Mitochondrial Dynamics Play a Key Role in Brown Adipocyte Activation

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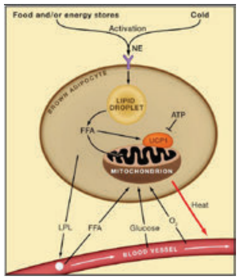
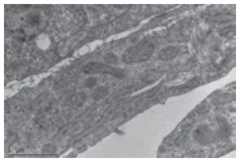
Introduction

Mitochondria continuously go through fusion and fission events. This process, termed mitochondrial dynamics, is essential for the maintenance of mitochondrial bioenergetic functions. While chronic disruption of mitochondrial fusion results in the deterioration of respiratory capacity, it is unclear whether mitochondrial dynamics directly influence respiratory functions. With its ability to sharply increase uncoupled respiration upon stimulation, brown adipose tissue (BAT) presents an intriguing system in which the interdependence between mitochondrial morphology, dynamics and function can be studied. To study the relationship between dynamics and bioenergetics in brown adipocytes, interscapular BAT preadipocytes were isolated from mice and differentiated in vitro.

Confocal microscopy revealed that mitochondria were highly networked, as demonstrated by lumen connectivity and by frequent fusion events. Upon stimulating cells with a combination of norepinephrine and free fatty acids we found a synergistic effect which caused a dramatic increase in oxygen consumption rates using the Seahorse XF 24 platform. Depolarization of mitochondrial membrane potential ($\Delta\psi m$) was accompanied by fragmentation of the mitochondrial network, while individual mitochondria appeared sphere-like and exhibited disengagement from fusion events. After removal of norepinephrine, cells regained normal function, mitochondrial morphology and $\Delta\psi m$ within 24h. Given that BAT mitochondria fragment when activated, we went on to elucidate the significance of mitochondrial fission in the induction of uncoupled respiration by blocking fission using the dominant negative form of DRP1. Inhibition of fission resulted in reduced respiratory response to norepinephrine and palmitate. We conclude that mitochondrial fission is therefore essential but insufficient for induction of uncoupled respiration in brown adipocytes.

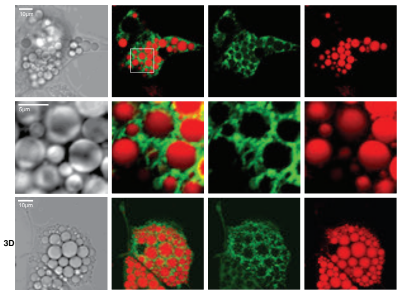




Materials and Methods

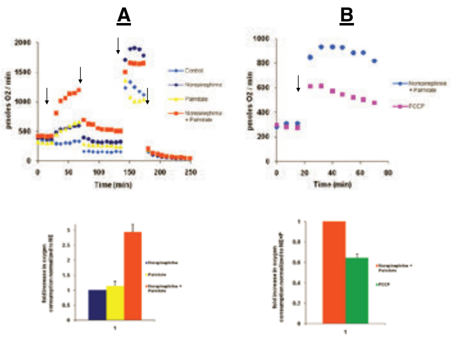
BAT was harvested from 3 to 4-week-old wild-type male C57BL6/J mice. Brown adipocytes were differentiated in vitro. A LSM 710 laser scanning confocal microscopy (Zeiss) was used for imaging of mitochondrial morphology using several fluorescent dyes and proteins. Oxygen consumption was measured using the XF24 platform (Seahorse Bioscience). The pro fission protein Drp1 was inhibited with adenoviral expression of its dominant negative form.

Confocal Imaging with Lipid and Mitochondrial Dye



Mitochondrial morphology and lipid droplets. Differentiated BA were stained with the mitochondrial inner membrane dye, MTG, and the lipid droplet dye, Nile Red. Images are single sections and 3D projections from z-stacks of multiple images. Note the close proximity of lipid droplets (red) and mitochondria (green). BF=brightfield. White square shows area that was zoomed in on.

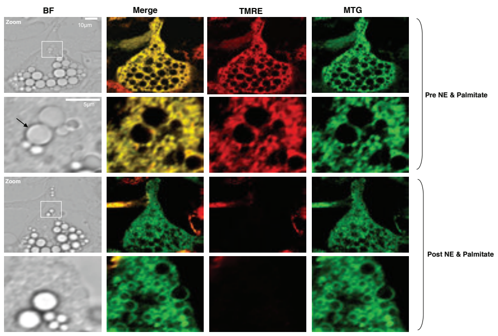
Synergistic Effect of NE and Palmitate Includes Reversible Mitochondrial Depolarization and Fragmentation



A) Synergistic increase in oxygen consumption rates by the combination of NE and palmitate. Basal rates of oxygen consumption were first recorded followed by measurements under stimuli indicated by the first arrow. Then measurements were performed in the presence of the following drugs: oligomycin (5μM) (inhibits F1F1ATPsynthase), FCCP (1μM) (uncoupler) and Rotenone (5μM) Myxothiazol (5μM) (inhibits complex I/III). Data is normalized to basal rates. Note the large increase due to NE (1μM) and palmitate (0.4mM) combination. Arrow indicates application of indicated stimuli.

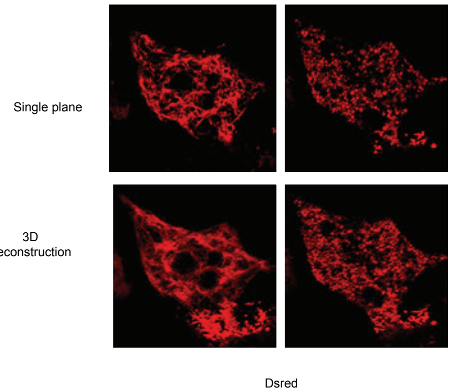
B) Comparison of oxygen consumption response to NE and palmitate vs. FCCP. Data is normalized to basal rates. Arrow indicates application of NE (1μM) and palmitate (0.4mM) or FCCP (1μM).

BAT Mitochondria Depolarize when NE+P is Added

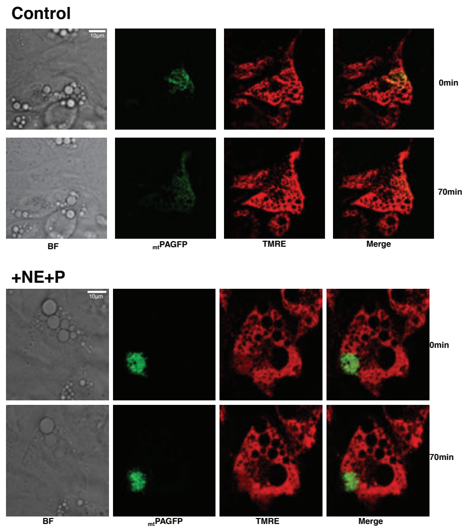


NE and palmitate induce mitochondrial fragmentation and swelling. Cell stained with TMRE/MTG, imaged before and after NE and palmitate stimulation. Note transition from filamentous polarized mitochondria to fragmented ring-like depolarized mitochondria. Arrow indicate lipid droplet in the bright field image.

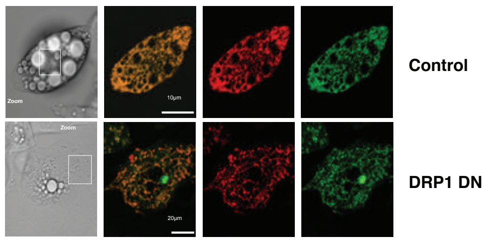
BAT Mitochondria Fragment when NE+P is Added



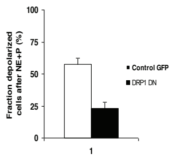
BAT Mitochondria Exhibit Decreased Fusion when Activated with NE+P



DRP1 D/N Mitochondria Are Hyperfused and Do Not Readily Depolarize

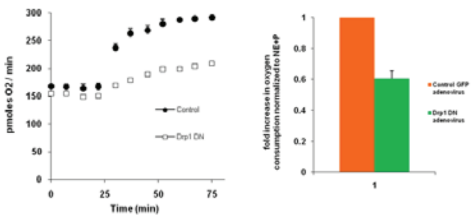


Mitochondrial morphology of Control and Drp1 DN cells. Cells stained with TMRE/MTG. Note the normal mitochondria in control and the hyperfused mitochondria in Drp1 DN cells.



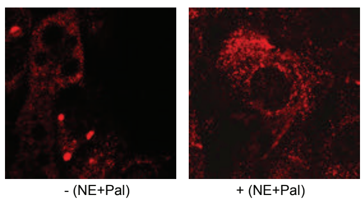
Fraction of Drp1 DN cells that depolarize in response to NE (1μM) and palmitate (0.4mM). N=19 (control), 22 (Drp1 DN). Control cells were infected with equivalent amounts of mtPAGFP adenovirus. ** p<0.0001.

DRP1 D/N Mitochondria Exhibit Lower Levels of Respiration



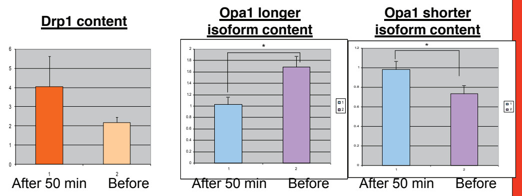
Drp1 DN cells show a lower level of oxygen consumption in response to NE (1μM) and palmitate (0.4mM). Control cells were infected with mtPAGFP adenovirus. Data is normalized to basal rates.

Increase in DRP1 with NE (1μM) and Palmitate (0.4mM)

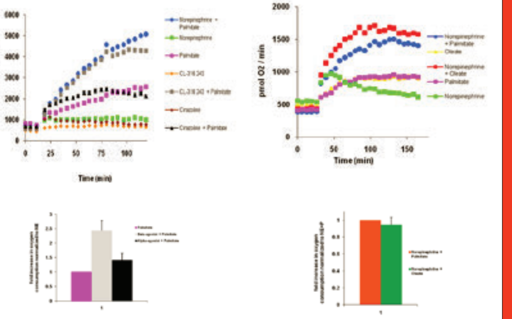


Immunostaining for DRP1 (red color).

BAT Drp1 and Opa1 contents change when activated with NE+P



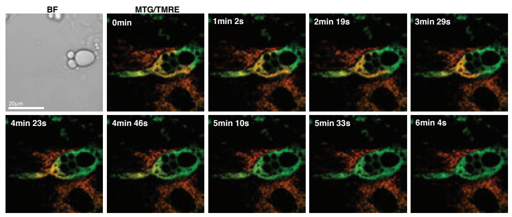
NE activates respiration, depolarization and fragmentation through β-adrenergic receptors that synergize with either palmitate or oleate



Synergistic response through β-adrenergic pathway. Measurements of basal rates were followed by measurements under stimuli as indicated, including CL-316243 (selective β3 agonist) and cirazoline (selective α1 agonist). Note that only CL-316243 and palmitate (0.4mM) lead to a synergistic response similar to NE (1μM) and palmitate. Data is normalized to basal rates.

A synergistic response is induced when NE is combined with either palmitate (0.4mM) or oleate (0.4mM). Basal rates of oxygen consumption was first measured followed by measurements under stimuli as indicated. Note that oleate (0.4mM) and NE (1μM) is similar to palmitate (0.4mM) and NE. Data is normalized to basal rates.

Depolarization Occurs as a Wave



$\Delta\psi m$ depolarization wave in control cell. Cell stained with TMRE/MTG and stimulated with NE (1μM) and palmitate (0.4mM). Time lapse imaging was started after the cell started to depolarize on one side. Mitochondria that depolarize loose TMRE staining and become green.

Conclusions

In this study we present novel data on mitochondrial structure and function in live BA by utilizing state of the art microscopy and oxygen consumption methods. Mitochondrial morphology was found to be highly networked and dependent on mitochondrial dynamics proteins. In addition, two mitochondrial subpopulations were discovered, peridroplet and cytoplasmic mitochondria, exhibiting differences in $\Delta\psi m$ and fusion capacity. When stimulating cells with a combination of NE and FFA we found a synergistic response that included a marked increase in oxygen consumption rates and $\Delta\psi m$ depolarization as well as a massive mitochondrial fragmentation. The fragmented mitochondria appeared sphere-like and had dampened fusion; however cells regained normal mitochondrial function and morphology within 24h. Interestingly, $\Delta\psi m$ depolarized and mitochondria fragmented in a wave-like fashion where depolarization preceded fragmentation. Furthermore, inhibition of the pro-fission protein Drp1 was found to inhibit BA function. We also found the synergistic response to go through the β-adrenergic pathway. Together, these data reveals that mitochondrial fission is essential for the activation of uncoupled respiration in brown adipocytes and its transition from bioenergetic efficient to inefficient metabolism.

Seahorse Bioscience Travel Award Winner