Author's response to reviews

Title: Alterations in proton leak, oxidative status and uncoupling protein 3 content in skeletal muscle subsarcolemmal and intermyofibrillar mitochondria in old rats

Authors:

RAFFAELLA CRESCENZO (rcrescen@unina.it)
FRANCESCA BIANCO (francibianco@yahoo.it)
ARIANNA MAZZOLI (arimazzoli@hotmail.it)
ANTONIA GIACCO (antonia.giacco@hotmail.it)
GIOVANNA LIVERINI (liverini@unina.it)
SUSANNA IOSSA (susiossa@unina.it)

Version: 3 Date: 14 May 2014

Author's response to reviews: see over
Reviewer: Fernando Goglia

At the end of the introduction, we have added a sentence on the reason why we have measured UCP3 content (see page 4, line 6-8). The citation to the paper of Lombardi et al., 2010 has been added (ref. 11).
Reviewer: Carlo Cervellati

1) As for statistical analysis, at least in animal studies, the number of “subjects” is usually set at n=6/8 in the vast majority of the published studies. In addition, t-test can be used even if n is very small, i.e. <4 (Using the Student’s t-test with extremely small sample sizes. J.C.F. de Winter, *Practical Assessment, Research & Evaluation, Vol 18, No 10 p.1-12*). However, we have also checked and confirmed the level of significance obtained with t-test by applying non parametric Mann Whitney test. As for two-way ANOVA, we have used PASS Software for determining the minimum number of “subjects” required, on the basis of the difference between the means and the degree of standard deviation and we have obtained N ranging from 6 to 8 (Neter, J., Kutner, M., Nachtsheim, C., and Wasserman, W. 1996. *Applied Linear Statistical Models*. Richard D. Irwin, Inc. Chicago, Illinois; Winer, B.J. 1991. *Statistical Principles in Experimental Design*. Third Edition. McGraw-Hill. New York, NY.) (see page 8, line 19-20 and 23-25).

2) Row values for SOD and TBARS are now reported in the text, section results (page 10, line 9-12). The fold increase in UCP3 content is now reported in fig. 2C.

3) The link between UCP3 and oxidative damage has been clarified (page 13, line 9-14).

4) The need for a deeper investigation on the oxidative damage in SS and IMF mitochondria has been cited in the discussion (page 13, line 12-14).

Minor:

Xanthine has been corrected.

In the test it has been reported that SOD units were normalized for mg of proteins (page 7, line 25-26).

Details on UCP3 units have been added in methods (page 8, line 15-16).