**Editorial comments:**  
Changes to be made by the Author(s):  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.  
2. Author License Agreement (ALA): Please check Standard Access or Open Access. Please note that in the Questionnaire Responses Standard Access is selected. Please then scan and upload the completed ALA to your Editorial Manager account.  
3. Please revise lines 132-134, 136-141, 142-146, 150-154 to avoid textual overlap with previously published text.  
4. Figures: Please line up the panels better. Some panels are off-set in Figure. Please ensure that the panels are of the same dimensions if possible. Please use consistent font size among panels in the same figure, if possible.  
5. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text.  
6. Keywords: Please provide at least 6 keywords or phrases.  
7. Please rephrase the Introduction to include a clear statement of the overall goal of this method.  
8. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.  
9. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s; etc.  
10. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution’s human research ethics committee.  
11. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).  
12. 1.3.2: What are the inclusion/exclusion criteria?  
13. Please revise the protocol so that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.”  
14. 2.1.6, 2.1.8, 2.2.1, 2.2.5-2.3.2, 2.3.6, 3.1.1-3.1.3, 3.2.1-3.2.12, 3.3.2-3.3.7, 3.3.9.1-3.3.13.3, 4.0: Please write the text in the imperative tense. Any text that cannot be written in the imperative tense may be added as a “Note.”  
15. There is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.  
16. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.  
17. Please revise the Acknowledgements section to include any acknowledgments and all funding sources for this work.  
18. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s).  
19. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al.  
20. References: Please do not abbreviate journal titles.  
21. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.

We have addressed these changes in the manuscript.   
  
**Reviewers' comments:**  
  
  
  
**Reviewer #1:**  
Manuscript Summary:  
This is a relevant article describing 2 different exercise testing protocols where traditional physiological measurements can be combined with either blood samples (or as suggested in the end of the article, saliva, urine and sweat) to further understand the exercise induced biological mechanisms improving health.  
  
With minor revision this article can be published.  
  
Major Concerns:  
First of all, in the protocol section, I really miss a description of the safety arrangement around, especially, the maximal exercise test procedure as well as a description of indications for termination a maximal exercise test, including absolute, relative indications and subjective symptoms. Also a part of the expertise knowledge for those supervising the tests should be included. (For detailed information and reference see ACSM's Guidelines for exercise testing and prescription). Please add this to ensure that the protocol presented also account for the patients safety!

We apologize for this oversight. We have added a section regarding safety and included the reference to ACSM Guidelines.  
  
Also, the attempt to explore the dynamic response of immunological or growth factors or other biomarkers is not new. We have published a series of article where maximal exercise testing was combined with measurements of markers from blood sample taken at submaximal workload and at maximal workload, see references at the end. Please add information in your Introduction section and also in your Discussion section, compare your protocol with information from our studies.

Yes we understand that that what our attempt to measure biological samples before and after exercise is not new and we did not state that in our manuscript. We were aware of the work that was suggested to us as by the reviewer. However, we are not sure that comparing these protocols is appropriate in this manuscript because it does not fit the scope of this manuscript. Additionally, there are many studies that have looked at measuring responses after exercise and are too numerous to adequately cite them all. In the references provided, the main method for exercise and measured biological outputs were different, so we are not sure a direct comparison would be fruitful at this point. That being said, we did include a couple suggested references into the discussion.  
  
Title  
add  
Conduction maximal and submaximal endurance exercise testing….  
Comment; Using the term submaximal exercise test usually refer to a 6-10 min test with constant workload, where the steady-state heart rate combined with the workload are combined to estimate VO2max. This is different from the submaximal exercise test that you present in your article. Thus, in your article please ensure that you include endurance whenever you refer to your submaximal exercise test  
  
Minor Concerns:  
Abstract  
83 add and rearrange … increasing cardiopulmonary and metabolic response (heart rate, stroke volume, ventilation, oxygen consumption and carbon dioxide production).  
86 add…., but are not limited, heart rate, slow….  
  
Introduction  
97 delete The World Heath Organization defines  
change to Physical activity is defined as any bodily….. (Reference: Caspersen et al. Physical activity, exercise and physical fitness: definitions and distinctions for health-related research. Public Health Rep. 1985, 100 (2) 126-31).  
120 add Exercise testing is, for example, …. (Reference: ACSM's Guidelines for Exercise Testing and Prescription)  
129 change peripheral to metabolic  
131 add … long-term moderate to high….  
Comment; The current guidelines for physical activity is 150 min weekly aerobic exercise at moderate intensity or 75 min weekly at high or vigorous intensity  
133 add… not fully clear  
142 add…. exercise tests output  
143 add and delete; or work load ….. but are not limited to, mode, the test protocol (workload, inclination, duration of the exercise test) standardized methods to measure the output in response….  
In the protocol section the test procedure is well described  
311-312 add…(RPE) scale where indicating 6 is the exertion perceived at easy….and indicating 20 is the exertion perceived at the hardest….  
360 see 311-312 above  
380 Reviewers question; Why have you chosen 60% of the subject's maximal workload? Why not a % of VO2max or of HRmax or HRRmax, which is a more common way to guide intensity? See Norton K, Norton L, Sadgrove D (2010) Position statement on physical activity and exercise intensity terminology. J Sci Med Sport 13: 496-502. Please add some comment on this in your discussion section!

Please see the second paragraph of the discussion as we addressed these questions.

393-95 Reviewers question; Why have you chosen not to measure using ergospirometry during the whole 45 min test? Please add some comment on this in your discussion section!

Intermittent gas exchange was chosen to make the test more comfortable for the participants as breathing on a mouthpiece for long periods is uncomfortable as the mouth becomes dry, saliva can accumulate and holding the mouthpiece in the mouth can be tiring for the jaw. Since the primary outcome for this particular study was changes in peripheral blood leukocytes not changes in cardiopulmonary response to submaximal endurance exercise, intermittently monitoring gas exchange to ensure the exercise test was remaining a steady-state was sufficient. We have made this note in the methods and commented on this in the discussion.

424, 428, 431,, 437 add submaximal endurance exercise test  
428 add …duration (short/long respectively) and intensity (high/low respectively)  
429 add…a study with exercise testing, ….  
435 add and rearrange …cardiopulmonary and metabolic response (heart rate, stroke volume, ventilation, oxygen consumption and carbon dioxide production).  
449 add…. to respond, as well as by the metabolic function.  
469 Delete From our experience, the exercise protocols outlined here provide relatively reproducible results. However, we have found that  
Comment; You have not shown that in this article, no test-retest are presented, thus re-write.  
Suggestion re-write; We have outlined exercise protocols and have found that additional steps….  
474 add… proper calibration of all test equipment, especially the metabolic…  
475 change subject population to individual response  
476 add food intake and also a line of standardization of the room temperature, test conditions, time (if test are going to be repeated)  
  
Figure legends  
511, 519 add… Data for Maximal and Submaximal Endurance…  
514 change Subscribe 2, that is VO2 and VCO2  
516 Comment and change; If using peak oxygen uptake, then remove max and use VO2peak  
change last sentence to; Peak oxygen consumption (VO2peak) and test duration are shown in panel A. Submaximal oxygen uptake (VO2) and workload are shown I panel B.  
522 add…and time (minutes) for the submaximal endurance test (B)….  
  
Figure 1  
change Subscribe 2, that is VO2 and VCO2 (A and B in several places) and change VO2max to VO2peak  
add in heading B, Submaximal Endurance Test  
add in B, Work = 192 W (60% Wmax from maximal test)  
Comments;  
1. It is not relevant to have 37 yo male in the heading, please remove in A and B  
2. If you are using the unit ml at the y-axis, also give the corresponding value of VO2peak in ml (and also in ml/kg/min as you have) in A and B  
Table 2

add RER in both columns

We have addressed all of the minor concerns as sufficiently as we could.

References that might be useful to include in introduction and or in discussion section  
  
Using maximal exercise test with a submaximal continuous workload included  
Blood samples at rest, submax, max and recovery  
  
1. Gustafsson A. Ventorp F. Wisén A. Ohlsson L. Ljunggren L. Westrin Å.  
Effects of acute exercise on circulating suPAR in patients with major depressive disorder. Biomarkers Insights. 12:1-6 (2017)  
<http://insights.sagepub.com/effects-of-acute-exercise-on-circulating-soluble-form-of-the-urokinase-article-a6290>  
2. Wisen AGM, Ekberg,K, Wohlfart B, Ekman R, Westrin A. Plasma ANP and BNP during exercise in patients with major depressive disorder and in healthy controls. J Affect Disord 129, 371-375 (2011) <http://ac.els-cdn.com/S0165032710005707/1-s2.0-S0165032710005707-main.pdf?_tid=24105f86-d1ae-11e6-b3d8-00000aab0f6c&acdnat=1483445834_9fdeba48c8b6a3f351e9ac1e686a9395>  
3. Hallberg L, Janelidze S, Engstrom G, Wisén AGM, Westrin A, Brundin L. Exercise-induced release of cytokines in patients with major depressive disorder. J Affect Disord 126, 262-267 (2010) <http://ac.els-cdn.com/S0165032710002715/1-s2.0-S0165032710002715-main.pdf?_tid=0d8e2612-d1ae-11e6-90b0-00000aacb360&acdnat=1483445796_01da72121e7da3e9dd19fce2e5c17831>  
4. Gustafsson G, Lira CM, Johansson J, Wisén AGM, Wohlfart B, Ekman R, Westrin Å . The acute response of plasma brain-derived neurotrophic factor as a result of exercise in major depressive disorder. Pshychiatry Res. 244-8 Oct 30 (2009) <http://ac.els-cdn.com/S0165178108001984/1-s2.0-S0165178108001984-main.pdf?_tid=3db2cf22-d1af-11e6-9d7e-00000aacb360&acdnat=1483446306_ace4c70d87add49bffaf3c79085be28a>  
Using maximal exercise test, blood samples at rest, max  
5. Marie E. Bengtsson-Lindberg M. Wilke L. Vestberg S. Jacobsson H. Wisén A. Exercise-induced release of cytokines/myokines in a single exercise test before and after a training intervention in patients with mild cognitive impairment. International Journal of Physical Therapy & Rehabilitation.  
<http://www.graphyonline.com/archives/archivedownload.php?pid=IJPTR-138>  
  
  
**Reviewer #2:**  
Manuscript Summary:  
This manuscript presents the proposal of two different protocols of exercise (maximal and submaximal exercise testing) of potential interest to measure different physiological and biological responses to acute exercise in humans. The paper is overall well written and technically correct, and illustrate step by step the procedure of two cardiopulmonary exercise tests.  
  
Major Concerns:  
- The procedure of the maximal cardiopulmonary exercise test (and the spirometry that should be performed before it) is largely available within the published guidelines of different International societies.

Yes we agree with the statement. Upon invitation from the journal, our desire was to submit a manuscript to fit the scope of the journal for video demonstrations of protocols which facilitate scientific reproducibility and productivity using an exemplar study that was previously published by our group (*J Immunother Cancer, 2017 5:30).*

- The step-by-step procedure is exclusive only for a specific brand (device and software), and follows the manufacturers' procedures. [Editor's Note: This is okay, but it would be useful to add a note stating that the protocol would need to be adapted if other instruments are used]

We have put in a note in the table description that other instruments can be used.

- It is unclear to me why you use a specific timing of blood draws. Actually, I understand why, but I believe that this timing is not generalizable and appropriate for any biological response to be recorded.

We are not sure how to respond to this comment. Our timing of pre, post, 3 hours, and 24 hours as outlined in our *J Immunother Cancer* paper was chosen as a balance of what has been done previously, our estimations of capturing data that would reflect changes, and what was practical for healthy volunteer subjects availability for blood draws. We did in fact see many leukocyte changes/ biological responses at each time point. In our system, the peripheral immune system returned to normal homeostasis after 24 hours, so that time point was also helpful to gain context as well. We certainly acknowledge and encourage that depending on your system and methodology, one has to empirically determine the optimal timing of measuring responses. Thus we added a comment in protocol step 4.1.

- Considering your example, the athlete's VAT 1 (First Ventilatory Anaerobic Threshold) is above the proposed intensity of the prolonged, constant load, exercise (at 60% of maximal power). However, it may happen that untrained and sedentary people have a VAT 1 below this exercise intensity, thus developing a premature fatigue. By the way, for these reasons, the use of a fixed percentage respect to the maximal power could also elicit different true physiological strains.

The reviewer is correct, this is why we monitored the “steady-state” as described in 3.3.9 to ensure fatigue wasn’t occurring or the intensity increasing with time, if we observed signs of fatigue we dropped the workload by 5-10% to ensure that the entire 45min exercise bout could be completed.

Minor Concerns:  
- Gas calibration 1.2.2: while within the room air O2 should be 20.93%, CO2 calibration is not mentioned (room air CO2: 0.03%)  
- Please correct a few spelling mistakes (lines 98, 452, Table).  
- Exercise Test 3.1.2: V5 position is unclear  
- Exercise test 3.2.1 You could specify which should generally be a correct positioning of the seat  
- Exercise test 3.2.7: why here you don't initially use an unloaded pedalling?  
- Exercise test 3.2.9: no mention about Borg's Scale?

We have addressed all of the minor concerns as sufficiently as we could.

We would like to thank both reviewers for their comments that helped improve the quality of the manuscript. We appreciate their efforts greatly.