

## Title

Hypoxia-reoxygenation induces neutral lipid accumulation in cardiomyocytes

## Authors

Juliette Bréhat, Alexis Nguyen, Leeyah Issop, Nario Tomishige, Toshihide Kobayashi, Sandrine Pons, Bijan Ghaleh, Didier Morin

## Key words

Lipids, Hypoxia-Reoxygenation

**Introduction** Circulating lipids are well established cardiovascular risk factors but, independently from dyslipidemia, their direct physiopathological role on cardiomyocytes is poorly known. We previously demonstrated that reperfusion of an ischemic myocardium induced accumulation of both cholesterol and oxysterols in mitochondria. Pharmacological inhibition of this phenomenon with the use of 4'-chlorodiazepam protected mitochondrial function and reduced infarct size. Nevertheless, the distribution and the trafficking of lipids in cardiomyocytes, especially cholesterol, during cardiac ischemia-reperfusion is still ill known. **Objective** The goal of this study is to investigate the dynamic of lipids during hypoxia-reoxygenation in a cellular model. **Method** AC16 human cardiomyocytes were submitted to 14h hypoxia (1% O<sub>2</sub>) followed by 2h reoxygenation (21% O<sub>2</sub>). Mortality was assessed with crystal violet assay. Cytosolic lipid droplet formation and plasma membrane cholesterol were monitored by fluorescent microscopy using Bodipy and D4-mCherry probes, respectively. Whole cell neutral lipids were quantified by thin layer chromatography. **Results** 14h hypoxia followed by 2h reoxygenation induced 41±3% of cell mortality compared to normoxia, (n=5, p<0.05). Hypoxia-reoxygenation also induced lipid droplet formation (+2368±878% vs. normoxia, n=4, p<0.05) and increased cholesterol-rich domains in plasma membrane (258±29% vs. normoxia, n=5, p<0.05). These results were confirmed by thin layer chromatography, showing an increase in triglycerides, cholesterol esters and free fatty acid in hypoxia-reoxygenation compared to normoxia (+291±30%, +657±306% and +315±81%, respectively; n=5, p<0.05). **Conclusion** Hypoxia-reoxygenation induces cytosolic neutral lipid and plasma membrane cholesterol accumulation in cardiomyocytes. Whether this change of lipid distribution is responsible for mitochondrial cholesterol accumulation previously described at reperfusion needs to be determined.