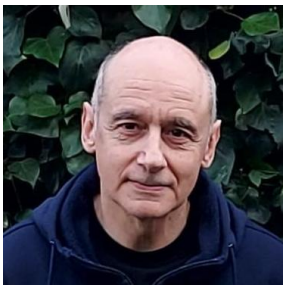
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Short Communication

Adaptions of oxidative phosphorylation to acute and chronic exercise



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Introduction

Skeletal muscle is a highly adaptable tissue that continuously remodels in response to physiological stimuli, particularly exercise-induced contractile activity. Because muscle contraction imposes substantial energetic demands, mitochondrial function and oxidative phosphorylation (OXPHOS) play central roles in maintaining ATP production, metabolic flexibility, and overall muscle performance. Physiological mitochondrial adaptions, including improved respiratory capacity, are key mechanisms underlying enhanced exercise performance (Garcia-Roves et al 2025), whereas mitochondrial dysfunction is associated with aging, obesity, and metabolic disease (Georgiev et al 2022).

High-resolution respirometry (HRR) provides sensitive and direct measurements of oxygen consumption. The use of permeabilized muscle fibers (pfi) offers advantages compared to isolated mitochondria, including preservation of mitochondrial architecture and maintenance of both subsarcolemmal and intermyofibrillar mitochondrial populations. Recent technological advances expand mitochondrial phenotyping beyond respiration, enabling simultaneous measurements of mitochondrial membrane potential ($\Delta\psi_{mt}$), ATP production, reactive oxygen species generation, and coenzyme Q (CoQ) redox state.

The Oroboros NextGen-O2k Q-Module enables real-time monitoring of both oxygen consumption and CoQ redox status. As CoQ acts as the central electron carrier linking Complexes I and II to Complex III, its redox state provides valuable information about electron transfer system activity and mitochondrial metabolic status. This approach has been successfully applied in isolated mitochondria (Komlódi et al. 2021).

The feasibility of combining HRR, CoQ redox measurements, and membrane potential assessment in skeletal muscle pfi was a challenge faced in the present study.

First, we optimized an integrated platform for the simultaneous determination of mitochondrial respiratory capacity, CoQ redox state, and membrane potential in pfi. Second, applying this platform we investigated mitochondrial adaptions induced by acute and chronic exercise. This approach provides new insights into the mechanisms underlying exercise-induced improvements in mitochondrial function.

Methods

Experiments were conducted on young adult male C57BL/6J mice maintained under standardized laboratory conditions. Extensor digitorum longus (EDL) muscles were excised, mechanically separated into fiber bundles, and chemically permeabilized with saponin. This procedure provided direct access to the mitochondrial network while preserving muscle structure and mitochondrial organization (Doerrier et al 2024).

Male mice were assigned to three experimental groups: sedentary control (n=18), acute exercise (n=6), and chronic exercise training (n=6). The chronic group underwent a 4-week running training protocol at constant speed for one hour, with an every other day gradual increment of speed (from 0.17 $m \cdot s^{-1}$ and flat -day 1- to reach a final speed of 0.33 $m \cdot s^{-1}$ and a slope of 10 degrees, last 2 days) (Gonzalez-Franquesa et al 2022). The acute exercise protocol consisted of treadmill running at a 5° incline for 10 min at 0.17 $m \cdot s^{-1}$, followed by 10 min at 0.2 $m \cdot s^{-1}$ and 10 min at 0.23 $m \cdot s^{-1}$. Thereafter, animals performed repeated 2-min intervals at progressively increasing speeds (0.27, 0.3, and 0.33 $m \cdot s^{-1}$, each repeated twice), interspersed with 2-min recovery periods at 0.2 $m \cdot s^{-1}$. EDL and *tibialis anterior* muscles where dissected 16 hours after last exercise bout.

Mitochondrial respiration was assessed in EDL permeabilized muscle fibers using the Oroboros NextGen-O2k with the substrate-uncoupler-inhibitor titration protocol SUIT-008 (https://www.bioblast.at/index.php/SUIT-008_O2_pfi_D014), allowing sequential evaluation of leak respiration, OXPHOS capacity, electron transfer system (ETS) capacity, and residual oxygen consumption. Oxygen consumption rates were corrected for instrumental background and residual oxygen consumption and normalized to tissue mass.

The redox state of the mitochondrial CoQ pool was monitored using the NextGen-O2k Q-Module and a short-chain CoQ analogue (CoQ_2). The oxidation and reduction peak potentials were determined by cyclic voltammetry at +70 ± 12 mV and −259 ± 10 mV, respectively. Changes in electrical current generated by oxidation of reduced CoQ_2 are indirect indicators of CoQ redox status (Komlódi et al 2021).

Mitochondrial membrane potential ($\Delta\psi_{mt}$) was measured by safranin fluorescence coupled to the O2k Fluo-Module. Fluorescence changes across respiratory states were used to evaluate relative changes in membrane potential (Krumschnabel et al 2014).

Mitochondrial protein content and markers of mitochondrial dynamics were analyzed by SDS-PAGE and immunoblotting using standard procedures. To evaluate respiratory complexes and supercomplex organization, blue native electrophoresis and in-gel activity assays were performed in isolated mitochondrial preparations (Acín-Pérez et al 2020).

Data were analyzed using DatLab 8 and GraphPad Prism. Statistical comparisons were performed using non-parametric tests when appropriate.

Results

Optimization of simultaneous measurements

Previously reported CoQ_2 concentrations interfered with respiration in pfi. Therefore, a concentration-response analysis was performed. Concentrations ≥40 nM significantly inhibited oxygen consumption, whereas 20 nM CoQ_2 exerted no detectable effect. Validation using the SUIT-008 protocol confirmed that 20 nM CoQ_2 did not alter leak respiration, OXPHOS, or ETS capacity and was selected for subsequent experiments.

The suitability of 1 µM safranin for membrane potential measurements was evaluated. Safranin produced robust and reproducible fluorescence changes across respiratory states, confirming adequate sensitivity for $\Delta\psi_{mt}$ determination. Importantly, safranin did not significantly affect oxygen flux under most conditions, although a slight inhibitory effect was observed on NADH-linked OXPHOS respiration, consistent with previous reports (Krumschnabel et al 2014).

Together, these results established a platform for simultaneous quantification of mitochondrial respiration and either CoQ redox state or mt-membrane potential in pfi.

Effects of exercise on mitochondrial respiration

Mitochondrial bioenergetics was compared among sedentary controls, acutely exercised mice, and chronically trained mice. Both acute and chronic exercise significantly increased mitochondrial respiratory capacity. The enhancement was particularly evident in OXPHOS respiration when NADH- and succinate-linked pathways were simultaneously activated (Control: 138.7±9.67; Acute: 204.09±28.10; Chronic: 195.95±13.80 $pmol \cdot s^{-1} \cdot mg^{-1}$) and in ETS capacity upon uncoupler titrations (Control: 132.1±8.63; Acute: 207.67±22.66; Chronic: 190.06±15.08 $pmol \cdot s^{-1} \cdot mg^{-1}$).

Interestingly, the relative contribution of NADH-linked respiration to maximal oxygen flux was reduced significantly after acute but not chronic exercise (Control: 0.759±0.019; Acute: 0.635±0.014; Chronic: 0.705±0.030 $pmol \cdot s^{-1} \cdot mg^{-1}$), suggesting exercise-induced remodelling of the ETS.

Effects of exercise on mitochondrial membrane potential

Mitochondrial membrane potential displayed the expected pattern across respiratory states, reaching the highest values during leak respiration and the lowest in fully decoupled conditions. Despite exercise-induced increases in respiratory capacity, neither acute nor chronic exercise significantly altered $\Delta\psi_{mt}$ in any respiratory

state. These findings indicate that mt-membrane potential remains tightly regulated despite substantial changes in oxidative capacity.

Effects of exercise on CoQ redox state

Analysis of CoQ redox status revealed transient exercise-induced alterations. Acute exercise made the CoQ junction more oxidized during states selected OXPHOS (NADH and succinate-linked respiration - Control: 0.764±0.026; Acute: 0.668±0.023 reduced Q fraction) and ETS states (reduced Q fraction of NADH and succinate-linked respiration - Control: 0.796±0.023; Acute: 0.702±0.023; and reduced Q fraction of succinate-linked respiration - Control: 0.835±0.020; Acute: 0.733±0.021), indicating temporary changes in electron flow through the respiratory system. This was largely associated with an improved capacity of succinate-derived electron input into the Q-junction under both substrate-saturated (reduced Q fraction OXPHOS succinate-linked respiration - Control: 0.972±0.033; Acute: 0.763±0.040) and submaximal (reduced Q fraction of leak succinate-linked respiration - Control: 0.941±0.039; Acute: 0.721±0.042) conditions. However, these differences were no longer evident after chronic training, with CoQ redox values comparable to controls.

The improvements in respiratory capacity may involve post-translational mechanisms. Protein levels of the mitochondrial dynamics markers MFN2 and DRP1 were unchanged among groups but reaching a significant increase of OPA1 after chronic exercise training. However, muscles from both acute and chronic exercise groups exhibited increased concentrations of $I+III_2$ supercomplexes compared with controls, suggesting that OXPHOS reorganization contributes to the observed improvements in mitochondrial bioenergetic performance.

Overall, these findings suggest that acute exercise induces a transient redistribution of redox balance within the ETS, whereas long-term adaptions, including increased mitochondrial content, restore redox homeostasis.

Discussion

The present study demonstrates the successful implementation of a novel experimental approach integrating HRR with simultaneous assessment of mitochondrial membrane potential and CoQ redox state in skeletal muscle pfi. Compared with respiration measurements alone, this methodology provides a more comprehensive evaluation of mitochondrial fitness.

The improvements in mitochondrial respiratory function following chronic exercise are consistent with extensive evidence showing enhanced oxidative capacity after endurance training (Garcia-Roves et al 2025).

A particularly notable finding is the increase in respiratory capacity after a single exercise bout. Although mitochondrial biogenesis generally requires repeated exercise stimuli, acute improvements in mitochondrial function may arise through more rapid mechanisms, including post-translational regulation, enhanced respiratory supercomplex assembly, altered mitochondrial dynamics, or increased efficiency of existing respiratory complexes (Garcia-Roves et al 2025).

The analysis of CoQ redox state provides additional insights into these adaptive processes. CoQ serves as a key junction of electron transfer and its analysis allows to infer which part of the ETS if affected. The transient decrease in the fraction of reduced CoQ observed after acute exercise may reflect improved electron transfer efficiency downstream of CoQ or acute reorganization of OXPHOS activity. The restoration of control-like redox values following chronic training suggests the establishment of a new metabolic equilibrium associated with the well-described increase in mitochondrial content.

In contrast, mitochondrial membrane potential remained remarkably stable across all groups. This finding suggests that exercise-induced enhancements in respiration occur without major alterations in mt-membrane potential and that mitochondrial energy transformation remains tightly regulated.

Collectively, these findings support the concept that exercise enhances mitochondrial respiratory capacity through both quantitative and qualitative improvements in ETS efficiency rather than through persistent changes in membrane potential or CoQ redox balance.

Overall, these findings provide novel mechanistic insights into exercise-induced mitochondrial adaptions and establish an integrated methodological framework for future studies of mitochondrial physiology in health and disease.

Conflicts of interest: EG holds the position of Editor-in-Chief for BEC and LHDC holds the position of co-Editor-in-Chief for BEC. Both are blinded from reviewing or making decisions for the manuscript. The other authors declare no conflicts of interest.

Author contributions: JA-L, KK, PG-P, SC-T and PMG-RR performed HRR, mtMP and CoQ redox evaluation in pfi. LHDC, TK contributes technical support to stablish mtMP and CoQ redox experimental procedures. JA-L, KK, PG-P, PMG-R, LHDC, TK performed data analysis. JA-L, AS, BI, PMG-R contributed with the protocol and optimization of skeletal muscle mitochondrial isolation and pfi preparation. JA-L, KK, PG-P, SC-T, RB, NB and PMG-R train mice (acute and chronic). KK, NB, RB, LN performed determinations of RNA expression (PCR) and protein content (WB). JA-L, PF-M, BL, performed mitochondrial supercomplexes assessments. PMG-R wrote the manuscript. JAE, EG, PMG-R contributed to experimental design, data discussion, financial support and editing the manuscript.

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