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

Communication between skeletal muscle and adipose tissue: Extracellular Vesicles (EVs) as modulators of lipid metabolism and potential regulators of adipocytes phenotype

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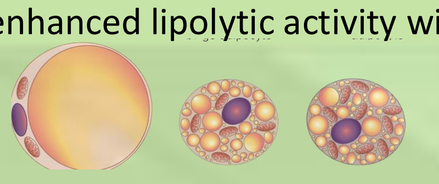
Introduction

It is well known that exercise induces adaptations in both skeletal muscle and white adipose tissue (WAT), mainly through the release of myokines from muscle and adipokines from WAT. Specifically, myokines may function to alter the phenotype of WAT, including the 'browning' of WAT (increased presence of multilocular adipocytes with enhanced lipolytic activity within the tissue)[1].

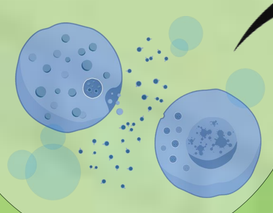


Moreover, recent advances in nanotechnology have led to the identification of small particles secreted during muscle contraction, known as SkM-EVs, which are capable of transporting bioactive molecules such as nucleic acids, proteins, and lipids. Although it is known that EVs are involved in the regulation of a wide range of physiological processes, their mechanism of action on adipocytes still remains to be fully elucidated.

Aim

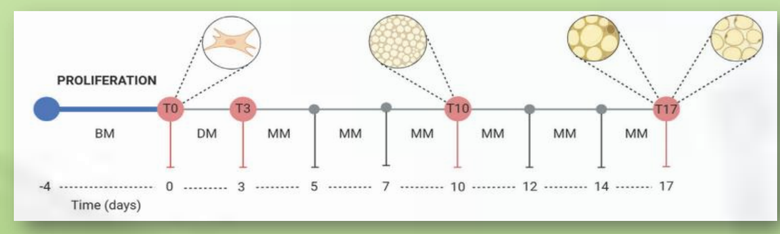
Investigating the subcellular and molecular mechanisms underlying the action of EVs on adipocytes. Specific biomarkers involved in lipid droplets and mitochondria dynamics will be employed to evaluate potential modulation of energy metabolism upone SkM-EVs treatment. Cell differentiation aspects characteristic of white, brown, and beige phenotype will be studied on differentiating adipocyte.

EVs

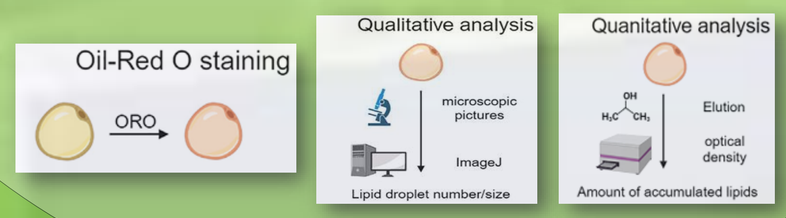


Method

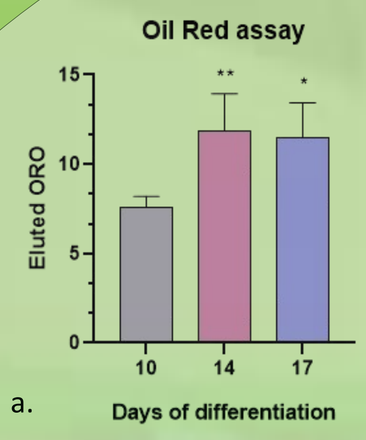
3T3-L1 cells were used as a model of murine adipocytes and were treated with EVs obtained from differentiated C2C12 cells, representing the physiological released by mature myotubes. EVs were collected and characterized at the University of Urbino. The differentiation of 3T3-L1 cells was optimized in 6-well plates as pilot experiments by testing two different protocols[2,3]; subsequently, the cells were differentiated in 24-well plates to increase replicates.



Adipocytic commitment was assessed by spectrophotometric quantification of Oil Red O extracted from cells on days 10, 14, and 17 of differentiation. Cytotoxicity of 5 and 10 µg of EVs per µg of total protein was evaluated using the Presto Blue assay on differentiating adipocytes (days 3-5). Additionally, the total area and average size of lipid droplets on day 14 were measured by ImageJ.



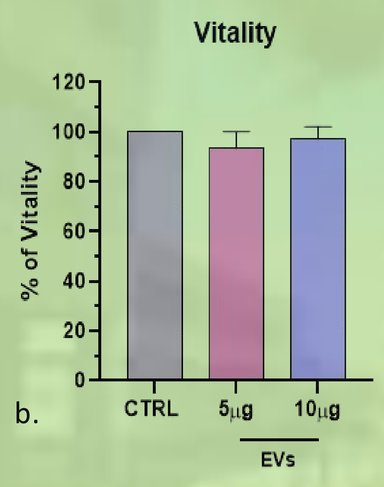
Results



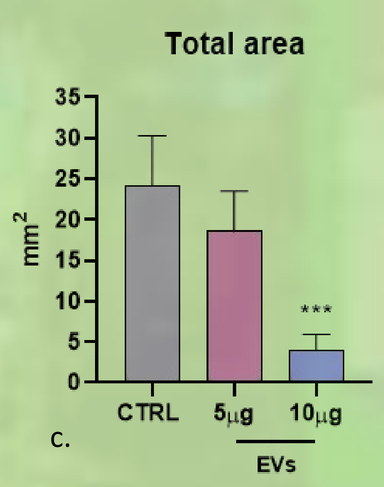
a. **Lipid differentiation increases over time.** *p< 0.05, **p<0.01 vs day 10 (ANOVA).

b-c-d . **The tested EV concentrations are non-cytotoxic and effectively inhibit lipid differentiation.** ***p<0.001 vs CTRL (ANOVA).

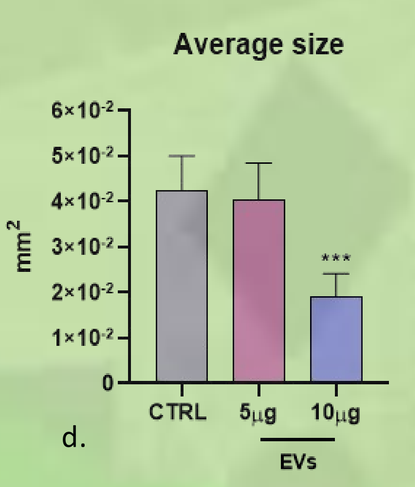
e-f-g. **EVs inhibit lipid differentiation commitment:** Oil Red O staining on adipocytes treated or untreated with EVs on day 14 of differentiation.



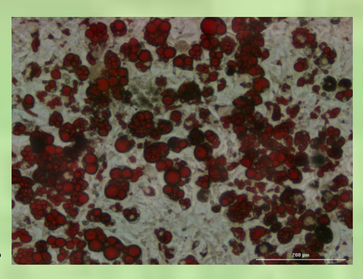
b.



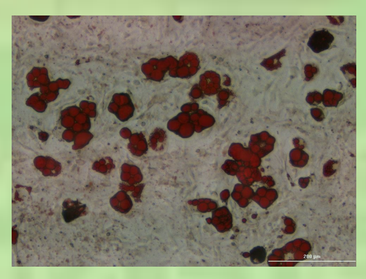
c.



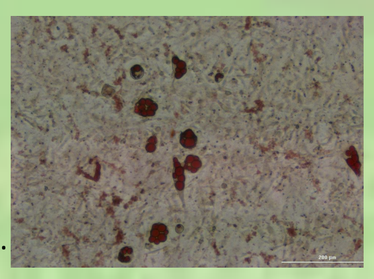
d.



e. *CTRL*



f. *5 µg*



g. *10 µg*

In coming

The Cytena bioreactor, operating under fully physiological conditions, will enable to asses the metabolic profile of differentiating adipocytes upone EVs treatment by monitoring parameters such as dissolved oxygen in the medium, OCR and pH changes.

The expression of several genes, including PPARγ, PRDM16, PGC1α and CIDEA will also be evaluated to assess differential adipocytes phenotype commitments upon treatment with EVs.

Bibliography

[1] **Bond, S. T.**, et,al. *Adipose-Derived Extracellular Vesicles: Systemic Messengers and Metabolic Regulators in Health and Disease.* [2] **Zebisch**, et,al. (2012). *Protocol for effective differentiation of 3T3-L1 cells to adipocytes.* [3] **Zoico**, et,al. (2023). *Senescent adipocytes as potential effectors of muscle cells dysfunction: An in vitro model.*