EC.13 - Time-course of redox status, redox activity-related and mitochondrial dynamics-related gene expression after acute bout of different physical exercise protocols

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The magnitude of exercise-induced adaptations depends on exercise parameters, such as intensity, duration and execution mode (continuous or with intervals). In this context, the present study aimed to investigate the expression of redox activity-related and mitochondrial dynamics-related genes in mice skeletal muscle along 24 hours after an exercise session carried out with different protocols. Sixty-five male Swiss mice were allocated into a control group (5 animals) and others 3 groups (20 animals/group), which were submitted to a forced swimming bout with the follow protocols: low-intensity continuous (LIC), high-intensity continuous (HIC) and high-intensity interval (HII). Five animals from each group were euthanized immediately (0h) and at 6h, 12h and 24h after the exercise session. Gastrocnemius muscle was removed for analysis of expression of mitochondrial dynamics-related genes: Ppargc1a (mitochondrial biogenesis), Mfn2 (fusion), Dnm1L (fission), and Park2 (mitophagy); and redox activity-related genes: Nos2 Nfe2l2 and GPx1. Within-group and between-group comparisons were performed with ANOVA, with significance level set as $p \leq 0.05$. Despite opposites in exercise intensity and duration, similar temporal behavior was observed in the expression of the Ppargc1a in LIC and HII, with greater expression at 6 and 24h, while HIC showed significant increases only at 0h and 6h after exercise. Mfn2 was significantly higher at 0h only in HIC and HII, remaining high at 6h only in HII. Only HII exhibited repression of Dnm1L and Park2 genes along 24h after exercise, while HIC was the unique with significant increase. Nos2 was significantly higher only in HIC (0h) and HII (6h). Nfe2l2 increased along the 24h in LIC and HII. GPx1 was significantly higher only in HIC (0h) and HII (24h). The use of intervals during high-intensity exercise could suppress the expression of fission and mitophagy-related genes, and enhances the molecular profile related to mitochondrial biogenesis and fusion, as well as antioxidant defense.
Keywords: activity redox, exercise, mitochondrial dynamics. **Supported by:** FAPESB

EC.14 - Neutrophil granules isolation: a new miniaturized method

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Neutrophils are the most abundant leukocytes in the bloodstream and play a key role in the immune system. The activation and functionality of neutrophils depends on the exocytosis of storage particles, that are divided in 2 groups: granules and secretory vesicles. The granules are subdivided in 3 subtypes: azurophil, specific and gelatinase. These particles are differentiated mainly through their protein content; therefore, abundant specific proteins can be used as markers for each storage particle. The markers are myeloperoxidase (MPO) for azurophil, lipocalin-2 (NGAL) and lactoferrin for specific, gelatinase for gelatinase granules and latent alkaline phosphatase (latent AP) for secretory vesicles. Characterization of granule's content is essential to comprehend the different functions of these cells, hence the importance of the granule's isolation. Currently, the protocols of isolation are performed in large density gradients, leading to the necessity of major cell quantities, about 3x108, that makes unfeasible to compare stimuli and biological replicates in the same experiment. Thus, the main goal of our work was to create a miniaturized isolation method based on a discontinuous percoll density gradient. In order to achieve this goal, 9 x 106 neutrophils were isolated, lysed, put on top of a 3-layer percoll density gradient and centrifuged. The resulting gradient (~940µL) was collected from the bottom to the top of the tube in 19 fractions. The protein markers were then analyzed for each fraction using western blot (MPO, lactoferrin and NGAL), gelatin zymography (gelatinase) and enzyme assay (latent AP). Our results showed we successfully isolated the granules and secretory vesicles using a gradient with less than 1 mL of total volume. The miniaturized method allows new experiments to be conducted, including a comparative study of neutrophill's response to diverse stimuli.
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