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Mitigating Exercise-Induced cellular damage in Skeletal Muscle: The Role of CoQ_{10} Supplementation in Overtraining Conditions

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INTRODUCTION

Regular physical activity is a powerful shield against the age-related weakening of muscles. This natural decline meets its match in exercise, which not only slows down the molecular and metabolic aging processes but also sparks a beneficial oxidative stress, leading to the body's adaptation. However, the double-edged sword of exercise reveals itself in overtraining, leading to oxidative damage. Antioxidants exhibit inconsistent results in battling these negative effects. Mitochondrial nutrients as Coenzyme Q_{10} are a viable substitute for overtraining in order to improve mitochondrial resilience. Unfortunately, skeletal muscle is very refractory to exogenous CoQ_{10} uptake, however a novel formulation of CoQ_{10} in phytosomes (Ubiqsome®) has shown improved muscle uptake representing a potential game changer in sport nutrition.

AIM

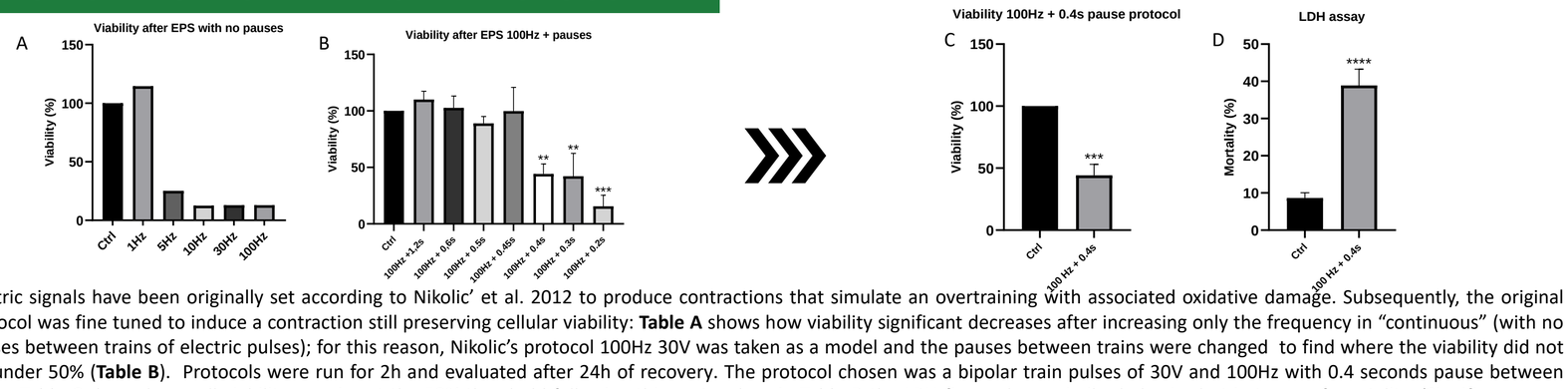
The aim of this project is to estimate the potential of CoQ_{10} in phytosome (Ubiqsome) to protect muscle cells from the oxidative stress caused by intense physical activity, focusing on mitochondrial preservation, oxidative stress reduction and cytotoxicity prevention.

METHODS

- C2C12 murine myoblasts were differentiated into myotubes as muscle model.
- Electrostimulation system composed by an electric generator and an amplifier connected to a multiwell plate (**Fig. 1 and 2**) was used to simulate an exhaustive physical exercise cells in cultures.
- HMG-CoA reductase inhibitor (Statin) was used to induce CoQ_{10} deprivation to impair mitochondria function.
- PrestoBlue Cell Viability Reagent, a resazurin-based method, and LDH assay which measures the release of the enzyme into the culture medium after cell membrane damage, were used to quantify vitality and mortality respectively.
- TBARS assay and DNPH assay were performed to measure respectively lipid peroxidation and protein oxidation.
- *In-vivo* supplementation with a formulation of phytosome containing CoQ_{10} (Ubiqsome) provided by Indena S.p.A. was performed as described in the relative paragraph of Results.
- HPLC with electrochemical detector was used to determined CoQ_{10} levels.

RESULTS

SET UP OF EPS HIGH INTENSITY PROTOCOL



Electric signals have been originally set according to Nikolic' et al. 2012 to produce contractions that simulate an overtraining with associated oxidative damage. Subsequently, the original protocol was fine tuned to induce a contraction still preserving cellular viability: **Table A** shows how viability significant decreases after increasing only the frequency in "continuous" (with no pauses between trains of electric pulses); for this reason, Nikolic's protocol 100Hz 30V was taken as a model and the pauses between trains were changed to find where the viability did not fall under 50% (**Table B**). Protocols were run for 2h and evaluated after 24h of recovery. The protocol chosen was a bipolar train pulses of 30V and 100Hz with 0.4 seconds pause between trains. **Table C** shows how cell viability remains at the 50% threshold following this protocol, as in **Table D** the LDH figure shows similar behavior but in terms of mortality. (Significance was expressed *vs* Ctrl).

Fig.1 Homemade multi-well plate used for the first stage of the project, to set the protocol of overtraining on myotubes in cultures.

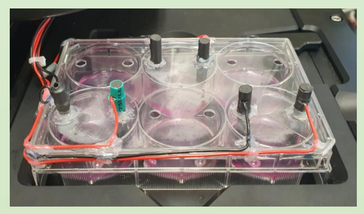
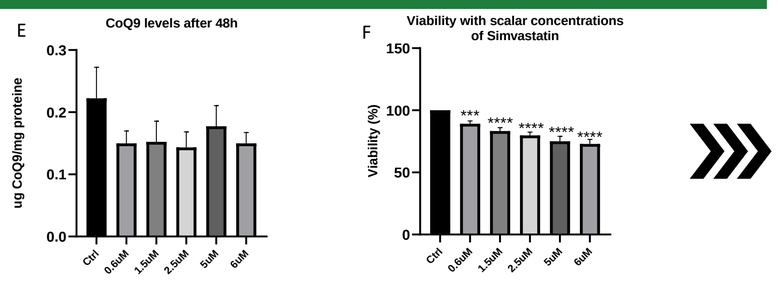


Fig.2 C-Dish multiwell plate from IonOptix®, more stable commercial system used for the experiments with statins

STATINS TREATMENT: MYOPATHY MODEL FOR EPS



To contain the high variability observed in myotubes viability after EPS protocol, a CoQ deprivation model was also developed. The rationale of this strategy was to verify that CoQ deprivation should lead to less functional mitochondria and hence myotubes contraction should be significantly affected in a way clearly recognizable from untreated control cells. Following treatment with a statin range, CoQ_9 levels already drop from 0.6 µM of simvastatin, as shown in **Table E** (not statistically significant, but the p value is 0.08); at the same time, vitality also falls as statin concentration increases, as shown in **Table F**.

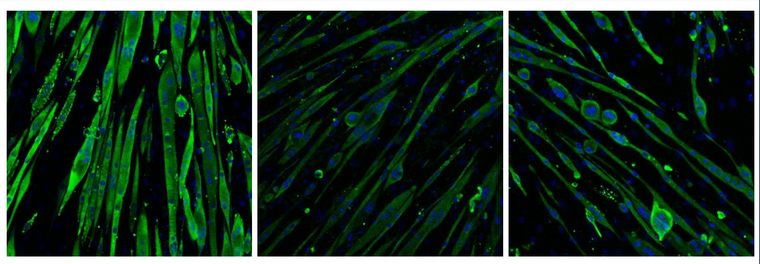
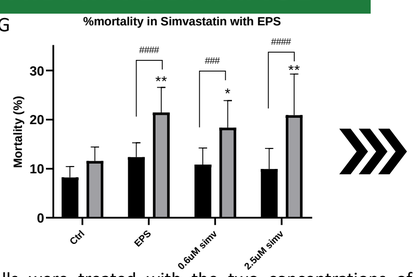


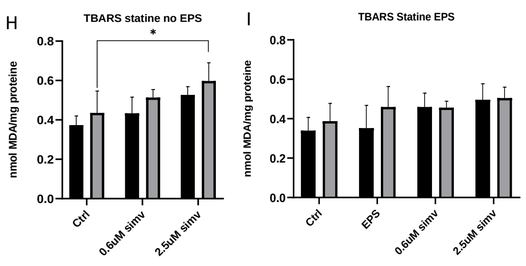
Fig.3 Ctrl **Fig.4 0.6 µM Simvastatin** **Fig.5 2.5 µM Simvastatin**

Since a sufficient decrease in CoQ levels already occurs at the lowest simvastatin concentrations, 0.6 µM and 2.5 µM simvastatin have been selected. Interestingly, the morphology of the myotubes after statin treatment was also highly impaired, as shown in **Fig. 3, 4 and 5**. Images were taken with myosin heavy chain (MF20) labeling with confocal microscopy.

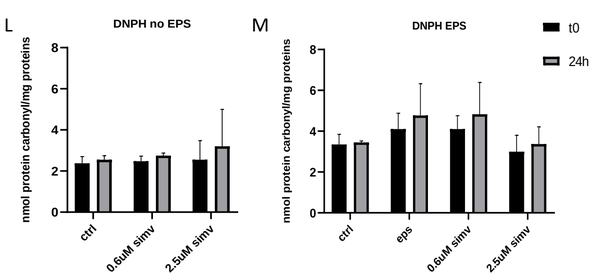
OXIDATIVE DAMAGE



Cells were treated with the two concentrations of Simvastatin for 48h, then the culture media was changed before starting EPS. **Table G** shows how, when the additional stress is applied to statins, i.e. EPS, mortality is higher not only at 24h compared to its own T0 control, but also compared to the non-EPS control.



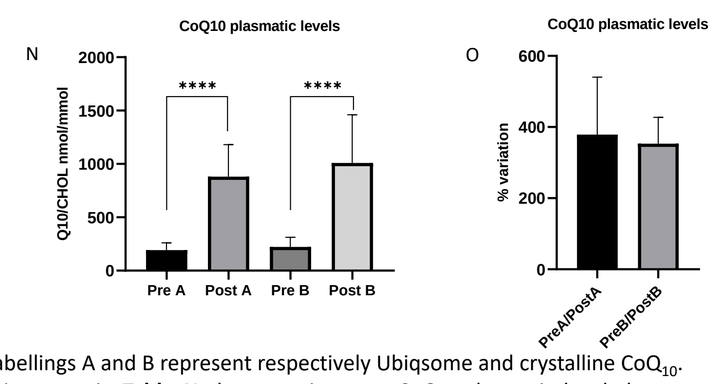
TBARS was performed to measure lipid oxidation on samples treated with statins alone **(Table H)** and with statins and EPS simultaneously (**Table I**). Only the difference between control at 24h and 2.5 µM statin at 24h without EPS was significant.



DNPH assay was also performed to measure variations in protein oxidation with statins alone (**Table L**) and statins and EPS simultaneously (**Table M**). There is no statistically significant difference between conditions.

UBIQSOME SUPPLEMENTATION

IN-VIVO EXPERIMENTAL DESIGN:
Randomized crossover study involving 10 subjects (5M/5F) aged <40 years, not assuming medications, with a BMI between 18.5 and 24.9kg/m^2. Volunteers, following a baseline blood withdrawal were randomized to 200mg daily of either to Ubiqsome or crystalline CoQ_{10}, for two weeks when they were sampled. Following two weeks wash-out the study was reproduced changing the type of CoQ taken [1]. Blood samples were collected under fasting conditions and LDLs were extracted from plasma. CoQ_{10} levels were determined with HPLC with electrochemical detector.

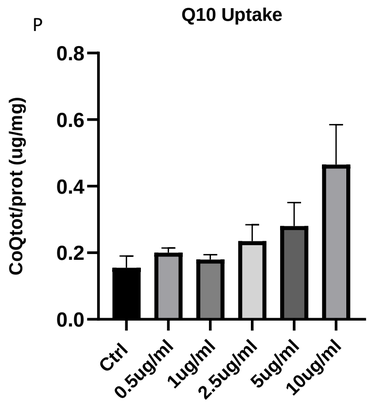


The labellings A and B represent respectively Ubiqsome and crystalline CoQ_{10}.
The histogram in **Table N** shows an increase CoQ_{10} plasmatic levels between pre and post supplementation of each formulation; however, there are no statistically significant differences between the percentage changes of the two formulations, as shown in **Table O**.

CoQ_{10} SUPPLEMENTATION

ONGOING WORK

Currently, ubiquinol in cremophor is being administered to murine myotubes to assess the effects of CoQ supplementation. This phase precedes the treatment with CoQ-enriched LDL mentioned in the previous section, which will involve human cells.
Modulation of CoQ_{10} content is crucial for understanding its role in muscle contraction as well as in preventing and curbing oxidative stress and its associated damage.
In this **Table P** is evident how the CoQ level increases only up to 5 µM.
This section is still ongoing.



DISCUSSION and CONCLUSIONS

- We have successfully achieved the EPS protocol to mimic exhaustive physical exercise in murine C2C12 myotubes.
- We have described cellular viability as well as cell damage in control *vs* EPS and found significant differences.
- In parallel, we have measured the same parameters in a model of CoQ deprivation to evaluate if impaired mitochondrial function could potentially lead in differences in the contractility of myotubes.
- We have identified the proper condition of treatment to achieve a trend of reduction in CoQ and verify that myotubes were still morphologically intact and able to sustain the EPS protocol.
- We have applied the EPS protocol to this deprivation model and found significant differences in oxidative stress parameter (TBARS).
- We are now working to find the optimal condition to supplement CoQ in order to reduce oxidative damage and restore functionality of myotubes
- We have also completed the first part of the *in-vivo* component of study by collecting enriched LDL collected by volunteer's subjects supplemented with Ubiqsome. The LDL will be used to treat human cell model exposed to EPS protocol.

References:
[1] Marcheggiani, F.; Orlando, P.; Silvestri, S.; Cirilli, I.; Riva, A.; Petrangolini, G.; Orsini, F.; Tiano, L. CoQ_{10} Phytosomes Improve Cellular Ubiquinone Uptake in Skeletal Muscle Cells: An Ex Vivo Study Using CoQ_{10}-Enriched Low-Density Lipoproteins Obtained in a Randomized Crossover Study Antioxidants (2023).
[2]Nikolić N, Siril Skaret Bakke,Eili Tranheim Kase,Ida Rudberg,Ingeborg Flo Halle,Arild C. Rustan,G. Hege Thoresen,Vigdis Aas. Electrical Pulse Stimulation of Cultured Human Skeletal Muscle Cells as an In Vitro Model of Exercise. PLoS ONE (2012).
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