Abstract

Skeletal muscles not only perform mechanical functions but also play an important role in regulating numerous metabolic processes. In various muscular disorders such as dystrophies and myopathies, alterations are observed in both the molecular and elemental composition of muscle tissue. This study aimed to describe these changes occurring in muscle tissue using human material - i.e. patients examined for the diagnosis of neuromuscular disorders. Material was obtained by surgical biopsy, originally used for neuropathological diagnostics and subsequently used for this research with the use of several complementary experimental methods.

The Micro Particle Induced X-ray Emission μPIXE was used, along with imaging aided by Scanning Transmission Ion Microscopy STIM, Secondary Ion Mass Spectrometry SIMS, and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry MALDI-TOF. Additionally, for the MALDI method, a fragmentation using the Laser-Induced Fragmentation Technology LIFT method was performed.

The μPIXE technique, supported by STIM imaging, enabled the quantitative determination of the concentration and distribution of selected elements (including Ca, K, Fe, and Zn) while preserving the morphological context of the tissue. MeV-SIMS provided high-resolution spatial information on the molecular composition, especially lipids, small-molecular metabolites and ions. The MALDI-TOF analysis allowed for the identification of specific lipid molecules mostly from the group of phosphatidylcholines (PC) including their adduct forms ($M+H^+$, $M+Na^+$, $M+K^+$) and their fragmentation patterns for structural identification.

The results showed that all discriminative peaks between the study groups corresponded to PC species. These molecules varied in acyl chain length, number of unsaturated bonds, and the ionization type. Such differences may influence the physical and chemical properties of biological membranes, including membrane fluidity, ion-binding capacity, and the function of membrane-associated proteins. In muscle tissue, these features are particularly relevant to calcium handling, which is essential for proper contraction and relaxation of muscle fibers. Changes in lipid composition were also found to be associated with alterations in elemental distribution, particularly of calcium, potentially indicating impaired sarcoplasmic reticulum function. This finding is especially relevant in the context of muscular conditions, where decreased ATP-ase activity and reduced calcium-binding capacity are frequently reported. The performed analysis allowed for a better understanding of these dysfunctions thanks to the combination of molecular and elemental analyses, which also suggested possible markers of muscle functional status.

The data obtained suggest that phosphatidylcholine may serve not only a structural role in membranes but also a regulatory one, affecting cellular metabolism, signaling, and tissue adaptation in pathological states. The presented approach may facilitate future studies on the characterization of

pathological changes in skeletal muscle by integrating findings from different analytical methods. While the identified molecular and elemental differences require further validation in larger and clinically diverse study groups, they may provide a foundation for future biomarker discovery. The results also offer a valuable starting point for translational research aiming to link alterations in lipid composition and elemental distribution with practical diagnostic or therapeutic strategies applicable in a clinical setting.

This study indicates that the use of advanced microscopic, spectrometric and spectroscopic methods may be a significant step in analyzing complex pathomechanisms such as those occurring in neuromuscular disorders.

Patrycja Śliż-Szptyma
12.11.2025