# The Effects of Palmitate and Milrinone on Cardiomyocytes

Andrei Caymo[2,6], Philip Kawalec[2,5,6], Matthew Martens[1,2,3,6], Simone Da Silva Rosa[1,2,3,6], Joe Gordon[1,2,3,4,6]

[1]Department of Human Anatomy and Cell Science, [2]Children's Hospital Research Institute of Manitoba (CHRIM), [3]Rady Faculty of Health Sciences, [4]College of Nursing, [5]College of Medicine
[6]University of Manitoba, Winnipeg MB, Canada.

## Background

- Diabetes affected approximately 460 million people globally in 2019, with 90% of these cases being attributed to Type 2 Diabetes (T2D).
- T2D is a type of diabetes that results from insulin resistance in target tissues.
- Lipotoxicity is a form of cellular stress involving lipid metabolite accumulation and is implicated with the development of insulin resistance and mitochondrial dysfunction
- Lipid metabolite accumulation has also been implicated with the expression of certain cellular proteins in the cell
- Previous projects in our lab has demonstrated an increase in the cell death protein Nix during a high-fat diet (HFD) and that a Nix-dependent pathway was involved with mitochondrial dysfunction and subsequent cardiac dysfunction.
- Inhibition of PDE-3 can lead to Nix inactivation via phosphorylation by Protein Kinase A (PKA).
- Milrinone is a drug classified as a Phosphodiesterase-3 inhibitor (PDE3i) and is clinically used as an ionotropic agent used to treat patients with cardiac failure

**Hypothesis:** Milrinone can inhibit the effects of lipotoxicity on cardiomyocytes

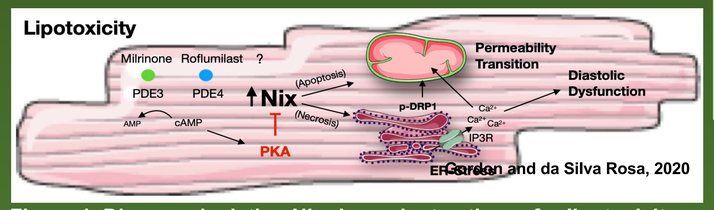


**Figure 1. Diagram depicting Nix-dependent pathway for lipotoxicity and relevance of Phosphodiesterase-3**

## Materials and Methods

**Models:**

- H9C2 rat cardiomyocytes were the cell lines used for all experiments

**Treatments:**

- *200 µM Palmitate* – treatment overnight to induce lipotoxicity
- *10 µM Milrinone* – treatment overnight to alleviate effects of palmitate

**Live Cell Imaging:**

- Following treatments, cells were stained with MitoTracker, LysoTracker, Calcien-AM, and Ethidium homodimer to evaluate the physiological activities in the cardiomyocyte.

## Results

### Mitochondrial Dysfunction is induced by Lipid Exposure

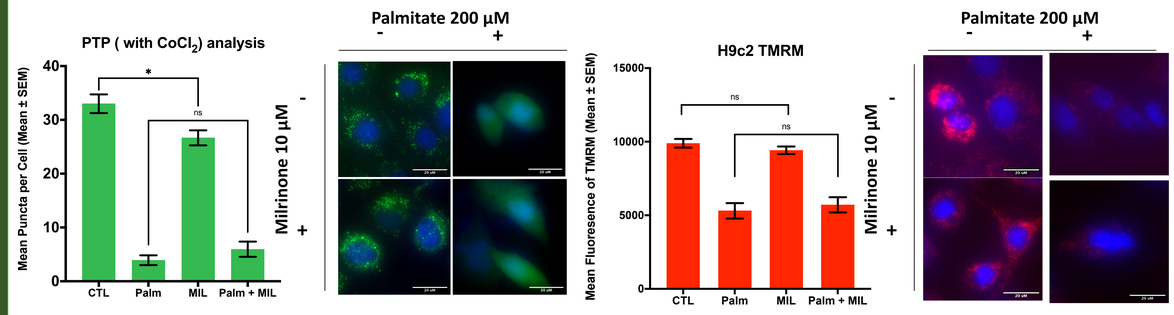


**Figure 2.** Lipid exposure on cardiomyocytes induces: (A) Permeability Transition Pore (PTP) opening; (B) Mitochondrial membrane depolarization. (*, p <0.05)

### Lipid Exposure influences Lysosomal Activity

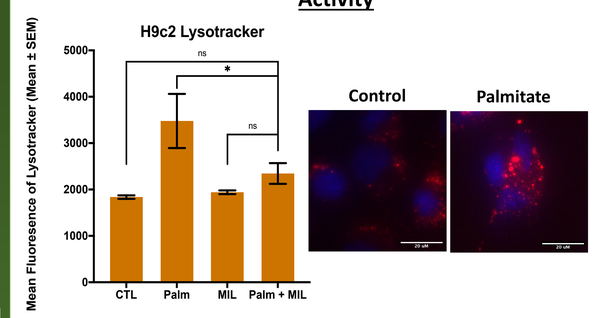


**Figure 3.** LysoTracker dye depicts greater lysosomal activity in lipid-exposed cells compared to controls. (*, p<0.05)

### Lipid Exposure is concurrent with changes in Mitochondrial Morphology

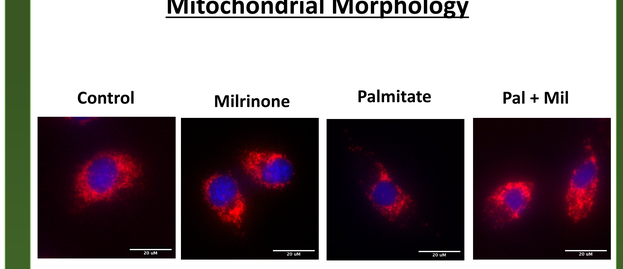


**Figure 4.** MitoTracker changes in mitochondrial morphology in lipid-exposed cells compared to controls.

### Palmitate exposure is concurrent with cell death while Milrinone treatments show minimal cell death

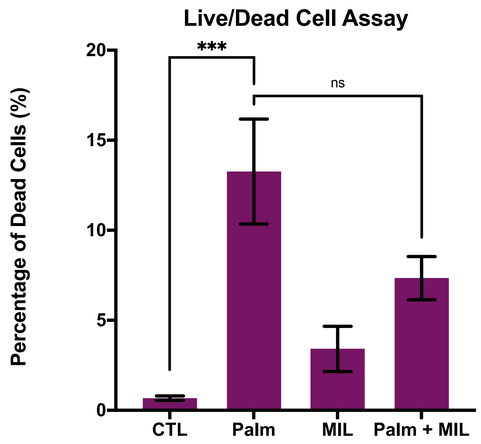


**Figure 5.** Lipid exposure results in significantly greater amounts of cell death compared to both control and milrinone treatments. (***, p< 0.001)

## Conclusions

- Lipid exposure is concurrent with mitochondrial dysfunction in the cardiomyocytes
- Minimal rescuing capacity was observed after a 24-hour exposure co-treatment of a lipid metabolite and milrinone
- Milrinone demonstrated minimal toxicity to the cardiomyocytes over a 24-hour exposure period at 10 µM
- Mitochondrial morphological change is observed in lipid-exposed cardiomyocytes

## Future Directions

- Western blot to evaluate Nix expression
- Evaluate the effects of a pre-treatment with milrinone followed by the addition palmitate
- Observe the time-dependent effects of Milrinone on cardiomyocytes
- Comparison of Roflumilast vs Milrinone effects
- Conduct similar experiments on primary rat or human cardiomyocytes
- Rhod-2 experiment to determine the presence of calcium release

## Acknowledgements