VISUALIZING MITOCHONDRIAL NETWORK DYNAMICS WITH CONFOCAL AND STED MICROSCOPY

Sabine Daemen[1], Anne Gemmink[1], Helma J.H. Kuijpers[2], Gert Schaart[1], Patrick Schrauwen[1], Marc A.M.J. van Zandvoort[2,3], Matthijs K.C. Hesselink[1]

[1]Department of Human Biology and Movement Sciences, NUTRIM School for Nutrition and Translational Research in Metabolism, and [2]Department of Genetics & Cell Biology, division Molecular Cell Biology, Cardiovascular Research Institute Maastricht; Maastricht University Medical Centre+, UNS50, Maastricht, The Netherlands. [3]Institute for Molecular Cardiovascular Research IMCAR, Universitätsklinikum Aachen, Pauwelstrasse 30, Aachen, Germany.

Email: sabine.daemen@maastrichtuniversity.nl

Mitochondria play an essential role in cellular energy homeostasis and dysfunction of mitochondria has been implicated in the pathophysiology of obesity and related metabolic disorders. Although classically being viewed as individual organelles, mitochondria in cells with a wide range in metabolic activity (like muscle cells) nowadays are considered to undergo dynamic processes of fusion and fission to maintain a mitochondrial network or reticulum to deal with fluctuations in energy supply and demand. Mitochondrial network dynamics has emerged as a potentially important factor in mitochondrial function and energy homeostasis. Here we aimed to develop confocal and STED microscopy as tools to study mitochondrial network dynamics. Confocal imaging of mitochondrial outer membrane protein TOMM20 in primary human myotubes revealed a clear mitochondrial network within these cells. Moreover, using dual-color STED we were able to distinguish between the outer and inner membranes of mitochondria, when additionally staining for mitochondrial inner membrane OXPHOS proteins (Figure 1). We applied these techniques to study the lipotoxic effects of the saturated fatty-acid palmitate relative to the unsaturated fatty acid oleate on the mitochondrial network in primary human myotubes. We observed a clear disruption of the mitochondrial network upon palmitate incubation with confocal microscopy. STED microscopy allowed us to visualize the remaining mitochondrial fragments after palmitate incubation in more detail. In conclusion, both confocal and STED microscopy can be powerful tools to study mitochondrial network dynamics and its role in metabolic disease.

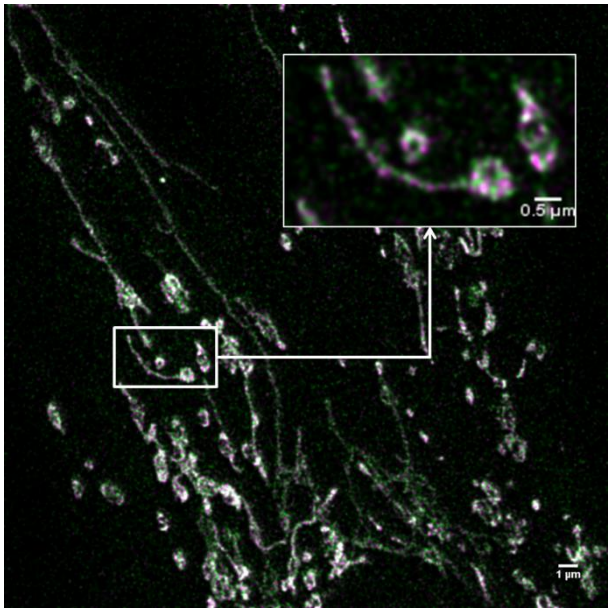

Figure 1: Dual color STED image of the mitochondrial network in a cultured human myotube stained for TOMM20 (green) for the outer mitochondrial membrane and OXPHOS proteins (magenta) for the inner mitochondrial membrane.