Lifelong exercise training modulates cardiac mitochondrial phosphoproteome in rats

Rita Ferreira^{1#} and Rui Vitorino^{1#}, Ana Isabel Padrão¹, Guadalupe Espadas², Francesco M. Mancuso², Daniel Moreira-Gonçalves^{3,4}, Gonçalo Castro-Sousa⁴, Tiago Henriques-Coelho³, Paula A. Oliveira⁵, António S. Barros¹, José Alberto Duarte⁴, Eduard Sabidó^{2,*}, Francisco Amado^{1,6,*}

[#] equally contributors

* corresponding authors

¹QOPNA, Department of Chemistry, University of Aveiro, Portugal
²Proteomics Unit, Centre for Genomic Regulation (CRG) and Universitat Pompeu Fabra (UPF), Barcelona, Spain
³Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine, University of Porto, Portugal
⁴CIAFEL, Faculty of Sports, University of Porto, Portugal
⁵Department of Veterinary Science, UTAD, Portugal
⁶School of Health Sciences, University of Aveiro, Portugal

Supplementary information

Figure S1: Gene ontology analysis of subcellular locations of the identified proteins using the ClueGo bioinformatic tool (45).

Figure S2: Gene ontology analysis of subcellular locations of the identified proteins using the WebGestalt bioinformatic tool (46).

Figure S3: Venn diagram of the identified mitochondrial proteins from five subjects in each group evidencing inter-individual variability.

Figure S4: Venn diagram of the identified mitochondrial phosphopeptides from five subjects in each group.

Figure S5: Comparative ClueGo (45) and CluePedia analysys (47) of unique phosphorylated proteins in each group according to biological processes. Green nodes refer to exercised group whereas red nodes refer to control group. Large nodes represent the biological processes whereas the small nodes denote gene products. Green hedges are associated with activation, blue edges to binding, yellow edges to expression, and pink edges to the *ptmod*.

Figure S6: Manhattan plot of –log10 (p-values) highlighting the significance quantitation levels of phosphopeptides between groups.

Figure S7: Normalized sorted frequency analysis of phosphorylated proteins modulated by exercise training.

Supplementary Table S1: A) List of identified proteins and peptides in the control and the exercised group, including biological and functional annotations of the identified proteins, and quantitative peptide peak area, and protein abundance estimates using the median of the three most abundant peptides per protein; B) List of identified proteins and peptides in the control and the exercised group; and C) Biological and functional enrichment analysis of the identified proteins.

Supplementary Table S2: A) List of identified phosphorylated peptides in the control and the exercised group, including quantitative peptide peak area, abundance comparison between control and exercise groups, and statistical assessment; B) List phosphorylated peptides newly identified in rat mitochondria; C) List phosphoproteins newly identified in rat mitochondria; D) Funtional domains of differentially phosphorylated proteins identified in controls and/or exercised animals; E) List of predicted kinases responsible for the phosphrylation of the identified phospho-peptides based on sequence motifs; F) List of predicted kinases responsible for the phosphorylation of the identified exclusively in one group, either exercised (E) or control (C) mice; and G) List of peptide-spectrum matches identified in the phosphorylation dataset.













