Lipid Dynamics in mosquito ovaries using Mass Spectrometry Imaging

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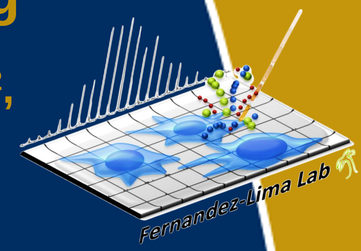

Overview

The mobilization of nutrient reserves within the mosquito *Aedes aegypti* ovaries during previtellogenic development plays a vital role in female's reproductive maturation. In the present study, a mass spectrometry tools are applied to probe the lipid composition and distribution within the ovarian follicle as a function of diet-induced phenotypes.

Introduction

New approaches are needed to control the vectors of mosquito-borne diseases that constitute critical threats to public health in many parts of the world. Understanding the molecular and biochemical basis of ovary development is a central aspect in mosquito reproductive biology. [1,2] In particular, lipid metabolism based on carbohydrate feeding is very important for mosquito female survival and reproductive success [3]. Here, Time-of-Flight Secondary Ion Mass Spectrometry Mass Spectrometry (TOF-SIMS) an ideal tool for the imaging of lipids in small samples, [4] is to better understand lipid mobilization and metabolism during diet-induced phenotypic changes. Complementary mass spectrometry techniques and fluorescence labeling are used to confirm molecular assignments and verify sample integrity.

Experimental Methods

- *Aedes aegypti* of the Rockefeller strain are lab grown and raised on either a sugar or water diet
- On the fourth day, insects are dissected and their ovaries are harvested and subjected to TOF-SIMS imaging, MALDI FT-ICR MS, MALDI-TOF, and LC-MS/MS analysis
- To verify morphology for imaging, ovaries are DAPI stained
- Imaging samples are freeze-dried in 10mM ammonium acetate

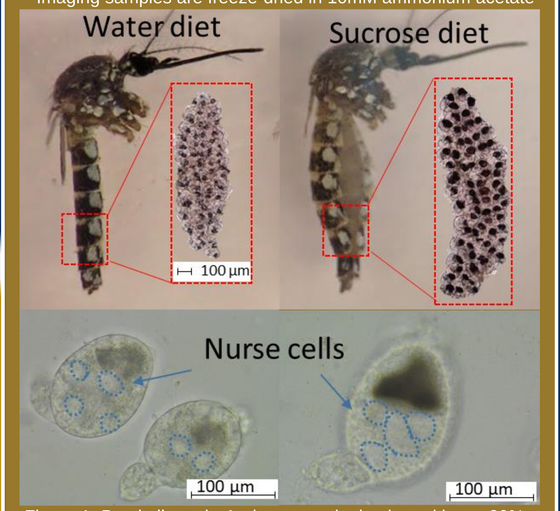

Figure 1. Previtellogenic *Aedes aegypti* raised on either a 20% sucrose solution or water diet and dissected at four days.

TOF-SIMS Imaging

- ION-TOF TOF-SIMS V
  - 25keV Bi_3^+ primary ion beam
  - 20keV Ar_{1500}^+ sputter beam

$$Y_{SI} = \frac{Secondary\ Ion\ count(Area)}{Region\ of\ Interest(cm)^2 \times Primary\ Ion\ fluence\ \left({}^{primary\ ions}/_{cm^2}\right)}$$

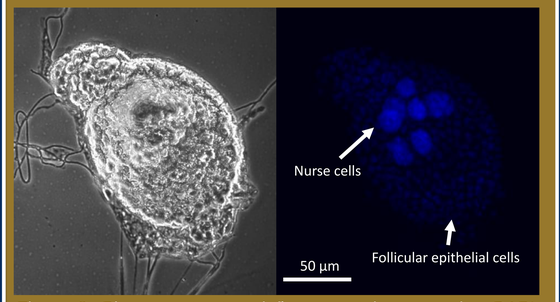

Figure 2. Phase contrast and fluorescent images using DAPI stained follicle prior to Ar_{1500}^+ sputtering. The ~12 µm fluorescent structures are nurse cells, which assist in the development of the oocyte. Smaller epithelial cells which form the membrane of the follicle are also visualized with DAPI.

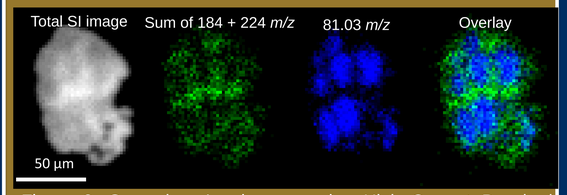

Figure 3. Secondary Ion images using High Current Bunched mode (HCBU) in positive polarity of a 20% fed follicle.

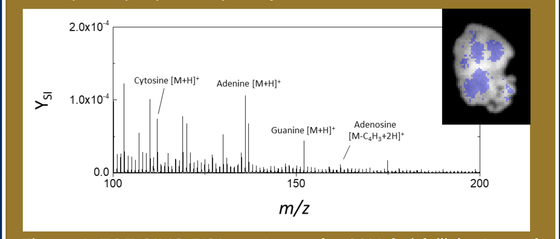

Figure 4. TOF-SIMS ROI spectrum of a 20% fed follicle. Ions of cytosine, guanine, adenine, and adenosine are consistent with the biological role of the nurse cells, which is to synthesize RNA.

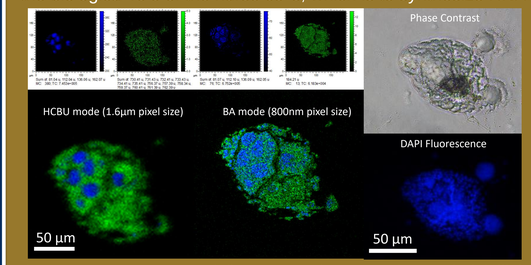

Figure 5. TOF-SIMS and fluorescent images of 20% fed follicles. Notice the distribution of the nurse cells.

SIMS vs MALDI Results

Abbreviations: PC Phosphocholine; PE Phosphoethanolamine; PE-Cer PE Ceramide; PS; Phosphoserine; PI Phosphoinositol; TAG Triacylglycerol

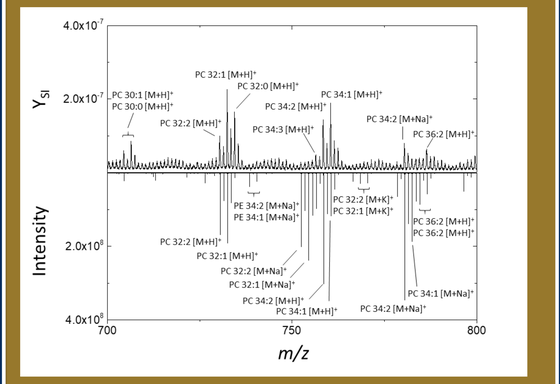

Figure 6. Positive polarity analysis using TOF-SIMS (top) and MALDI FT-ICR MS (bottom) of a 20% fed follicle. All assignments are confirmed by MS^2.

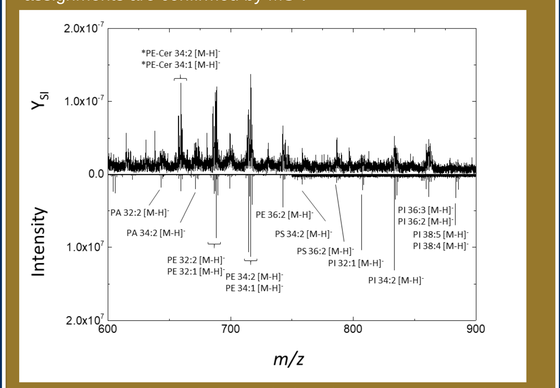

Figure 7. Negative polarity analysis using TOF-SIMS (top) and MALDI FT-ICR MS (bottom) of a 20% fed follicle. Those denoted with an * are not confirmed by MS^2.

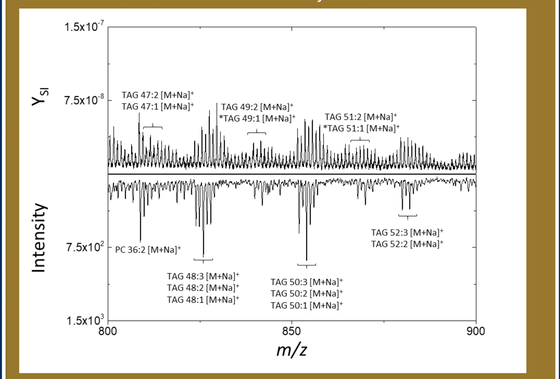

Figure 8. Positive polarity analysis using TOF-SIMS (top) and MALDI-TOFMS (bottom) of a 20% fed follicle. Those denoted with an * are not confirmed by MS^2.

Sugar vs water diet

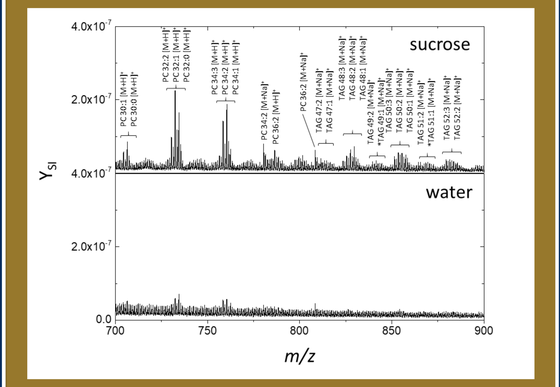

Figure 9. Positive polarity TOF-SIMS analysis comparison between a 20& fed (sucrose) and water fed insect follicle. Lipid reserves are depleted in as a result of starvation.

Conclusions

Mass Spectrometry is demonstrated to be the tool of choice for identifying and localizing lipid species in a challenging ~100 µm biological sample. Under the experimental conditions of these diet-induced phenotypes, phospholipids and neutral lipids are relatively depleted in sugar-deprived *Aedes aegypti* ovarian follicles. Phospholipid species are generally distributed in the membrane of the follicle, while neutral lipids are stored in the oocyte. Secondary ions related to ribose and nucleotide bases [5] are observed to be localized to nurse cells within the follicle, consistent with their biological function.

While the fold changes observed here are qualitative at the present, they serve as a proof of concept to demonstrate that the methods used are sensitive to changes in the biology of the developing insect.

References

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Acknowledgments

This research was supported by the NSF grant HRD-1547798 through FIUs Centers of Research Excellence in Science and Technology (CREST) program by way of fellowship, and by National Institute of Health AI135469 to FFL and AI045545 to FGN