Strategies in ToF-SIMS Analysis of Liver Tissue – Critical Impact on Comparative Molecular Profiling in a Diabetic Rat Model

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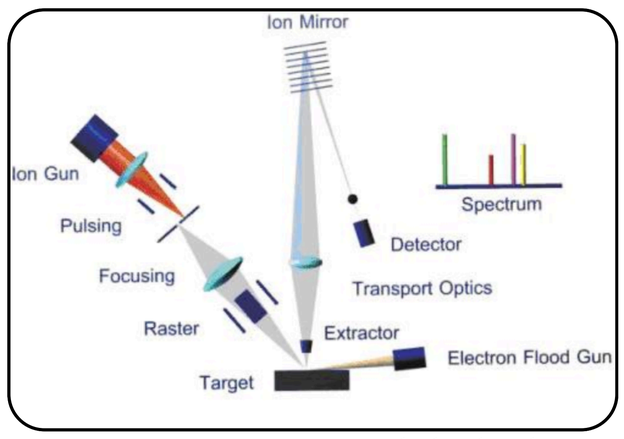
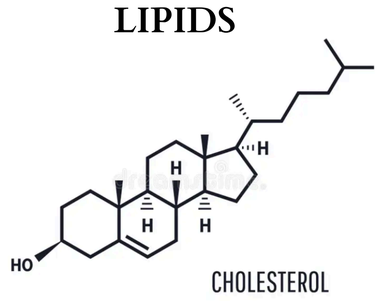
Introduction

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a high-resolution molecular imaging technique that reveals chemical composition of biological tissues. Here, we applied ToF-SIMS to liver samples from diabetic rats treated with metformin and flaxseed mucilage to assess how different data-normalization strategies influence spectral outcomes. Accurate normalization is essential for meaningful interpretation as it minimizes instrumental and matrix effects while enhancing sensitivity to subtle biological changes.

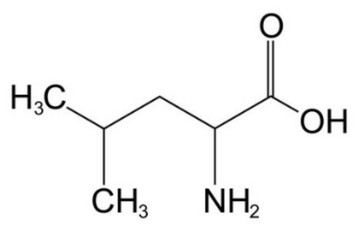


LIPIDS



CHOLESTEROL

AMINO ACIDS



LEUCINE

Objective

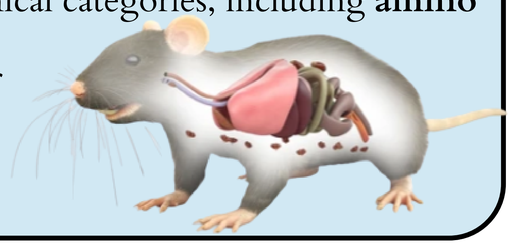
The aim of the research is to **determine the role of normalization strategies** in improving the reliability of tissue profiling results obtained with the ToF-SIMS method and their application in comparative pharmacological studies.

Methodology

- Study groups were divided into:

Group	Description
Lean	Lean control
P1	Diabetic control
P2	Treated metamorfin
P3	Treated lower dose of linseed slime
P4	Treated higher dose of linseed slime

- Signal intensity was calculated from average values, using three normalizations: **Raw Data ,Total Ion Counts (TIC) and Total Ion Dose.**
- Detected ions were grouped into two, major chemical categories, including **amino acids and lipids.**
- Statistical analysis included the percentage share of each group, as well as the calculations of **standard deviation and standard error.**



Results

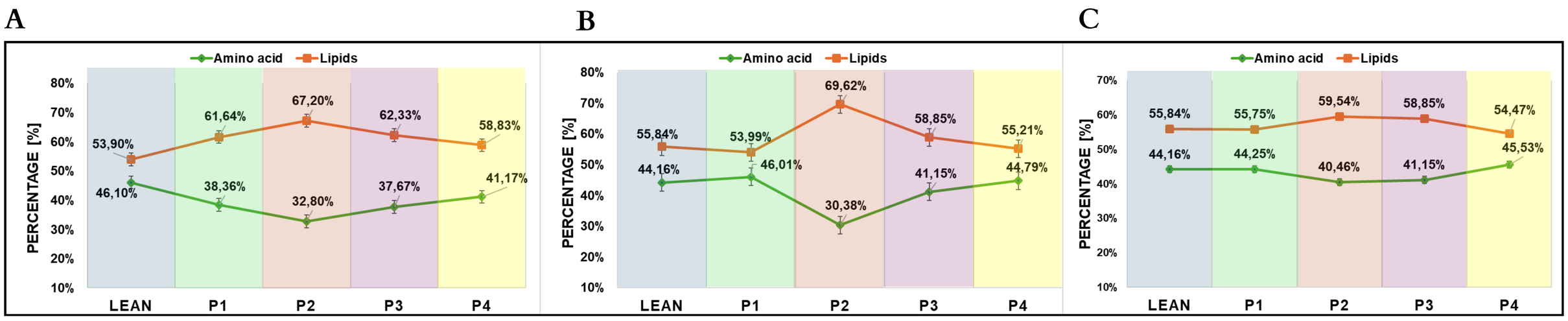


Fig.1. Percentage of amino acids and lipids for negative ions A) Raw Data, after B) Total Ion Counts (TIC), C) Total Ion Dose normalizations.

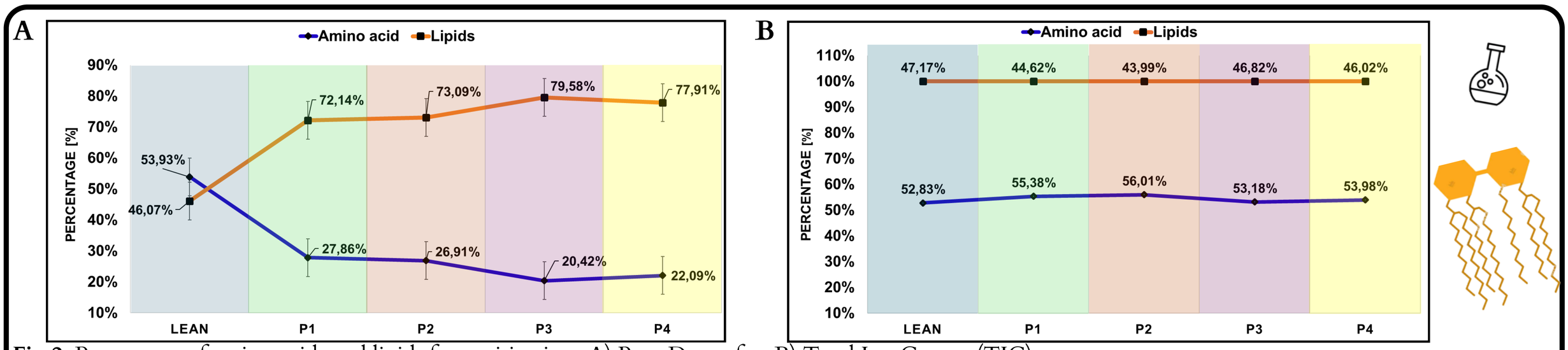


Fig.2. Percentage of amino acids and lipids for positive ions A) Raw Data, after B) Total Ion Counts (TIC).

Conclusion

Does Normalization Really matter?

Yes, normalization strategy strongly influences biological interpretation.

- Raw Data reflect native compound proportions.
- Total Ion Count (TIC) normalization **improves** comparability but can obscure subtle metabolic changes.
- Primary Ion dose normalization **preserves** spatial trends while reducing group discrimination.

Take home message - careful selection of normalization is essential for reliable ToF-SIMS profiling in pharmacological and metabolic studies.