A127 Using High Resolution Mass Spectrometry Analysis to Investigate Trabecular Bone Metabolomics for Postmortem Interval (PMI) Estimation

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Learning Overview: After attending this presentation, attendees will better understand the potential of quantifying lipid breakdown in trabecular bone for PMI for long periods of time following soft tissue decomposition (years to decades).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing the preliminary results of lipidomic analysis of bone biopsy samples from 115 individuals with postmortem intervals that range from less than 1 year to 30 years.

This study expands on previous research that established quantitative approaches for examining skeletal muscle and trabecular bone metabolites using high resolution mass spectrometry to examine long-term preservation lipid candidates in bone. As lipids have shown to be preserved in bone marrow for long-term intervals, from several months to several decades postmortem, further quantification of lipid degradation in bone marrow will aid in building and validating regression equations using metabolite ratios to estimate the postmortem interval of skeletonized remains, which is notoriously difficult for forensic practitioners.[1-3]

Bone biopsy samples of fresh and skeletal donors with varying postmortem intervals (<1 year–30 years) were subjected to high resolution mass spectrometry to identify preserved lipid biomolecules in bone, which, upon further analysis, will be used to test their capacity to accurately predict long-term postmortem intervals (e.g., years or decades) from skeletal remains.

Twenty fresh human donors were placed at the University of Tennessee Anthropological Research Facility in two cohorts. Ten fresh donors were placed in late January 2018, and an additional ten fresh donors were placed in mid July 2018. Ninety bone biopsy samples have so far been obtained from this experimental cohort to track any significant changes in lipid content that may be correlated with soft tissue decomposition. These donors will continue to decompose, and bone biopsy samples will be collected every six months through the end of 2019. Bone biopsy samples were additionally taken from a cross-sectional sample represented by dry skeletal material of 115 individuals curated at the William M. Bass Donated Skeletal Collection, with postmortem interval ranges of 1 to 30 years.

A total of 435 bone biopsies were taken from 135 individuals from three skeletal sites with high trabecular bone content (calcaneus, proximal tibia, and vertebral body) from the combination of experimental and cross-sectional samples (fresh and skeletal, respectively). Of the 435 bone biopsy samples taken from 135 individuals (this *n* refers to the fresh and skeletal donors), bone biopsy material from 99 donors have undergone an organic extraction process and have been subjected to mass spectrometry analysis. Relative intensities (ratio of lipid classes identified in samples compared to internal standards) have been recorded and banked in a Microsoft® Access® database.

In a previously reported pilot study, the diversity of a class of lipid mediators and bone metabolism regulators, N-acyl amino acids, were investigated in a dry human calcaneus with a PMI of approximately seven years.[4] Utilizing a high-resolution electrospray ionization lipidomics analytical platform, 76 potential N-acyl amino acids were identified in the seven-year PMI sample, providing the impetus for a large-scale study. The structural identities of palmitoyl and oleoyl serine were validated via generation of the MS^2 product ion for serine (<1ppm mass error), and 20 additional lipid class candidates are currently being validated using Tandem Mass Spectrometry (MS/MS) using the 435 bone biopsy samples from 115 skeletal donors. Upon validation, the ratio of lipid intensity compared to internal lipid standards allows for standardization and interpretation of degradation of lipids and associated metabolites over time.

This study expands the previous research on skeletal muscle metabolites to bone[5], and provides a destructive, yet minimally invasive, bone biopsy sampling technique that will ultimately be used to build a validated method for estimating PMI of skeletonized remains based on lipid degradation in bone over significant time periods following soft tissue breakdown.[5]

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Postmortem Interval, Metabolomics, Mass Spectrometry

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