Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVII

Exercise-induced extracellular vesicles in skeletal muscle-adipose tissue crosstalk

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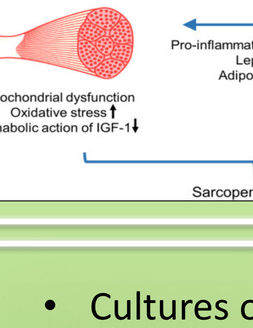


INTRODUCTION

Sarcopenia, the progressive reduction of muscle mass and function typical of aging, is frequently accompanied by an increase of deposition of ectopic adipose tissue, causing the so-called Sarcopenic Obesity (SO). This condition is characterized by increased inflammation and oxidative stress, mitochondrial disfunction and a reduction of insulin sensitivity both in muscle and fat; these alterations impact the signalling between these two organs involved in metabolic regulation (1). Physical exercise has been proved to be effective in modulating molecular pathways impaired during aging and thus curbing detrimental cellular events responsible of SO. Several mechanisms have been proposed but among these the endocrine role of muscle and the release of EVs are under the magnifying glass as potential modulators of the beneficial cross talk between exercised-muscle and adipose tissue function.

AIM

The aim of this project is to investigate the crosstalk between muscle and adipose tissue in order to characterize the endocrine and paracrine effect of exercise-induced EVs on models of insulin resistance metabolic syndrome. The goal is to understand the type of EVs and the molecules able to modulate and potentially restore insulin sensitivity, reduce oxidative stress in myotubes and regulate lipid metabolism and adipocytes differentiation.

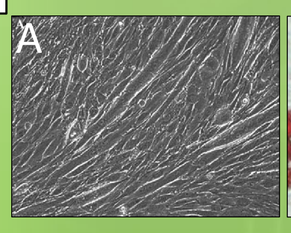
EXPERIMENTAL DESIGN

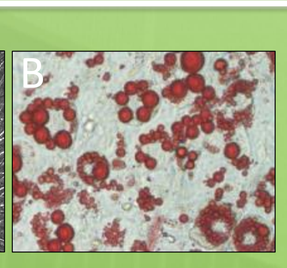
- Cultures of myotubes and adipocytes
- Development of Electrical Pulse Stimulation system (EPS) (Urbino and DiSVA laboratories)
- Contraction on myotubes with different protocols based on high and low frequency to mimic different types of exercise training

→ Isolation and characterization of exercise-induces extracellular vesicles (EVs) content (2)

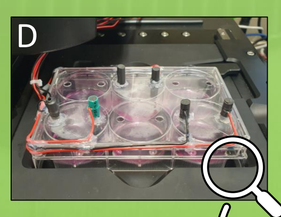
→ Evaluation of morphological and functional changes in myotubes after EPS stimulation

RESULTS

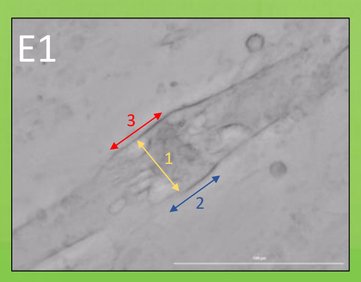


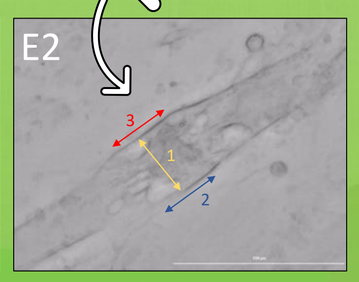


(A) C2C12 Myoblast and differentiated myotubes, phase contrast image. (B) 3T3-L1 adipocytes fully differentiated, Oil Red O staining show the lipid droplets storage inside differentiated adipocytes.



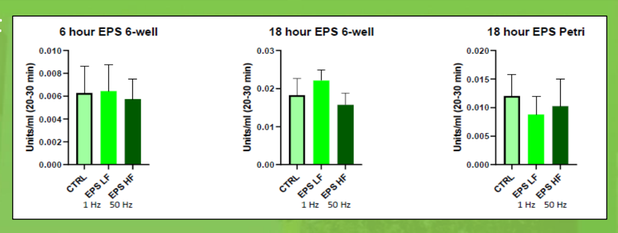
(C) Petri EPS system developed by University of Urbino: single couple of electrodes.
(D) 6-well EPS system developed in DiSVA laboratories: three couples of graphite electrodes can be stimulated together or individually and maintained under control conditions into the Lionheart microscope for visualization.

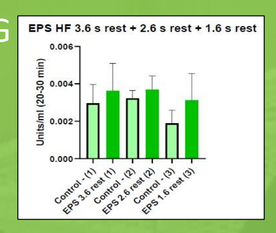




(E) Myotubes contraction morphometry

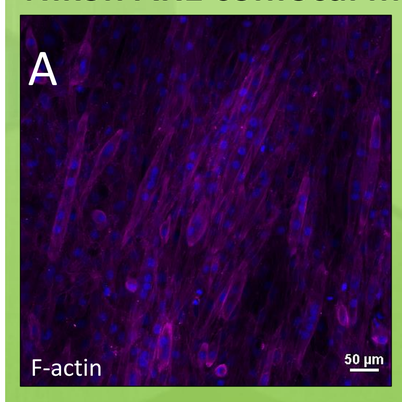
	E1	E2
Length 1	211 µm	206 µm
Length 2	126 µm	133 µm
Length 3	148µm	152 µm

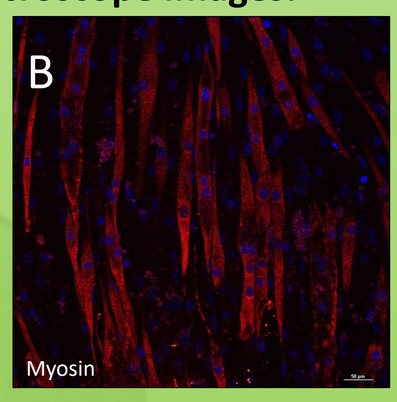




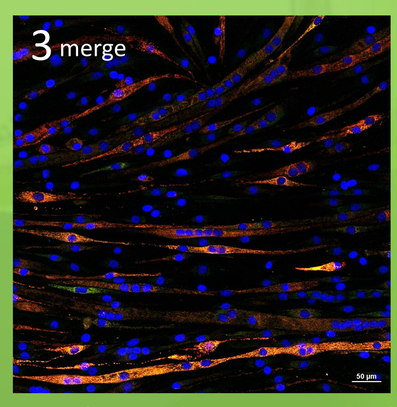
(F) LDH levels after stimulation of myotubes with two different protocols of EPS, High Frequency (HF) and Low Frequency (LF), in 6-well after 6 and 18 hours and in Petri dish after 18 hours of stimulation. No changes relative to cell damage were detected.
(G) Load curve: EPS stimulation for 1 hour and 3.6 sec rest (1), 2.6 sec rest (2), 1.6 sec rest (3) to establish the better condition for myotube contraction.

Nikon AR1 confocal microscope images:





(A) Fluorescence image of differentiated myotubes stained for F-actin filaments using Phalloidin and nuclei with DAPI.
(B) Immunostaining image of differentiated myotubes: cells are labelled with MF20 antibody and DAPI.



(C) C2C12 cell line permanently transfected with MitoTimer: the probe, constitutively expressed by myotubes, targets the mitochondrial matrix and its fluorescence shifts from green to red as the protein matures, as an expression of mitochondrial turnover rate. 1. Green channel shows myotubes with higher mitochondrial biogenesis; 2. Red channel shows myotubes with higher mitophagy; 3. Merge of the two fluorescence, Nuclei stained with DAPI. This tool will help to analysed potential remodelling of the mitochondrial network under EPS stimulation.

NEXT STEPS

TO BE COMPLETED FOR THIS YEAR:

- Treatment of 3T3-L1 fully differentiated with EVs isolated from myotubes after LF and HF protocols and evaluation of differences in lipolytic activity
- Development of insulin-resistant myotubes model and obese-diabetic adipocytes to be treated with EVs for amelioration of metabolic parameters in pathophysiological conditions

FUTURE PROSPECTIVES:

- Assessment of the effects of EVs treatment on adipocytes differentiation. Modulation on the expression of white adipocytes genes such as PPARγ, Fabp4, Adipoq and PLIN1; or brown genes (Ucp1, Pgc1α, Cidea, and Elovl3) will be evaluated at different time points of 3T3L1 differentiation. Linoleic acid and Cd36 expression will be also investigated
- Co-cultures of adipocytes and myotubes to better investigate on potential candidates involved in the cross talk between the two tissue types (3)

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2. Guescini, M.; Maggio, S.; Ceccaroli, P.; Battistelli, M.; Annibalini, G.; Piccoli, G.; Sestili, P.; Stocchi, V. Extracellular Vesicles Released by Oxidatively Injured or Intact C2C12 Myotubes Promote Distinct Responses Converging toward Myogenesis.
3. Nintou, E.; Karligiotou, E.; Vliora, M.; Fatouros, I.G.; Jamurtas, A.Z.; Sakellaridis, N.; Dimas, K.; Flouris, A.D. Effects of In Vitro Muscle Contraction on Thermogenic Protein Levels in Co-Cultured Adipocytes. Life 2021