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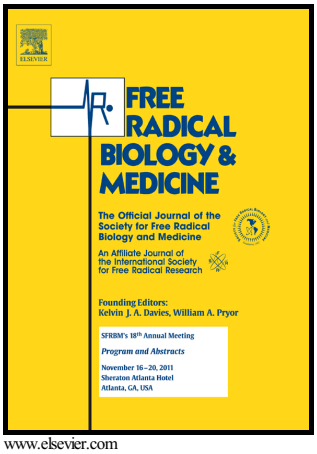

www.elsevier.com

PII: S0891-5849(18)31161-4
DOI: https://doi.org/10.1016/j.freeradbiomed.2018.06.041
Reference: FRB13837

To appear in: *Free Radical Biology and Medicine*

Received date: 22 May 2018
Accepted date: 29 June 2018

Cite this article as: Guglielmina Chimienti, Anna Picca, Giuseppe Sirago, Flavio Fracasso, Riccardo Calvani, Roberto Bernabei, Francesco Russo, Christy S. Carter, Christiaan Leeuwenburgh, Vito Pesce, Emanuele Marzetti and Angela Maria Serena Lezza, Increased TFAM binding to mtDNA damage hot spots is associated with mtDNA loss in aged rat heart, *Free Radical Biology and Medicine,* https://doi.org/10.1016/j.freeradbiomed.2018.06.041

Increased TFAM binding to mtDNA damage hot spots is associated with mtDNA loss in aged rat heart

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Summary

The well-known age-related mitochondrial dysfunction deeply affects heart because of the tissue's large dependence on mitochondrial ATP provision. Our study revealed in aged rat heart a significant 25% decrease in mtDNA relative content, a significant 29% increase in the 4.8 Kb mtDNA deletion relative content, and a significant inverse correlation between such contents as well as a significant 38% decrease in TFAM protein amount. The TFAM-binding activity to specific mtDNA regions increased at those encompassing the mtDNA replication origins, D-loop and Ori-L. The same mtDNA regions were screened for different kinds of oxidative damage, namely Single Strand Breaks (SSBs), Double Strand Breaks (DSBs), abasic sites (AP sites) and oxidized bases as 7,8-dihydro-8-oxoguanine (8oxoG). A marked increase in the relative content of mtDNA strand damage (SSBs, DSBs and AP sites) was found in the D-loop and Ori-L regions in the aged animals, unveiling for the first time *in vivo* an age-related, non-stochastic accumulation of oxidative lesions in these two regions that appear as hot spots of mtDNA damage. The use of Formamidopyrimidine glycosylase (Fpg) demonstrated also a significant age-related accumulation of oxidized purines particularly in the D-loop and Ori-L regions. The detected increased binding of

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