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Imidazole-osmium reduces elution of lipids from cryofixed rat hepatic tissue for ultrastructural analysis

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Introduction

Transmission electron microscopy (**TEM**) is the main tool for studying the ultrastructural properties of metabolically diseased tissue. A semi-correlative approach using TEM and **NanoSIMS** (Nanoscale Secondary Ion Mass Spectrometry) enables localization of stable isotope labelled tracers within cellular compartments.

Depicting undamaged lipid droplets is an important factor for subcellular investigation of **fatty liver disease** in a rat model. **Imidazole**, a highly polar heterocyclic compound, is hypothesized to enhance the binding ability of osmium tetroxide [1]. This study aimed to investigate the effect of imidazole-osmium application during cryopreparation to prevent elution from lipid droplets.

Methods

Perfusion-fixed (4.5 % phosphate buffered formaldehyde, pH 7) **hepatic tissue** from male Sprague-Dawley rats (n=6; 8 weeks old) on high fat diet (60% of calories as fat; fed for 4 weeks) was processed for TEM.

Imidazole was applied during sample preparation in the following ways:

1) Osmification (1% OsO_4 in 0.1M imidazole) of rat hepatic tissue before cryopreparation* (**Fig. 1B**).
2) Additional inclusion of imidazole (1% OsO_4 and 0.1M imidazole) in the freeze substitution medium acetone (**Fig. 1C**).

*High-pressure freezing (HPM100, LEICA Microsystems) and freeze substitution (AFS2, LEICA Microsystems) with sample agitation [2].

Results and Conclusion

In contrast to approaches without the compound (**Fig. 1A**), imidazole-mediated preparation techniques resulted in electron dense content of lipid droplets (**Fig. 1B, Fig. 1C**). We suggest that osmium-imidazole prevented the elution of lipid content during the warm-up phase of the freeze substitution protocol. This procedure promises a major advantage in the ultrastructural study of fat accumulation in hepatic tissue. Further investigations, including NanoSIMS analysis, will be conducted to confirm this hypothesis.

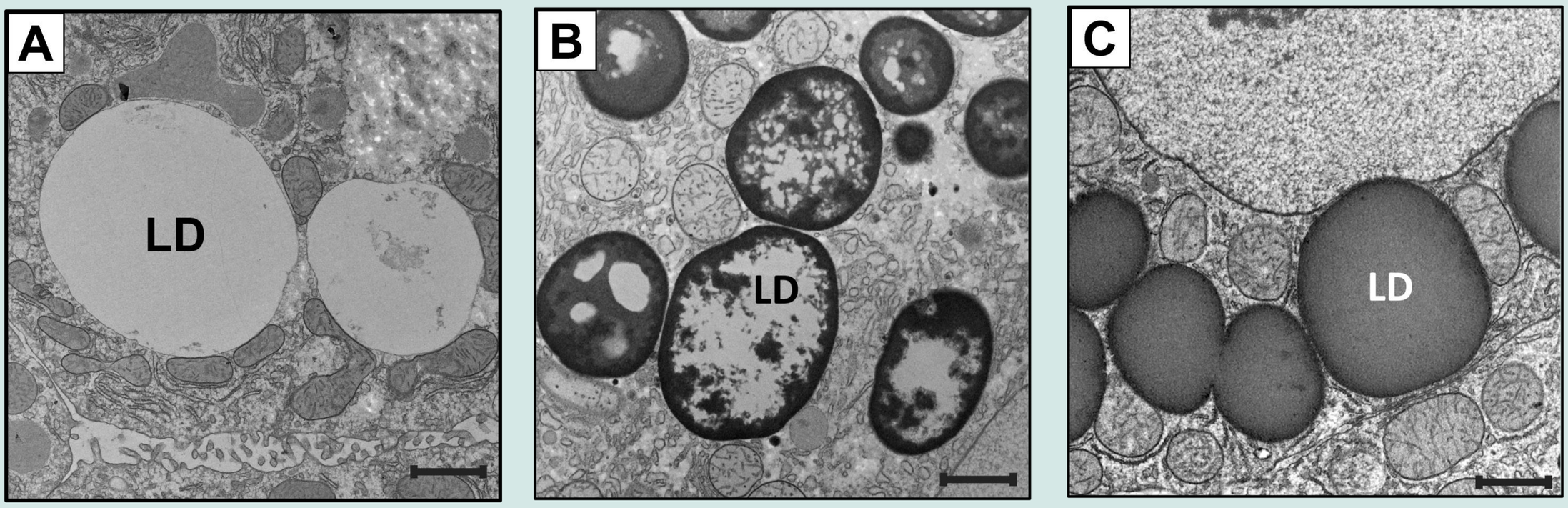

Figure 1. Steatotic rat hepatic tissue. A) Lipid droplets (LD) in tissue without imidazole treatment. Lipid content is apparently missing. **B)** Imidazole mediated osmification before conventional freeze substitution exhibiting heterogeneous, holey content. **C)** Entirely homogeneous lipid droplets after osmium-imidazole mediated freeze substitution. Scale bars, 1 μm.

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References

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