is the case, clarify it in the introduction.

2. Along whole text: remember to explain first the significance of "CK", "BMI", etc., at first appearance.

MATERIALS AND METHODS:
1. Lines 83-86: "We conducted this research for testing a hypothesis of identifying high versus low responders of NO kinetics in response to ERE. Moreover, we sought to investigate possible biochemical baseline differences between high and low responders" should be omitted, because these questions are clear in their right place, the last paragraph of the introduction.

2. Lines 89-92: Please, insert the specific references "National Institute of Diabetes and Digestive and Kidney Diseases" and "International Physical Activity Questionnaire" in the "references" section (J CTR has no restrictions on the number of references).

3. Lines 101-102: "Among 49 participants, 10 (20.4%) were classified as LR and the remaining as HR (n = 39)". Please, exclude this information, considering that it is correctly presented in the "results" section (lines 232-233).

4. Lines 107-109: The sentence "Moreover, the value of 24 μmol/L was an increase of approximately 50% in serum nitrite concentration following ERE, which was present in a reduced number of participants" is unclear. Please, rewrite and let it clearer.

5. Line 118: Change "reported" for "arrived".

6. Lines 125-133: What is the reference of this technique? Probably, you have the technique standardized by a Iranian authors (Zahedi Asl et al., 2008). Please, cite this work or another of the referred research group and modify a little this paragraph (not simply copy and paste).

7. In this "methods" section: stardardize as the informations about kits and/or devices are specified between parenthesis (City, country, state,

RESULTS:
1. The figure 1 seems misconfigured (in relation to line 249). Where is 94.4 μmol/L (the scale of the figure shows peak lower than 80 μmol/L) of HR and 66.5 μmol/L (the scale shows peak lower than 60 μmol/L) of LR in the figure?

2. The LR+HR levels should appear for nitrite and CK levels, or none them. Standardize it. Other question: Why the standard deviation of LR+HR levels are not presented in figure 1A?

3. Again, where really is 88.1 U/L (line 266) (the scale of the figure shows baseline levels higher than 90 U/L) in figure 1B?

4. Results: Despite the criticism, CK is really used to assess muscle damage and/or recovery day after exercise. Suggestion for next studies: evaluation of pain (example: visual analogue scale pain) and force production (time to recovery baseline levels of force) (example: using of the isoinertial machine for the evaluation of the rate of force production 24 and 48h after ERE).

DISCUSSION:
1. Discussion section is long and should be shortened. For example, mean age, BMI and specific details that do not explain the results of other studies should not be presented. Moreover, revise and delete some less relevant informations in "discussion" section.

2. You state that Zdrenghea et al (2003) considered 23.6 μmol/L as protective values. However, in your study, 24.1 μmol/L divides HR and LR classifications. Suggestion: in the final of the referred paragraph, state that different methods and techniques between the studies were utilized.

3. The authors state: "The results of the present study revealed that NO remained elevated up to 48 h following an ERE session, different from the immediate post exercise response found in previous studies (26). This suggests an important protective cardiovascular effects, while the chronic baseline elevation could reflect several metabolic disorders, such as diabetes, myocardium infarction, hyperlipaemia, rheumatoid arthritis and liver diseases".
What are the references of this last sentence? Does really the chronic baseline elevation of NO reflect several metabolic disorders? This last sentence should be omitted or attenuated. Of course, chronic elevation of NO may cause detrimental oxidative and endothelial damage through peroxynitrite formation (Lei et al., 2013) and/or increased activity of inducible nitric oxide synthase, which would inhibit endothelial nitric oxide synthase (Zahedi Asl et al., 2008). These two possible mechanisms responsible for a supposed harm effect which could result in metabolic disorders were not addressed in the discussion. On the other hand, it is known that NO exerts important vasodilator, antihypertensive, and antiatherosclerotic effects, should be considered (Maeda et al., 2004; Tsikas, 2007; Lei et al., 2013). In sum, there is a body of evidences showing that higher NO levels at baseline are desired to health.

4. Please, delete the lines 339-341, since these concerns are much better explained below.

5. Line 362: Reorganize the sentence (the form of reference #28 is incorrect).

6. Lines 392-409: Probably, the major limitation of the study was not cited. The ideal would be the checking or control of the intake of food rich in nitrite/nitrate (carrot, beet, kale, lettuce, spinach, radish, watermelon, strawberry, cheese, smoked, canned food, alcoholic beverages and corn) over 24 h ou 48h prior and in the following two days of blood collection (Dusse et al., 2005).

7. Please, exclude the obvious first sentence of conclusion paragraph (lines 410-411). Please, highlight in this paragraph that low responders showed higher body fat, cholesterol concentrations, LDL levels and lower upper limb fat-free mass, since this factors might affect the acute parameters along and after eccentric exercise.

WHOLE TEXT:
1. The writing of manuscript requires a careful revision by a native English speaker.

References:

DUSSE, L. M. et al. Does plasma nitrite determination by the Griess reaction reflect nitric oxide synthesis? Clin Chim Acta, v. 362, n. 1-2, p. 195-7, Dec 2005. ISSN 0009-8981 (Print)
0009-8981 (Linking).

LEI, J. et al. Nitric oxide, a protective molecule in the cardiovascular system. Nitric Oxide, v. 35, p. 175-85, Nov 30 2013. ISSN 1089-8611 (Electronic)
1089-8603 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24095696 >.

MAEDA, S. et al. Moderate regular exercise increases basal production of nitric oxide in elderly women. Hypertens Res, v. 27, n. 12, p. 947-53, Dec 2004. ISSN 0916-9636 (Print)
0916-9636 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15894835 >.

TSIKAS, D. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. J Chromatogr B Analyt Technol Biomed Life Sci, v. 851, n. 1-2, p. 51-70, May 15 2007. ISSN 1570-0232 (Print)
1570-0232 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16950667 >.

ZAHEDI ASL, S.; GHASEMI, A.; AZIZI, F. Serum nitric oxide metabolites in subjects with metabolic syndrome. Clin Biochem, v. 41, n. 16-17, p. 1342-7, Nov 2008. ISSN 1873-2933 (Electronic)
0009-9120 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18793628 >.

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' rebuttal:

Date: 31-May-2016

Ref.: Ms. No. JCTRes-D-16-00013
Responsiveness of nitric oxide to acute eccentric resistance exercise in elderly obese women
Journal of Clinical and Translational Research

Dear Michal Heger,

I am re-submitting the revised version of the paper entitled “Responsiveness of nitric oxide to acute eccentric resistance exercise in elderly obese women” numbered JCTRes-D-16-00013 to the Journal of Clinical and Translational Research. We are sending the manuscript including figure captions and 3 figures in black and white. Authors declare no conflict of interests. First, we would like to thank the reviewers’ comments. It is clear that all considerations were valuable and important. Moreover all suggestions made by the referees were accepted and are highlighted in the main text.

Best regards,
Jonato Prestes, PhD.
Catholic University of Brasilia
Brasília-DF, Brazil
E-mail: jonatop@gmail.com

Reviewer(s)'Comments to Author:
Reviewer: 1
Comments to the Author

TITLE:
1. The title is clear and represents the manuscript.

ABSTRACT:
1. Please, consider changing purpose, results and conclusion items (considering the more detailed commentaries below).
Response: All changes will be incorporated.

2. Please, present other important results at "results", as the equal levels o NO pre-exercise, and that LR group showed higher body fat, cholesterol concentrations, LDL levels (estimated) and lower upper limb fat-free mass, since this factors might affect the acute parameters along and after ERE. These concerns should be also included in the conclusions of abstract.
Response: The abstract was changed as requested.

3. Here and along the whole text: remember the using of "." rather than "," when reference is given to number (p value, numberS in tables and results section of manuscript) in English.
Response: Thanks for your comment, this was modified.

INTRODUCTION:

1. Lines 66-68. As previously cited, it is known the existence of higher inter-subject variability (references #4 and #12) for creatine kinase (CK). So, this should be omitted of the aim, except if this is specifically for elderly obese women and/or this model of eccentric resistance exercise. If is the case, clarify it in the introduction.
Response: This was not investigated in high versus low responders to nitrite. Thus, we will adjust the text and maintain. Thanks for your comment.

2. Along whole text: remember to explain first the significance of "CK", "BMI", etc., at first appearance.
Response: Thanks, these modifications were incorporated to the text.

MATERIALS AND METHODS:

Lines 83-86: "We conducted this research for testing a hypothesis of identifying high versus low responders of NO kinetics in response to ERE. Moreover, we sought to investigate possible biochemical baseline differences between high and low responders" should be omitted, because these questions are clear in their right place, the last paragraph of the introduction.
Response: We agree this was removed.

2. Lines 89-92: Please, insert the specific references "National Institute of Diabetes and Digestive and Kidney Diseases" and "International Physical Activity Questionnaire" in the "references" section (J CTR has no restrictions on the number of references).
Response: We agree this was removed.

3. Lines 101-102: "Among 49 participants, 10 (20.4%) were classified as LR and the remaining as HR (n = 39)". Please, exclude this information, considering that it is correctly presented in the "results" section (lines 232-233).
Response: We agree this was removed.

4. Lines 107-109: The sentence "Moreover, the value of 24 μmol/L was an increase of approximately 50% in serum nitrite concentration following ERE, which was present in a reduced number of participants" is unclear. Please, rewrite and let it clearer.
Response: The sentence was reformulated.

5. Line 118: Change "reported" for "arrived".
Response: Ok, changed.

6. Lines 125-133: What is the reference of this technique? Probably, you have the technique standardized by a Iranian authors (Zahedi Asl et al., 2008). Please, cite this work or another of the referred research group and modify a little this paragraph (not simply copy and paste).
Response: The reference was included and the sentence modified a little.

7. In this "methods" section: stardardize as the informations about kits and/or devices are specified between parenthesis (City, country, state).

Response: We agree. This modification was incorporated to the text.

RESULTS:
1. The figure 1 seems misconfigured (in relation to line 249). Where is 94.4 µmol/L (the scale of the figure shows peak lower than 80 µmol/L) of HR and 66.5 µmol/L (the scale shows peak lower than 60 µmol/L) of LR in the figure?
Response: The peak nitrite concentration does not represent the nitrite concentration at a specific time point. As we mentioned in the statistical analysis, the peak nitrite concentration represents the highest value achieved at 0, 3, 24, or 48 h for each subject. In this way, the highest value for each subject (regardless of time point) was used. We reformulated the sentence in the results in order to avoid confusion.

2. The LR+HR levels should appear for nitrite and CK levels, or none them. Standardize it. Other question: Why the standard deviation of LR+HR levels are not presented in figure 1A?
Response: We agree. We added the LR+HR for CK levels. We omitted the 95%IC for LR+HR because we just want to represent if the the regular response approximates better to HR or LR groups. No statistical analysis was made for HR+LR group.

3. Again, where really is 88.1 U/L (line 266) (the scale of the figure shows baseline levels higher than 90 U/L) in figure 1B?
Response: We agree. This was modified in the text. The figure was corrected.

4. Results: Despite the criticism, CK is really used to assess muscle damage and/or recovery day after exercise. Suggestion for next studies: evaluation of pain (example: visual analogue scale pain) and force production (time to recovery baseline levels of force) (example: using of the isoinertial machine for the evaluation of the rate of force production 24 and 48h after ERE).
Response: We totally agree with these suggestions and will include these parameters in the next studies.

DISCUSSION:
1. Discussion section is long and should be shortened. For example, mean age, BMI and specific details that do not explain the results of other studies should not be presented. Moreover, revise and delete some less relevant informations in "discussion" section.
Response: We agree. The discussion was shortened.

2. You state that Zdrenghea et al (2003) considered 23.6 µmol/L as protective values. However, in your study, 24.1 µmol/L divides HR and LR classifications. Suggestion: in the final of the referred paragraph, state that different methods and techniques between the studies were utilized.
Response: We agree. This was added.

3. The authors state: "The results of the present study revealed that NO remained elevated up to 48 h following an ERE session, different from the immediate post exercise response found in previous studies (26). This suggests an important protective cardiovascular effects, while the chronic baseline elevation could reflect several metabolic disorders, such as diabetes, myocardium infarction, hyperlipaemia, rheumatoid arthritis and liver diseases". What are the references of this last sentence? Does really the chronic baseline elevation of NO reflect several

metabolic disorders? This last sentence should be omitted or attenuated. Of course, chronic elevation of NO may cause detrimental oxidative and endothelial damage through peroxynitrite formation (Lei et al., 2013) and/or increased activity of inducible nitric oxide synthase, which would inhibit endothelial nitric oxide synthase (Zahedi Asl et al., 2008). These two possible mechanisms responsible for a supposed harm effect which could result in metabolic disorders were not addressed in the discussion. On the other hand, it is known that NO exerts important vasodilator, antihypertensive, and antiatherosclerotic effects, should be considered (Maeda et al., 2004; Tsikas, 2007; Lei et al., 2013). In sum, there is a body of evidences showing that higher NO levels at baseline are desired to health.
Response: We agree. The sentence was reformulated.

4. Please, delete the lines 339-341, since these concerns are much better explained below.
Response: We agree. The lines were deleted.

5. Line 362: Reorganize the sentence (the form of reference #28 is incorrect).
Response: The sentence was reorganized.

6. Lines 392-409: Probably, the major limitation of the study was not cited. The ideal would be the checking or control of the intake of food rich in nitrite/nitrate (carrot, beet, kale, lettuce, spinach, radish, watermelon, strawberry, cheese, smoked, canned food, alcoholic beverages and corn) over 24 h ou 48h prior and in the following two days of blood collection (Dusse et al., 2005).
Response: We agree. This was included in the study limitations.

7. Please, exclude the obvious first sentence of conclusion paragraph (lines 410-411). Please, highlight in this paragraph that low responders showed higher body fat, cholesterol concentrations, LDL levels and lower upper limb fat-free mass, since this factors might affect the acute parameters along and after eccentric exercise.
Response: We the paragraph was reformulated.

WHOLE TEXT:
The writing of manuscript requires a careful revision by a native English speaker.
Response: The manuscript was carefully reviewed by a nature English speaker. Please check for the supplementary letter attesting this information.

2nd editorial decision:

Date: 6-Jun-2016
Ref.: Ms. No. JCTRes-D-16-00013
Responsiveness of nitric oxide to acute eccentric resistance exercise in elderly obese women
Journal of Clinical and Translational Research

Dear Dr. Prestes,

Reviewers have submitted their critical appraisal of your revised paper. The reviewers'

comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by Jun 20, 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: Reviewer(s)' Comments to Author:

1. Please, present other important results at "results", as the equal levels o NO pre-exercise, and that LR group showed higher body fat, cholesterol concentrations, LDL levels (estimated) and lower upper limb fat-free mass, since this factors might affect the acute parameters along and after ERE. These concerns should be also included in the conclusions of abstract.
Response: The abstract was changed as requested.
The authors made the requests. However, it is necessary to change the expression "lower upper limb fat-free mass" to "lower and upper limbs fat-free mass" in whole text.

2. Lines 89-92: Please, insert the specific references "National Institute of Diabetes and Digestive and Kidney Diseases" and "International Physical Activity Questionnaire" in the "references" section (JCTR has no restrictions on the number of references). Response: We agree this was removed.
The authors didn't make the requests and didn't present compelling reasons. It is required to include the number and insert the specific references "National Institute of Diabetes and Digestive and Kidney Diseases" and "International Physical Activity Questionnaire" in the REFERENCES SECTION (JCTR has no restrictions on the number of references). Examples (only examples):
CRAIG, C. L. et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc, v. 35, n. 8, p. 1381-95, Aug 2003.
ECKEL, R. H.; BAROUCH, W. W.; ERSHOW, A. G. Report of the National Heart, Lung, and

Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on the pathophysiology of obesity-associated cardiovascular disease. Circulation, v. 105, n. 24, p. 2923-8, Jun 18 2002.

3. The figure 1 seems misconfigured (in relation to line 249). Where is 94.4 μmol/L (the scale of the figure shows peak lower than 80 μmol/L) of HR and 66.5 μmol/L (the scale shows peak lower than 60 μmol/L) of LR in the figure?
Response: The peak nitrite concentration does not represent the nitrite concentration at a specific time point. As we mentioned in the statistical analysis, the peak nitrite concentration represents the highest value achieved at 0, 3, 24, or 48 h for each subject. In this way, the highest value for each subject (regardless of time point) was used. We reformulated the sentence in the results in order to avoid confusion.
Read below.

4. Again, where really is 88.1 U/L (line 266) (the scale of the figure shows baseline levels higher than 90 U/L) in figure 1B?
Response: We agree. This was modified in the text. The figure was corrected.
Questions 3 and 4 were not resolved, since the figures continue misconfigured. As stated in first line of results sections, "the results are expressed as means (95% confidence interval)". So, these modifications are required in text and figures.

5. The LR+HR levels should appear for nitrite and CK levels, or none them. Standardize it. Other question: Why the standard deviation of LR+HR levels are not presented in figure 1A?
Response: We agree. We added the LR+HR for CK levels. We omitted the 95%IC for LR+HR because we just want to represent if the the regular response approximates better to HR or LR groups. No statistical analysis was made for HR+LR group.
Please, state in the manuscript that you omitted 95%IC for LR+HR data in the figure and no statistical analysis were performed in this group.

6. The authors state: "The results of the present study revealed that NO remained elevated up to 48 h following an ERE session, different from the immediate post exercise response found in previous studies (26). This suggests an important protective cardiovascular effects, while the chronic baseline
elevation could reflect several metabolic disorders, such as diabetes, myocardium infarction, hyperlipaemia, rheumatoid arthritis and liver diseases". What are the references of this last sentence? Does really the chronic baseline elevation of NO reflect several metabolic disorders? This last sentence should be omitted or attenuated. Of course, chronic elevation of NO may cause detrimental oxidative and endothelial damage through peroxynitrite formation (Lei et al., 2013) and/or increased activity of inducible nitric oxide synthase, which would inhibit endothelial nitric oxide synthase (Zahedi Asl et al., 2008). These two possible mechanisms responsible for a supposed harm effect which could result in metabolic disorders were not addressed in the discussion. On the other hand, it is known that NO exerts important vasodilator, antihypertensive, and antiatherosclerotic effects, should be considered (Maeda et al., 2004; Tsikas, 2007; Lei et al., 2013). In sum, there is a body of evidences showing that higher NO levels at baseline are desired to health.
Response: We agree. The sentence was reformulated.

Please, at line 305, state based in some studies (with references them) that elevated NOx levels up to 48h after ERE may be related to improved endothelial function and antiatherosclerotic effects.

Authors' rebuttal

Date: 8-Jun-2016
Ref.: Ms. No. JCTRes-D-16-00013R1
Responsiveness of nitric oxide to acute eccentric resistance exercise in elderly obese women
Journal of Clinical and Translational Research

Dear Michal Heger,

I am re-submitting the second revised version of the paper entitled "Responsiveness of nitric oxide to acute eccentric resistance exercise in elderly obese women" numbered JCTRes-D-16-00013 to the Journal of Clinical and Translational Research. We are sending the manuscript including figure captions and 3 figures in black and white. Authors declare no conflict of interests. First, we would like to thank the reviewers' comments. It is clear that all considerations were valuable and important. Moreover all suggestions made by the referees were accepted and are highlighted in the main text.

Best regards,
Jonato Prestes, PhD.
Catholic University of Brasilia
Brasília-DF, Brazil
E-mail: jonatop@gmail.com

Reviewer(s)'Comments to Author:
Reviewer: 1
Comments to the Author

The authors made the requests. However, it is necessary to change the expression "lower upper limb fat-free mass" to "lower and upper limbs fat-free mass" in whole text.
Response: Sorry, but the expression is correct, they lower (decreased) upper limb fat-free mass. The lower limb fat-free mass was not different between groups. However, we will change lower to decreased to avoid misconceptions.

The authors didn't make the requests and didn't present compelling reasons. It is required to include the number and insert the specific references "National Institute of Diabetes and Digestive and Kidney Diseases" and "International Physical Activity Questionnaire" in the REFERENCES SECTION (JCTR has no restrictions on the number of references). Examples (only examples):
CRAIG, C. L. et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc, v. 35, n. 8, p. 1381-95, Aug 2003.
ECKEL, R. H.; BAROUCH, W. W.; ERSHOW, A. G. Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working

Group on the pathophysiology of obesity-associated cardiovascular disease. Circulation, v. 105, n. 24, p. 2923-8, Jun 18 2002.
Response: Maybe this comment was not so clear. Please find the new references added.
National Institutes of Health (2001) Understanding adult obesity – National Institute of Diabetes and Digestive and Kidney Diseases. http://win.niddk.nih.gov/publications/PDFs/adultobesbw1201.pdf. Accessed 17 September 2014.
Craig C, Marshall A, Sjöström M, Bauman A, Booth M, Ainsworth B, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sport Exerc. 2003; 35: 1381 –95.

Again, where really is 88.1 U/L (line 266) (the scale of the figure shows baseline levels higher than 90 U/L) in figure 1B?
Response: We agree. This was modified in the text. The figure was corrected.
Questions 3 and 4 were not resolved, since the figures continue misconfigured. As stated in first line of results sections, "the results are expressed as means (95% confidence interval)". So, these modifications are required in text and figures.
Response: We agree that in the first version of the manuscript figure 1B was misconfigured. In the text, the value 88.1 U/L was written and in the figure scale the number was higher than 90 U/L. We changed the number 88.1 U/L in the text to 96.0 (95% CI: 56.3 – 135.7) during the first revision. The figure was correct and we did not change the figure.
Figure 1A represents nitrite concentration at the following time-points: pre exercise, 0, 3, 24 and 48 h after exercise. In the text, we described baseline serum nitrite concentration (line 258). As you can see, the text numbers are in accordance with the figure. Peak nitrite concentration was also described in the text (lines 262-263), while figure 1B does not present peak nitrite concentration values. Peak nitrite concentration was calculated using the highest value achieved by each subject between 0 and 48 h after exercise (the evaluation of peak nitrite concentration was described in the statistical analysis; line 227). For example, the mean peak nitrite concentration was higher than the nitrite concentration achieved after 3h of exercise for the HR group (figure 1A), because mean peak concentration was calculated with the highest value of each subject and not with the value of all subjects at the time-point 3 h after exercise. We added a sentence (line 261) to avoid confusion.
Finally, we described in the first line of the statistical analysis that the results in the text and in the figure are expressed as means (95% IC).

The LR+HR levels should appear for nitrite and CK levels, or none them. Standardize it. Other question: Why the standard deviation of LR+HR levels are not presented in figure 1A?
Response: We agree. We added the LR+HR for CK levels. We omitted the 95%IC for LR+HR because we just want to represent if the regular response approximates better to HR or LR groups. No statistical analysis was made for HR+LR group. Please, state in the manuscript that you omitted 95%IC for LR+HR data in the figure and no statistical analysis were performed in this group.
Response: We appreciate the appointment. We added a sentence in the text and in the figure legend with this information.

Please, at line 305, state based in some studies (with references them) that elevated NOx levels up to 48h after ERE may be related to improved endothelial function and antiatherosclerotic effects.
Response: Two new references and information were added lines 339-342.
Sun MW, Zhong MF, Gu J, Qian FL, Gu JZ, Chen H. Effects of different levels of exercise volume on endothelium-dependent vasodilation: roles of nitric oxide synthase and heme oxygenase. Hypertens Res. 2008 Apr;31(4):805-16.
Maeda S, Iemitsu M, Miyauchi T, Kuno S, Matsuda M, Tanaka H. Aortic stiffness and aerobic exercise: mechanistic insight from microarray analyses. Med Sci Sports Exerc. 2005 Oct;37(10):1710-6.

3rd editorial decision

Ref.: Ms. No. JCTRes-D-16-00013R2
Responsiveness of nitric oxide to acute eccentric resistance exercise in elderly obese women
Journal of Clinical and Translational Research

Dear Dr. Prestes,

Your manuscript has passed the final round of peer review and I am pleased to inform you that your work has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your manuscript as soon as possible.

Thank you for submitting your work to JCTR.

Kindest regards,

Rowan van Golen
Associate Editor
Journal of Clinical and Translational Research

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