

Studies of the Interaction of Cardiac Lipid Droplets with Mitochondria Using Electron Microscopy

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In the United States and many developed countries, obesity is a rising health problem. Obesity is the result of an imbalance in lipid homeostasis and is evidenced by the excessive accumulation of cytoplasmic lipid droplets (CLDs) in cardiac muscles. The CLD is a dynamic organelle that functions in maintaining cellular lipid storage and metabolism [1]. It interacts with various cell organelles such as the endoplasmic reticulum, endosomes and mitochondria for lipid transport, storage and beta oxidation to generate energy. To date, only a few lipid droplet-associated proteins have been identified; among these, perilipin proteins are the most abundant. In cardiac myocytes, perilipin 5 has been shown to play a role in the recruitment of mitochondria to CLDs and is a negative regulator of lipolysis [2]. Our ultimate goal is to understand how CLDs in cardiac muscle cooperate, spatially and metabolically with mitochondria and the role of perilipin 5 in the dynamic interactions between these two organelles. Due to the small size of CLDs, electron microscopy (EM) techniques are required to characterize their physical and molecular interactions with mitochondria. However, lipids are prone to be extracted during traditional EM and light microscopy sample processing steps. Using fluorescence microscopy, it has been shown previously that methanol and acetone fixation can induce lipid droplets fusion and alter the size and morphology of CLDs [3]. In the electron microscope, lipid droplets can be seen both as electron dense or electron opaque vacuoles in cells or tissues prepared by different fixation and staining methods. Here we summarize our findings on the effect of various fixation and staining methods on the morphology of CLDs in cardiac muscles.

Cardiac muscle from transgenic mice overexpressing perilipin 5 under the α -myosin heavy chain promoter were used in this study. For room temperature processing, muscles were vibrotomed or manually trimmed into thin slices and embedded in spurs epoxy resin or in uncryl by using the progressive lowering of temperature (PLT) method. For cryo-EM processing, muscle slices were high pressure frozen and subsequently freeze substituted and embedded in either uncryl or spurs resin.

Our results indicate that aldehyde fixation with subsequent osmium tetroxide post-staining is sufficient to provide good structure preservation and staining contrast for both mitochondria and CLDs. CLDs showed homogeneous electron dense staining pattern. Attempts to enhance membrane or protein staining by employing osmium-thiocarbohydrazide-osmium (OTO) staining or further en bloc uranyl or lead staining often resulted in lipid extraction (Figure 1 red arrowhead) or lipid droplet fusion (Figure 1, red arrow). Furthermore, immunolabeling of perilipin 5 was drastically reduced when the heart muscle specimen showed signs of lipid extraction. This suggests that perilipin 5 is closely associated with lipids within the CLDs. Further experiments are in progress to characterize the cause of the CLD extraction and fusions.

References:

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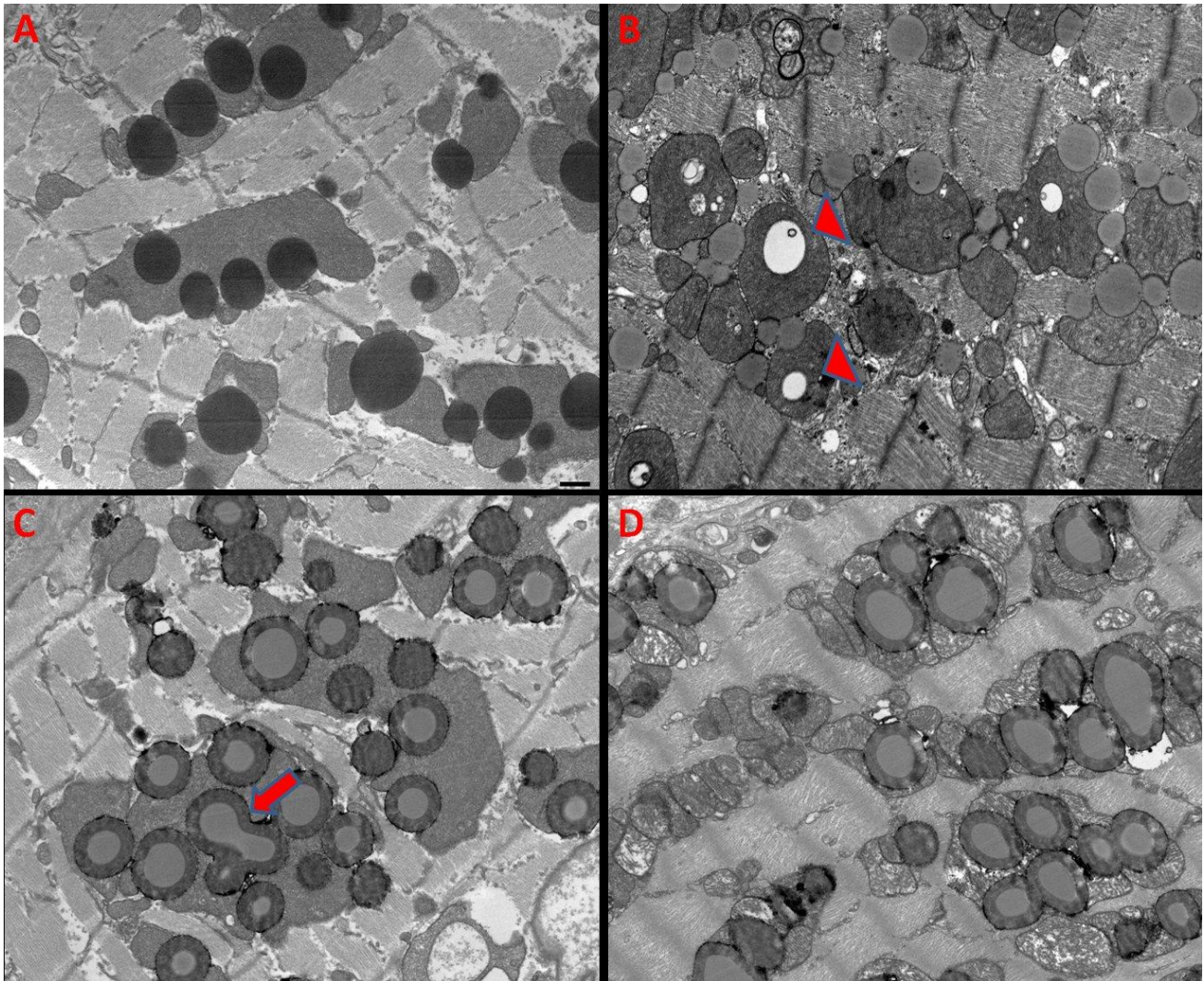


Figure 1. Comparison of CLD morphology in cardiac muscle processed using different staining method. Cardiac muscle specimen were fixed in buffered aldehyde fixative and post stained with 1% osmium tetroxide and 0.25% potassium ferrocyanide (A), 1% osmium tetroxide and 1% uranyl acetate en bloc staining (B), osmium-thiocarbohydrazide-osmium (C) and osmium-thiocarbohydrazide-osmium and 1% uranyl acetate staining (D). Bar = 500nm