

**Responses to the reviewers' concerns relating to our manuscript entitled:  
'Concepts of hypoxic NO signaling in remote ischemic preconditioning'**

**Name of journal: World Journal of Cardiology**

**ESPS Manuscript NO: 20184**

We thank all reviewers for their thorough revision of our manuscript and their very important concerns, which we address on a point-to-point basis below.

**Reviewer 1**

The manuscript is well written, very clear. I would eventually recommend the manuscript for publication after minor revision according to the suggestions described below.

1) Abstract more quantitative as possible, would favour the interest of the paper.

*Response: We thank the reviewer for this very important aspect. The abstract was revised accordingly and extended as requested. (page 2, lines 7-10 and 21-23 of the revised version of the manuscript)*

2) Figure is very good, and it should reflect as possible the main message. Eventually the last panel (or another figure describing the main targets of ROS on cell cardiac muscle would improve and go deeper to the message that the authors aimed to pursue.

*Response: We thank the reviewer for raising this aspect. We have extended the last panel of the figure to contain the targets of ROS. In order to explain this, the figure legend was extended accordingly. (page 23, lines 5-9 and 15-18 of the revised version of the manuscript)*

3) Previous papers described the effects of RNS and particularly peroxynitrite on myosin, and also in calcium homeostasis and cytoskeletal structures as putative early effects of ROS, before targeting mitochondria. If there is ONOO association with ischemia/reperfusion insults and inflammation, it was also described a link between calcium concentration changes and cytoskeleton disruption. In our opinion some additional references should be include that would turn more sound and solid the present paper (please see for example: Tiago et al, Cell Calcium 49 (2011) 174-183,; Tiago et al, Biochemistry 2006, 45, 3794-3804; Tiago et al, BBRC 342 (2006) 44-49), namely for the understanding of the biochemical effects of ROS and RNS.

*Response: The reviewer is right that this aspect was previously not included in the first version of the manuscript. We have added a new paragraph in the corresponding section to address the aspect of nitrosative stress (page 5, third paragraph, lines 6-12 and page 6 first paragraph lines 3-4 of the revised version of the manuscript) and updated the reference list.*

4) Ref 33 described the Kg of ATP per day. Several kg or it could be 700 kg!  
Kind regards

*Response: We thank the reviewer for his/her comment. The wording was revised accordingly. (page 7, paragraph 2, line 7 of the revised version of the manuscript)*

## **Reviewer 2**

The paper should revised in terms of English as well as the figure legend. For problems of English language, I have made revisions in the manuscript.

*Response: We thank the reviewer for the editing of the manuscript. We have accepted his/her changes in the manuscript. For further language editing, the manuscript was submitted to the language editing institution as required by the publisher.*

For the figure legend, please emphasize that the two major sources of nitrite (and nitrate) include the endogenous L-arginine-NO pathway, and the diet, with conversion of nitrate from diet into nitrite by oral commensal bacteria. A. The classical L-arginine-NOS-NO signaling pathway. NO is produced in mammalian cells by an oxygen-dependent oxidation of a guanidine nitrogen of L-arginine. This multistep reaction is catalyzed by the heme-containing protein NOS, which also requires two flavin molecules and tetrahydrobiopterin as cofactors. In most endothelial cells, eNOS is regulated by calcium-dependent binding of calmodulin and by tyrosine phosphorylation. B. Nitrite reduction to NO is favored by decreasing physiological oxygen tensions and low pH, via nonenzymatic pathways or enzymatic pathways catalyzed by metal-containing enzymes.

*Response: We thank the reviewer for raising this very important aspect. As requested we have revised the figure legend, which now refers to both pathways in detail as outlined by the reviewer. We also want to express our thanks for the kind consideration of our manuscript. ((page 23, lines 5-9 and 15-18 of the revised version of the manuscript)*

### **Reviewer 3**

The authors reviewed the effects of remote ischemic preconditioning (rIPC) applied by brief ischemic episodes to heart-distant organs tested in several clinical studies and discussed the possibly protective nitrite/NO signaling. It is suitable to the Journal and could be helpful in clinic study.

*Response: We thank the reviewer for his/her comment and the appreciation of our manuscript.*