

## Peer Review File

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### Reviewer A

The study determined the association between increases in muscle damage, as assessed with serum CPK and strength reductions following soccer games in elite soccer players. The novelty and practical application of the study is unclear.

Reply: Thank you for having given us the opportunity to revise the manuscript. We tried to address all your concerns. We are ready to further improve the quality of this article. In our opinion, the novelty of this investigation is that it is the first time that such a study has been performed in a cohort of professional football players. It would allow to develop effective and easily applicable methods to diagnose muscle microdamage. In practice, the present investigation details the association between increased levels of CPK and the functional condition of athletes during the competition period.

Line 32: “Adductor muscle group” – please be more specific on which adductor muscle group was assessed (hip? Shoulder?)

Reply: Thank you for this comment. We changed the text.

Changes in the text: Page 2 line 30: **adductor muscles of the hip**

In the title, it is implied that muscle fatigue was assessed, but you did not assess muscle fatigue. I suggest changing this to “muscle damage” .

Reply: We agree with the reviewer. We changed the title.

Changes in the text: Page 1 line1: **Markers of muscle damage and strength performance in professional football (soccer) players during the competitive period**

The purpose and importance of the study is not clear in the introduction. Why would it be important to assess serum CPK levels in soccer players? It is well-known that this reflects muscle damage and when muscle damage is increased, acute decreases in muscle strength occur. What novel information is proposed to be gathered from this study?

Reply: Thank you for pointing this out. We have clarified this in the introduction.

Changes in the text: Page 4 lines 91-93: **The aim of the study was to develop a relatively simple method to monitor the functional state of football players and potentially find a method to evaluate the adaptation to exercise and recovery in professional football.**

What was the test-retest reliability of the CPK measurement and the hip adductor strength measurement?

Reply: As specified in the text, measurements of CPK levels and hip adductor strength were carried out 3 times during the experimental period. The measurements did not significantly

differ from each other (Wilcoxon signed-rank test). Therefore, the measurements are fully reproducible, and the first cycle of measurements was chosen for further analysis.

Changes in the text: Page 7 line 166 **Wilcoxon signed-rank test**

Statistics: “Kruskal-Wallis one-way analysis of variance was used to compare independent samples.” Is this the appropriate statistical test to use (i.e. to compare independent samples)? Since you are doing repeated measures on the same individuals you should probably be using a statistical test for dependent samples.

Reply: Thank you for your question. In this research, we did not compare the same athletes at different times, but took only the first cycle of measurements to identify intergroup differences. We agree. According to your recommendations, it was calculated using the Wilcoxon test, which also showed no differences.

Changes in the text: Page 6, line 155 **Wilcoxon signed-rank test**

Lines 149-153: Activity levels during the matches are described here. How were these determined? This is not described in the methods.

Reply: Thank you for pointing this out. We specified how it was determined in the methods.

Changes in the text: Page 6, lines 150-151 **All the measurements of physical activity during matches were carried out using InStat® kinematic system (Moscow, Russia).**

Line 163-164: “Interestingly, the serum CPK level at 72 hours was associated with height.” Could this be just a spurious correlation because you have conducted many correlations (i.e. type I error)? You seem to have correlated all variables without any purpose or hypothesis behind these correlations.

Reply: Thank you for your question. We agree; we suggest that the correlation of serum CPK level after 72 hours and height, most likely can be attributed to random findings.

The importance and practical application of the study are unclear. As mentioned above, there are many studies showing that CPK levels increase with muscle damage and this is associated with decreased strength. What is unique about your study? You indicate the study can provide valuable results to coaches and trainers, but it is unclear how your results would influence training programs.

Reply: Thank you so much for this comment. The aim of the study was to develop a relatively simple method to monitor the functional state of football players. In this respect, CK and dynamometry are best suited for this purpose, since serum levels of CK is often used in clinical practice in football clubs. The main unique feature that this study was performed on elite professional soccer players during the competitive season.

## **Reviewer B**

### General Comments

The authors performed an interesting work with the purpose to study the association between CPK and isometric strength of the adductor muscle group in professional soccer. Post-match fatigue of the adductors muscle group and their temporal recovery are not so well described in

the literature as for the extensors and flexors muscle. Nevertheless, I believe some improvements can be made to the actual state of the manuscript.

#### Specific Comments

#### Introduction

1- Line 52. Better to spell "...release of CPK and other..." and put Myoglobin inside parenthesis "(for example myoglobin and lactic dehydrogenase..."

Reply 1: We agree with the reviewer. We changed this line.

Changes in the text: Page 3, lines 50-52: **with release of creatine phosphokinase (CPK) and other markers of such damage (for example myoglobin and lactic dehydrogenase (LDH)) in the bloodstream.**

2- Line 61-63...not clear for me. Are the authors suggesting that players with high intracellular CK may have also higher natural baseline plasma CK concentrations in the bloodstream?

Reply 2: Thank you for this comment. Yes, this is what we suggested. We have clarified this.

Changes in the text: Page 3 lines 60-61: **may reflect the level of enzyme tissue activity. In such individuals, the serum CPK may be higher**

3- Line 67. I believe is better to mention the link between CK levels and very high-intensity running during the matches...no need to specify values and the 100 m distance that was used as an indication of proportion in those studies (each additional 100 m will corresponds to an increase of 30 % of CK levels if I'm correct)

Reply 3: We agree with the reviewer. We changed this line.

Changes in the text: Page 3, line 65: **there is a link between the level of CPK and very high-intensity running during the match.**

4- The authors need to develop in the introduction the rational for investigating the adductors muscle group (e.g. epidemiological evidences, less investigation on the fatigue experience by this particular muscles)

Reply 4: Thank you for this comment. We have added more relevant information on the adductor muscles into the introduction.

Changes in the text: Page 3, lines 75-79: **Pain associated with adductors muscles is the most common clinical finding on palpation and isometric muscle contraction in professional athletes, and can be a serious problem affecting the functional state and physical performance. It is assumed that it is precisely the weakness of the adductor muscles that is associated with this clinical manifestation.**

Page 4, lines 80-83: **Every second football player suffers from pain in the adductor muscles during one football season, and every third football player begins the competition season with discomfort in the area of proximal adherence of adductor muscles, while a third of them have a problem from the previous competitive period.**

## Methods

5- Line 108. I believe that a sentence shouldn't start with a numeric expression?

Reply 5: Thank you for this comment. We changed this line.

Changes in the text: Page 5, line 119: **For the analysis we used 32  $\mu$ L of capillary blood.**

6- Clinical and Laboratory monitoring: Please provide manufacture reliability data for CK analysis and for strength testing measures (we may mention values from a reliability paper)

Reply 6: We agree with the Reviewer. We added needed citations supporting the validity and reliability of testing devices.

Changes in the text: Page 6, line 121: **(15, 26)**

*Horder M, Jorgensen PJ, Hafkenschied JC, Carstensen CA, Bachmann C, Bauer K, Neuwald C, Rosalki SB, Foo AY, Vogt W. Creatine Kinase Determination: A European Evaluation of the Creatine Kinase Determination in Serum, Plasma and Whole Blood With the Reflotron System. Eur J Clin Biochem. 1991;29(10):691-6.*

Page 6, line 138: **(27)**

*(Moreno-Perez V, Travassos B, Calado A, Gonzalo-Skok O, Del Coso J, Mendez-Villanueva A. Adductor Squeeze Test and Groin Injuries in elite Football Players: A prospective study. Physical Therapy in Sport. 2019. doi: 10.1016/j.ptsp.2019.03.001)*

Page 6, line 146: **(28,29)**

*(Jensky-Squires NE, Dieli-Conwright CM, Rossuello A, Erceg DN, McCauley S, Schroeder ET. Validity and reliability of body composition analysers in children and adults. Br J Nutr. 2008;100(4):859-65. doi: 10.1017/S0007114508925460)*

*(Nickerson BS, McLester CN, McLester JR, Kliszczewicz BM. Agreement Between 2 segmental Bioimpedance Devices, BOD POD, and DXA in Obese Adults. J Clin Densitom. 2020; 23(1):138-148. doi: 10.1016/j.jocd.2019.04.005)*

7- Strength testing: Please provide more information on strength testing methodology (e.g trunk and arms position) and if players performed some type of warm up before strength testing (please describe)

Reply 7: Thank you for this comment, we have provided more information on the methodology of strength testing. This information is presented in the materials and methods section, strength testing and figure1.

Changes in the text: Page 6, line 134-135: **hands along the body**

Page 6, lines 142-143: **Before the test, each player performed passive resistance contraction and passive manual stretching of the adductors as a warm up.**

8- If match external-load data was collected system of collection and data processing and collection needs to be described.

Reply: Thank you for this comment. We clarified that the physical activity during the match was collected using instat kinematic system

Changes in the text: Page 6 lines: All the measurements of physical activity during matches were carried out using InStat® kinematic system (Moscow, Russia) (30).

(Modric T, Versic S, Sekulic D, Liposek S. Analysis of the Association Between Running Performance and Game Performance Indicators in Professional Soccer Players. *Int J Environ Res Public Health*. 2019;16(20):4032. DOI: 10.3390/ijerph16204032)

## Results

9- Line 169-171 This needs to be mentioned in the methods and not in the results section

Reply 9: We agree with the reviewer. We moved these lines to the Statistical analysis section in the Methods.

10- Line 177-178 I appreciate the contextualization of the association values to the practical context. Sometimes the reader doesn't fully understand how the relation between A and B works. Please do the same for line 175...if not here in the discussion. Probably both need to be done in the discussion and not in the results section??

Reply 10: Thank you for your comment. We agree, and have discussed this issue.

11- Please include the strength values (mean and SD) at baseline and pre-match for the isometric testing. I believe is important to inform on these values as this help describing the training status of the players and help in further studies (comparation purposes, systematic reviews and meta-analyses)

Reply 11: Thank you for this comment. This study did not measure strength at baseline, as athletes were at the beginning of the preparatory period (preseason training camp) before the start of the season after the holidays. The authors considered that there are risks of injury during performing of the maximum contraction of the muscles for measurement. In any case, it is obvious that the strength values at baseline would be less than the values measured in the competitive season. We agree that the absence of these measurements is the weakness of this study.

However, we certainly agree that strength values (mean and SD) matter in determining the athlete's training status and are possible for use in future systematic reviews and meta-analyses. These values are given in the text. Thanks again for your comment.

Changes in the text: Page 7, line 159-160 The mean and SD pre-match strength values were: 0°: 53.2 ± 7.4 kg; 45°: 38.9 ± 7.5 kg; 90°: 45.7 ± 7.3 kg.

12- In the figures, the Y axis should have as Newtons the units of measure no??

Reply 12: Thank you for your question. In this research, the Smart Groin Trainer dynamometer (NeuroExcellence, Braga, Portugal) was used for the measurement. All the measurements were given in kilograms. We added the units (kg) in the text and figures legend of manuscript.

Changes in the text: Page 19, line 129, 159-160 (kg)

## Discussion

13- Line 190. Given that the authors describe the post-match temporal recovery of adductor strength more than seasonal alterations I may believe that authors findings may impact more training organization within the microcycle than during the season?

Reply 13: Thank you for this comment. In this study, the microcycle is implied as part of the competition period of the season. It means that the microcycle is the period between two matches. Depending on the competition calendar, the microcycle can be 5-6 days, or 3-4 days (in this case, POST 72 could coincide with PRE 1 or precede it).

14- Line 197-201. Better to include at the end of discussion as a limitation paragraph were studies limitations are acknowledged

Reply 14: Thank you so much for this comment. We move this to the end of the discussion.

Changes in the text: Page 11 lines 275-282: We did not study the pattern of motor activity and did not test physical qualities in the days of changes in physical performance and muscle strength, and this could be incorporated in future experimentations.

We also acknowledge that we studied only the relationship between the serum level of CPK and muscle strength, but we could not ascertain how the level of CPK and a decrease in the strength of the adductors are related to the dynamics of various other parameters of physical performance, and how the level of daily physical activity can affect the indicators studied. This could be interpreted as a weakness of our work.

15- Line 202 I believe that the authors should also make it clear that CK responses reflect systemic damage to the muscle (e.g. damage to knee flexors, extensors, gastrocnemius and other muscles subject to trauma (as such during contact for ball protection) and..not reflecting only micro-trauma of adductors muscles). As is the case for biopsies, and pointed by the authors that reflects the muscle investigated

Reply: We agree with the reviewer. We specified this in the text.

Changes in the text: Page 9 lines 218-219: Serum CPK levels may be also high due to systemic damage to the various muscles or a consequence of direct muscle injury.

16- Line 218 and line 221. Don't start two times a sentence with the same word. Please edit.

Reply 16: Thank you for this comment. We changed the line.

Changes in the text: Page 10 line 235: There is a number of biochemical serum

17- Line 261 -163 Not clear for me the authors statement. "The fitness coaches did not study the pattern of motor activity...". Were not the authors that performed the investigation.? As so, should be mentioned "We did not study the pattern of motor activity..."

Reply 17: We agree with the reviewer. We changed the text.

Changes in the text: Page 11 lines 280-287: We did not study the pattern of motor activity and did not test physical qualities in the days of changes in physical performance and muscle strength, and this could be incorporated in future experimentations.

We also acknowledge that we studied only the relationship between the serum level of CPK and muscle strength, but we could not ascertain how the level of

CPK and a decrease in the strength of the adductors are related to the dynamics of various other parameters of physical performance, and how the level of daily physical activity can affect the indicators studied. This could be interpreted as a weakness of our work.

18- I would like to see the authors providing some discussion/rational around the use of the ratio CK/ strength.

Reply 18: Thank you for this comment. We provided more discussion around the use of the ratio CK/strength.

Changes in the text: Page 10 lines 260-266: The present study showed a U-shaped CPK concentration/adductors isometric strength ratio trend. A significant decrease was observed for the POST1 measurements 24 hours after the match, 72 hours after the match, the ratio has returned to basal values. Other studies have evidenced a positive association between CPK peak activity and maximal isometric strength of lower extremity extensor muscles (46). This trend can be used to assess the normal recovery of the CPK level and adductors strength relative to each other in the competitive period, as well as for the competent construction of the training process in the microcycle.