Mitochondrial dysfunction and oxidative stress induced by palmitate in human muscle cell

V. DUDOME[1], M.-C. DABAUVALLE[2], F. COPPEE[1] and A.-E. DECLEVES[1]

1Laboratory of Metabolic and Molecular Biochemistry and Research Institute for Health Sciences and Technology, University of Mons (UMONS), Mons, Belgium.
2Division of Electron Microscopy, Biocenter, University of Wuerzburg Wuerzburg, Germany.

Introduction

FFA

↗ lipotoxicity and skeletal muscle dysfunction
→ Trigger by oxidative stress?
- Produced by mitochondria?
- Antioxidant response set up?

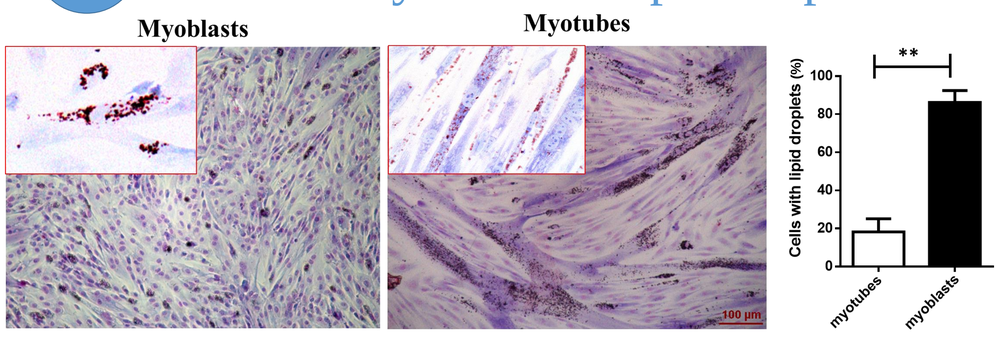
Methods

FFA: Palmitate treatment (PA-300µM-24h) on **human** myoblasts and myotubes (after 4 days of diff.).

Cells were treated with PA complexed to BSA and compared to cells treated with BSA only (control condition).

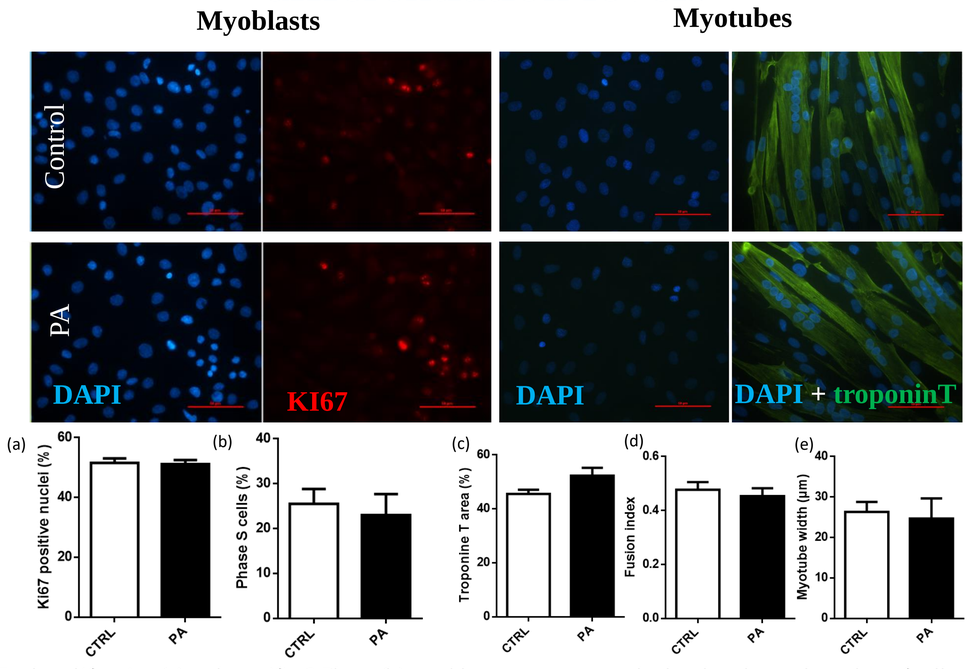
Results

1 Significant accumulation of intramyocellular lipid droplets



Neutral triglycerides and lipids inclusions were labeled by Oil red O (red) staining and cells counterstained with GIEMSA. Lipid Droplets were present in less myoblasts (20 ±8%) than myotubes (86 ±8%). Any lipid droplet was founded in control cells. Bar graphs represent mean ± SEM.

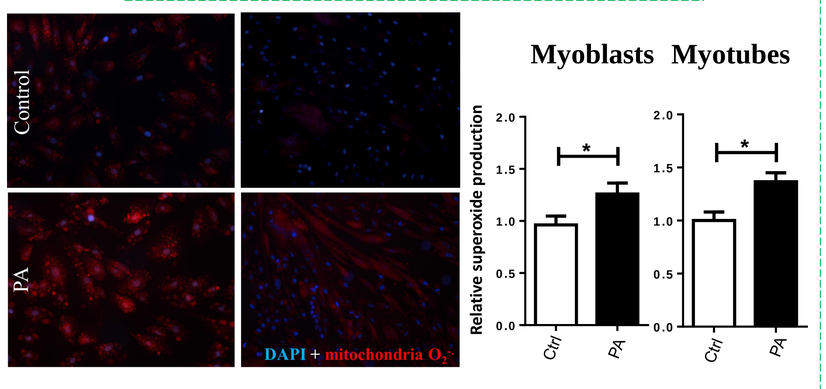
No impact of PA on proliferation and differentiation levels



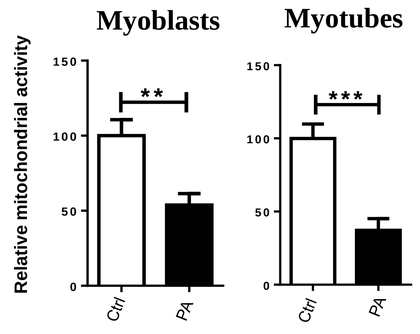
Total proliferative (a) and specific S Phase (b) myoblasts were count and related to the total number of cells. Area coverage of myotubes (c) was determined on the total cell culture area, fusion index (d) were calculated as the ratio between myotube nuclei and total nuclei; myotube width (f) were measured to detect morphologic changes. Our analyses showed no significant difference with or without palmitate treatment. Bar graphs represented mean ± SEM

2 Mitochondrial dysfunction in myoblasts and myotubes

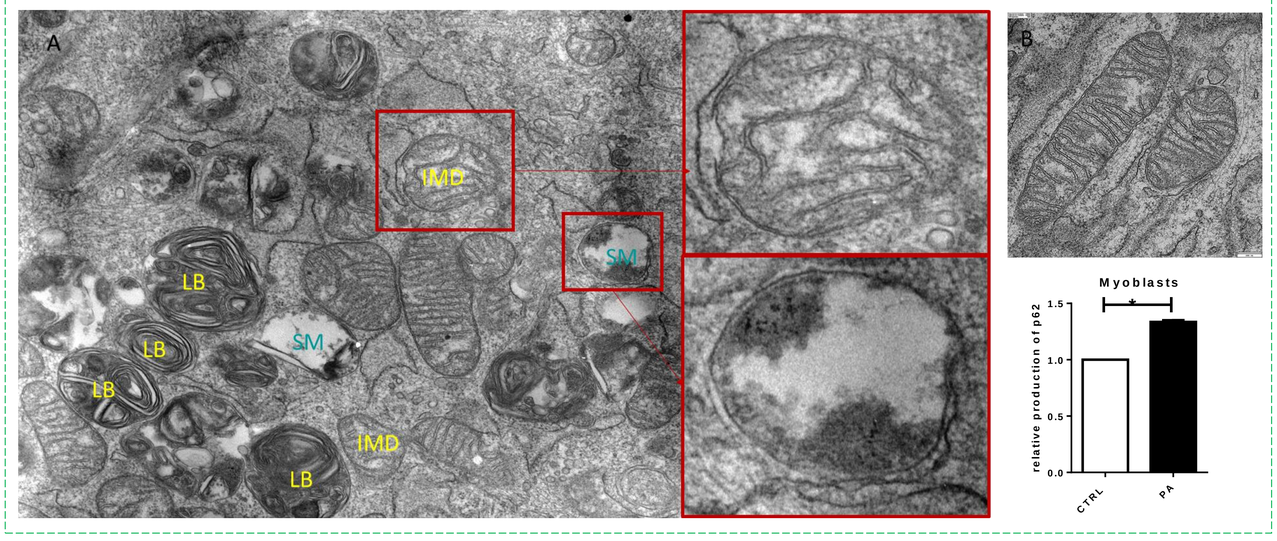
a) mt superoxide overproduction



b) Mitochondrial activity



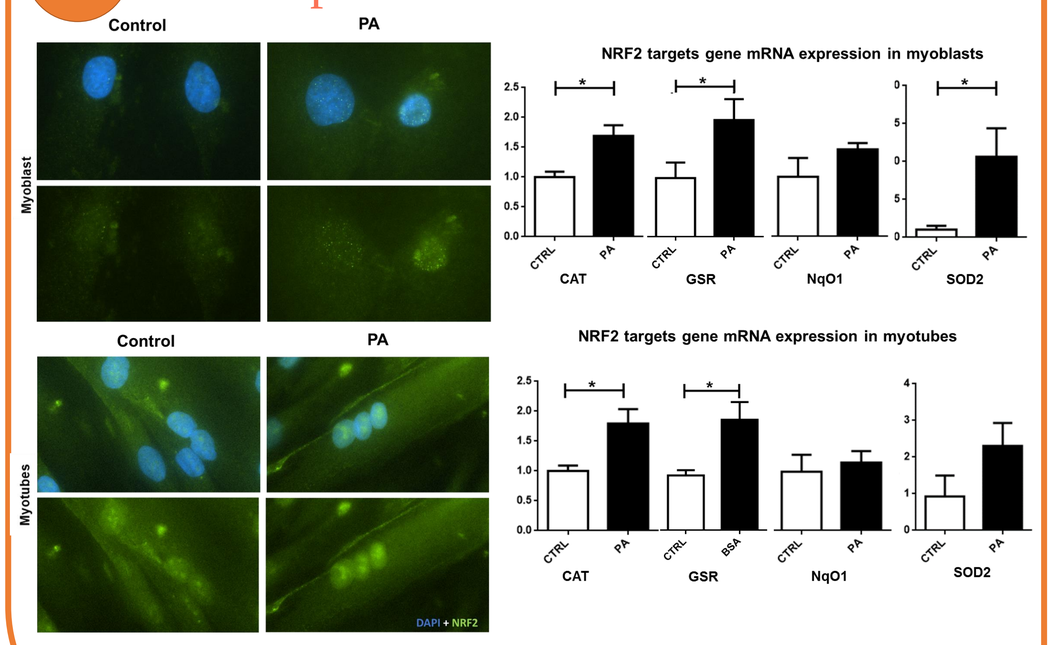
c) Mitochondrial swelling



(a) Production of mitochondria superoxide was detected using MitoSOX™ Red Mitochondrial Superoxide. (b). Vybrant® MTT Cell Assay was used to measured complex II activity, data suggest a decrease of mitochondria activity.(c) (A) Myoblast mitochondria treated with PA. (B) Control myoblast mitochondria. Inner membrane was disrupt fallow by a swelling of mitochondria and a increased of lamellar body.
LB: Lamellar body DM: Dead mitochondria INMD: Inner membrane disruption

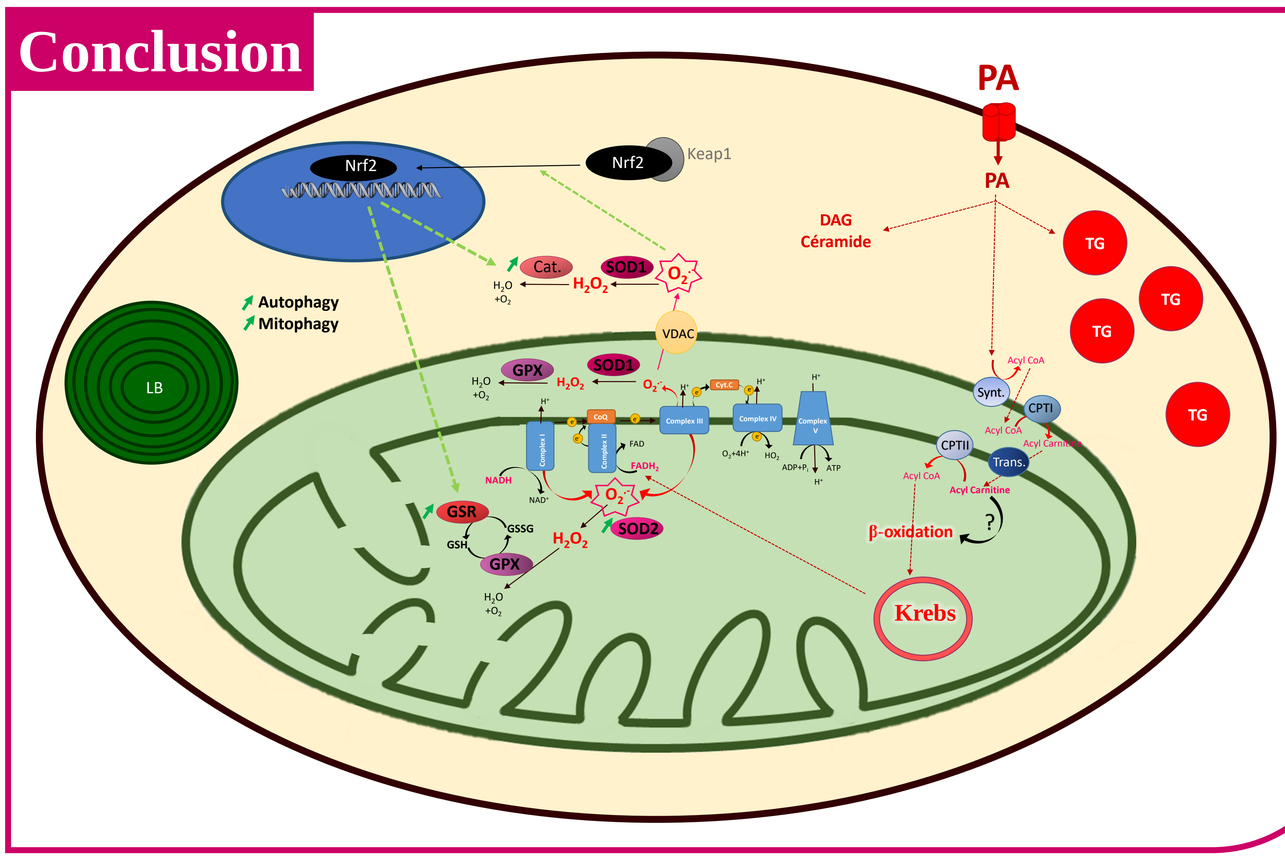
➔ **PA treatment induces an important alteration of mitochondrial activity along with increased production of mitochondrial $O_2^{\cdot-}$ and mitochondrial swelling.**

3 Nrf2 expression & Antioxidant balance



➔ **Nrf2 translocation was observed after PA treatment along with increased antioxidant response in PA-treated myoblasts and myotubes.**

Conclusion



Acknowlegments : We acknowledge the platform for immortalization of human cells from the Institute of Myology (Paris) for providing the immortalized myoblasts. This work was financially supported by a grant of the ABMM Téléthon.

UMONS

virginie.dudome@umons.ac.be