A61 Using Stable Nitrogen Isotope Ratios From Human Muscle Tissue for Postmortem Interval (PMI) Estimation

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Learning Overview: After attending this presentation, attendees will understand how changes in stable nitrogen isotope ratios ($\delta^{15}N$) of decomposing human muscle tissue may aid in the estimation of the PMI.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a novel application of stable isotope analysis of $\delta^{15}N$ ratios of decomposing human muscle tissue to use as a useful alternative to traditional PMI estimation techniques. Furthermore, this study explores the use of $\delta^{15}N$ ratios of maggots feeding on decomposing muscle tissue to assess if a systematic trophic level enrichment can be used for a PMI estimate after identifiable muscle tissue is no longer present.

To test how $\delta^{15}N$ ratios can be used as a PMI estimate, it is key to understand two basic principles: 91) what a $\delta^{15}N$ is measuring; and (2) what is happening to nitrogen during muscle tissue decomposition. Nitrogen has two stable isotopes with different masses, a heavy (^{15}N) and a light (^{14}N) isotope. In a chemical reaction, the isotopes with different masses will react at different speeds, resulting in a preferential selection of one isotope (^{14}N) compared to the other (^{15}N). During decomposition of muscle tissue (i.e., putrefaction), the breakdown of amino acids produces a variety of volatile compounds, including ammonia (NH_3) and lesser amounts of two other nitrogenous gases, cadaverine ($NH_2(CH_2)_5NH_2$) and putrescine ($NH_2(CH_2)_4NH_2$). As putrefaction progresses, the chemical reactions to produce the nitrogenous gases would preferentially incorporate the light isotope (^{14}N) because it will react faster in a chemical reaction, resulting in an enrichment of the heavy isotope (^{15}N) in the remaining muscle tissue. The impact to $\delta^{15}N$ ratios as putrefaction progresses would be higher values compared to the $\delta^{15}N$ ratios of the fresh muscle tissue.

This research was conducted at the Anthropology Research Facility in Knoxville, TN, an outdoor laboratory for the study of human decomposition. A total of 20 donors were enrolled in the study, and muscle and associated maggot samples were collected for as long as each were present on a donor. Donors were placed on the surface in two trials with ten donors each in the winter and summer season to test differential seasonality effects to the method as a PMI estimator. The $\delta^{15}N$ ratios from the muscle and maggot samples were analyzed. The raw $\delta^{15}N$ ratios are not of interest because each donor has a different baseline value. To normalize the assessment of change in $\delta^{15}N$ ratios, the $\Delta^{15}N$ was calculated and compared ($\Delta^{15}N = \delta^{15}N_{Day\ X} - \delta^{15}N_{Day\ 1}$, with Day 1 as the initial fresh tissue sample).

Initial results indicate that alteration of $\delta^{15}N$ ratios in decomposing muscle tissue can be used for PMI estimates when identifiable muscle tissue is still present. The general pattern follows expectations that $\Delta^{15}N$ values increase as putrefaction progresses, with as much as a 3.0‰ increase in muscle tissue values. The winter trial donors had identifiable muscle tissue for up to three months, while the summer trial donors had muscle tissue for only two weeks. When the winter trial $\Delta^{15}N$ values are grouped in 20-day increments, there is no significant difference between the initial Day 1 sample and the Day 2–20 group (p=0.258) or the Day 21-40 group (p=0.197). However, past Day 40, there is a significant difference between the initial Day 1 sample and the Day 41–60 group (p=0.003) or the Day 61–80 group (p=0.000). The associated maggot $\Delta^{15}N$ values will eventually be combined with the muscle data to assess the usefulness of using live maggots found associated with a body at a crime scene to estimate PMI after identifiable muscle tissue is no longer available.

This study revealed that change in the $\delta^{15}N$ ratios of decomposing muscle tissue can be used as an alternative method for PMI estimation, especially in the winter months when identifiable muscle tissue can be retained on a body for months. This East Tennessee pilot study indicates that the significant influence to when $\Delta^{15}N$ values start to increase is associated with a shift to increasing temperatures when putrefaction progresses faster. This initial study indicates there is value in the method as a PMI estimate for recently deceased individuals, but further work is needed in other environmental contexts.

Stable Nitrogen Isotope Ratios, Postmortem Interval Estimation, Muscle Tissue Decomposition