We thank Professor Cui for the letter, as well as the reviewers for the comments concerning our manuscript entitled “Testosterone Improves Erectile Function through the Inhibition of Reactive Oxygen Species Generation in Castrated Rats”. The comments were all valuable and very helpful for revising and improving our paper, and they were of important guiding significance to our study. The revised portions of the paper are marked in red. We truly appreciate the Academic Editor’s (Ranji Cui) and Reviewers’ thorough work and hope that the revised manuscript will meet with your approval. Once again, thank you very much for your comments and suggestions.

**Editor**

# Figure Readability
We recommend removing the patterns/gradients in your bar graphs in figures and using colors to distinguish between graphs bars.
 Response: This term has been corrected.
 # Figure Notations
Please make figures more readable. Notations that overlap elements of the image using the same color as the image (such as error bars and asterisks) should be changed to something more visible.
 Response: This term has been corrected.
 # Conflict of Interests
Please remove all competing interests information from the source file manuscript and make sure it is included in your Competing Interest Statement instead here <https://peerj.com/manuscripts/8306/declarations/#question\_17>.
 Response: We have removed all information on competing interests from the source file manuscript (manuscript: page19, line 333-334).
 # References
In the reference section, please provide the full author name lists for any references with et al. If you have used EndNote, you can change the references using the steps provided on our author instructions here <https://peerj.com/about/author-instructions/#reference-section>.

Response: We have updated the references using EndNote (manuscript: page20, line 340-451; clean revision: line 286-408).

# Funding Statement
Please remove all financial information from the Acknowledgments and add the information into the Funding Statement instead: <https://peerj.com/manuscripts/8306/declarations/#question\_18>.

Response: This term has been corrected (manuscript: page19, line 336-338).

**Reviewer 1**

1. Line 144, please clarify the testosterone concentration is blood testosterone concentration.

Response: We are sorry for this oversight. We have revised this text according to the reviewer’s suggestion (manuscript: page11, line 171; clean revision: line 150).

1. Fig 1a, please add the x-axis and unit, also the unit for Y-axis for ICP;

 Response: Thank you for this constructive comment. This term has been corrected (Figure 1).

1. In figure 2 D, the label for Y-axis is not correct, please update;

Response: This term has been corrected (Figure 2).

1. Fig 3 B, please correct the Y-axis. Also, ratio of p-eNOS/eNOS is enough, no need to show the total eNOS and p-eNOS;

 Response: This term has been corrected (Figure 3).

1. Fig 4, please also update the y-axis.

Response: This term has been corrected (Figure 4).

1. The manuscript need an English editing;

 Response: We had our revised manuscript edited by a professional English-speaking editor. The certification has been uploaded to Supplemental Files. If there are still language errors, then we can have the manuscript re-edited.

Reviewer 2

(1)It is still not clearly described how and when treat the rat with testosterone after castration? What is the dosage? Did the author monitor the plasma concentration during the testosterone replacement?

Response: These comments were valuable and helpful for improving our paper. The rats in the testosterone treatment groups received 100 mg kg-1 month-1 testosterone (Zhejiang Xianju Pharmaceutical Co., Ltd., Taizhou, Zhejiang, China, subcutaneous injection) for 1 month immediately after castration (Zhang MG, Shen ZJ, Zhang CM, et al. Vasoactive intestinal polypeptide, an erectile neurotransmitter, improves erectile function more significantly in castrated rats than in normal rats. *BJU Int* 2011; 108:440-446). It is well known that testosterone injection results in a superphysiological testosterone level in the body but that the level most likely decreases over time. The purpose of our study was to explore the effects and mechanism of testosterone in erectile dysfunction in castrated rats, and the change in testosterone concentration was not a focus of our study. Thus, we did not measure changes in its concentration, we only measured the plasma testosterone concentration after 1 month.

(2) What is the concentration of dihydrotestosterone after castration? Since dihydrotestosterone should be the most active form in serum than testosterone. Response: Thank you for this meaningful comment. Studies have shown that dihydrotestosterone (DHT) is the active androgen involved in the maintenance of nitric oxide-mediated penile erection in rats. (Lugg JA, Rajfer J, González-Cadavid NF. DHT is the active androgen involved in the maintenance of nitric oxide-mediated penile erection in the rats. Endocrinology 1995, 136(4):1495-501). We measured the plasma DHT concentration again, and the results are shown in Table 1.
(3) The raw data of eNOS/cGMP and COX-2/PTGIS/cAMP in all 40 rats should be uploaded as supplementary figures.

Response: We thank the reviewer for the rigorous evaluation of our manuscript. Unfortunately, because the rat penis is very small and there are such a large number of parameters (cAMP, cGMP, NOS, WB, and DHE), and we could not measure every parameter in each rat, we could only obtain as much information as possible for each parameter.
(4) The western blot results were not clearly shown. eg. Fig 3A , too many dots, even actin very near the membrane border. Fig2C. Fig 4A

Response: We completely agree with this valuable suggestion. We measured the protein expression again, but the image of p40phox and p67phox expression was the best image that we could obtain. Representative Western blots are shown in Figure 2C, 3A, and 4A.
(5) It was better shown the ICP/MAP and eNOS/cGMP and COX-2/PTGIS/cAMP change simultaneously.

Response: Thank you for this valuable comment. We have presented the changes in ICP/MAP, eNOS/cGMP, and COX-2/PTGIS/cAMP in Figures 1, 3, and 4, respectively. If these changes were presented together, then the image would have been too large to visualize; therefore, three figures were created. In addition, all of the changes are displayed in following table.

Erectile function, NOS activity, cGMP and cAMP concentrations, penile superoxide production, and immunoblotting data for the 4 experimental groups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Mean±SD(Co) | Mean±SD(So) | Mean±SD(Ca) | Mean±SD(Ct) |
| ICP/MAP (5V) | 0.83±0.04\*# | 0.85±0.08\*# | 0.42±0.04 | 0.73±0.06\* |
| NOS activity | 44.00±3.74\* | 44.00±4.90\* | 22.83±3.54 | 44.17±2.93\* |
| cGMP concentration | 6.02±0.62\* | 5.98±0.71\* | 2.53±0.35 | 5.93±0.66\* |
| cAMP concentration | 14.00±0.78\*# | 14.17±0.86\*# | 5.00±0.79 | 10.40±0.98\* |
| Penile superoxide production (% Co) | 0.99±0.22\* | 1.05±0.24\* | 3.75±0.29 | 1.11±0.29\* |
| Immunoblot:  |  |  |  |  |
| p40phox/β-actin | 0.046±0.006\* | 0.045±0.005\* | 0.094±0.006 | 0.057±0.006\* |
| p67phox/β-actin  | 0.065±0.006\* | 0.066±0.006\* | 0.237±0.015 | 0.059±0.006\* |
| p-eNOS/eNOS | 1.226±0.191\* | 1.212±0.156\* | 0.706±0.162 | 1.250±0.138\* |
| COX-2/β-actin | 0.653±0.045\*# | 0.680±0.040\*# | 0.157±0.015 | 0.273±0.025\* |
| PTGIS/β-actin | 0.583±0.051\* | 0.577±0.050\* | 0.397±0.015 | 0.627±0.098\* |

Co = control; So = sham-operated; Ca = castration; and Ct = castration-with-testosterone-replacement. \* *p* < 0.05 vs the castration group; # *p* < 0.05 vs the castration-with-testosterone-replacement group.

(6) Numerous language errors.

Response: We had our revised manuscript edited by a professional English-speaking editor. The certification has been uploaded to Supplemental Files. If there are still language errors, then we can have the manuscript re-edited.

Reviewer 3

1.Please discuss the potential clinical significance. What patients should be benefited from treatment with testosterone? For example: hypogonadism, diabetes-related ED, and cancer-related ED, etc. Can we use the treatment with testosterone for ED in patients with prostate cancer after castration therapy?

Response: These comments were valuable and helpful for improving our paper. We have re-written this text according to the reviewer’s suggestions (manuscript: page17, line 306-315; clean revision: 261-269).

Recent clinical trials suggested a significant improvement in ED and sexual function in hypogonadal men caused by metabolic syndrome, type 2 diabetes and postprostatectomy with testosterone treatment.

(1. Giltay EJ, Tishova YA, Mskhalaya GJ, Gooren LJ, Saad F, and Kalinchenko SY. 2010. Effects of testosterone supplementation on depressive symptoms and sexual dysfunction in hypogonadal men with the metabolic syndrome. *J Sex Med* 7:2572-2582.

2. Hackett G, Cole N, Bhartia M, Kennedy D, Raju J, and Wilkinson P. 2013. Testosterone replacement therapy with long-acting testosterone undecanoate improves sexual function and quality-of-life parameters vs. placebo in a population of men with type 2 diabetes. *J Sex Med* 10:1612-1627.

3. Khera M. 2009. Androgens and Erectile Function: A Case for Early Androgen Use in Postprostatectomy Hypogonadal Men. *Journal of Sexual Medicine* 6:234-238.

4. Zitzmann M, Mattern A, Hanisch J, Gooren L, Jones H, and Maggi M. 2013. IPASS: a study on the tolerability and effectiveness of injectable testosterone undecanoate for the treatment of male hypogonadism in a worldwide sample of 1,438 men. *J Sex Med* 10:579-588. )

The traditional view is a high serum testosterone level leads to an increased prostate cancer risk and invariably stimulates prostate cancer growth. However, several reports have uniformly demonstrated that total testosterone may be safely considered in patients with a history of successfully treated prostate cancer.

(1.Davilla H, Arison C, Hall M, et al. Analysis of the PSA response after testosterone supplementation in patients who previously received management for their localized prostate cancer. J Urol 2008; 179(Suppl):428, abstract 1247.

2.Khera M, Colen J, Grober E, et al. The safety and efficacy of testosterone replacement therapy following radical prostatectomy. J Urol 2007; 177(Suppl):384, abstract 1164.

3.Sarosdy MF. Testosterone replacement for hypogonadism after treatment of early prostate cancer with brachytherapy. Cancer 2007; 109:536–41.

4.Nabulsi O, Tal R, Gotto G, et al. Out-comes analysis of testosterone supplementation in hypogonadal men following radical prostatectomy. J Urol 2008; 179(Suppl):406; abstract 1181.)

Testosterone replacement is controversial in men with a history of prostate cancer. Since there is limited evidence suggesting that testosterone replacement may not pose an undue risk of prostate cancer recurrence or progression, testosterone replacement is contraindicated in patients with untreated prostate cancer (EAU guidelines). Hence, we do not recommend the use of testosterone for the treatment of erectile dysfunction in patients with prostate cancer after castration therapy.
2. Few recent discoveries and concepts may help in the discussion. For example, following literatures are not cited in this manuscript.
J Sex Med. 2011 Jul;8(7):1865-79.
J Sex Med. 2010 Mar;7(3):1116-25.
Mol Cell Endocrinol. 2009 May 6;303(1-2):67-73.

Response: We appreciate the reviewer’s familiarity with the literature in this field. We have added these references to manuscript according to this suggestion (manuscript: page3, line 30; clean revision: line 27).