

Untargeted metabolomics of murine wound samples



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Introduction

- A distorted skin microbiome (dysbiosis) may have a significant impact on wound healing.
- It is conceivable that there are differences in the wound microbiome of obese and healthy mice, influencing the wound healing process.
- This study aims to investigate whether significant differences of the metabolomic profile are present between these two groups.
- The identification of wound-associated metabolites may support the understanding of relevant biological pathways and contribute to a better understanding of wound healing mechanisms.

Methodology

- Untargeted GC-MS metabolomics was performed following the workflow described by Oliver Fiehn for mammalian sample preparation, quality control, and data processing [1]. In total, 22 murine wound samples from healthy (n = 14) and high-fat diet induced obese (n = 8) male and female mice were analyzed, including germ-free mice and short-term colonized mice (defined as one generation carrying the microbiota). The same mouse model has already been used in a previous study [2].
- For metabolite extraction, 10 mg wound tissue was homogenized using stainless steel beads and extracted with cooled acetonitrile/isopropanol/water (3:3:2, v/v/v).
- Following centrifugation and vacuum drying, samples were derivatized in a two-step procedure using MeOx in pyridine followed by a FAMES/MSTFA mixture.
- GC-MS analysis was performed using the ecTOF platform (Bruker Daltonics, Bremen, Germany), combining electron ionization (EI) and chemical ionization (CI) in a single analytical run. Chromatographic separation was achieved within a 25 min GC method. EI spectra were acquired at 70 eV, while ammonia was used as reagent gas for CI.
- EI data were processed using MS-DIAL and statistically evaluated in MetaboAnalyst. CI data were analyzed in MetaboScape (Bruker Daltonics) and used for complementary metabolite annotation.



1.) Extraction
2.) Evaporation
3.) Derivatization

Conflict of Interest

Sonja Klee and Steffen Bräkling are employees of Tofwerk AG. Heiko Neuweger, Ilmari Krebs, Nikolas Kessler and Mathew Lewis are employees of Bruker AG. Tofwerk AG/Bruker Daltonics provided access to the ecTOF GC/MS setup and the MetaboScape software. This study was conducted independently and the results presented reflect the authors' objective assessment.

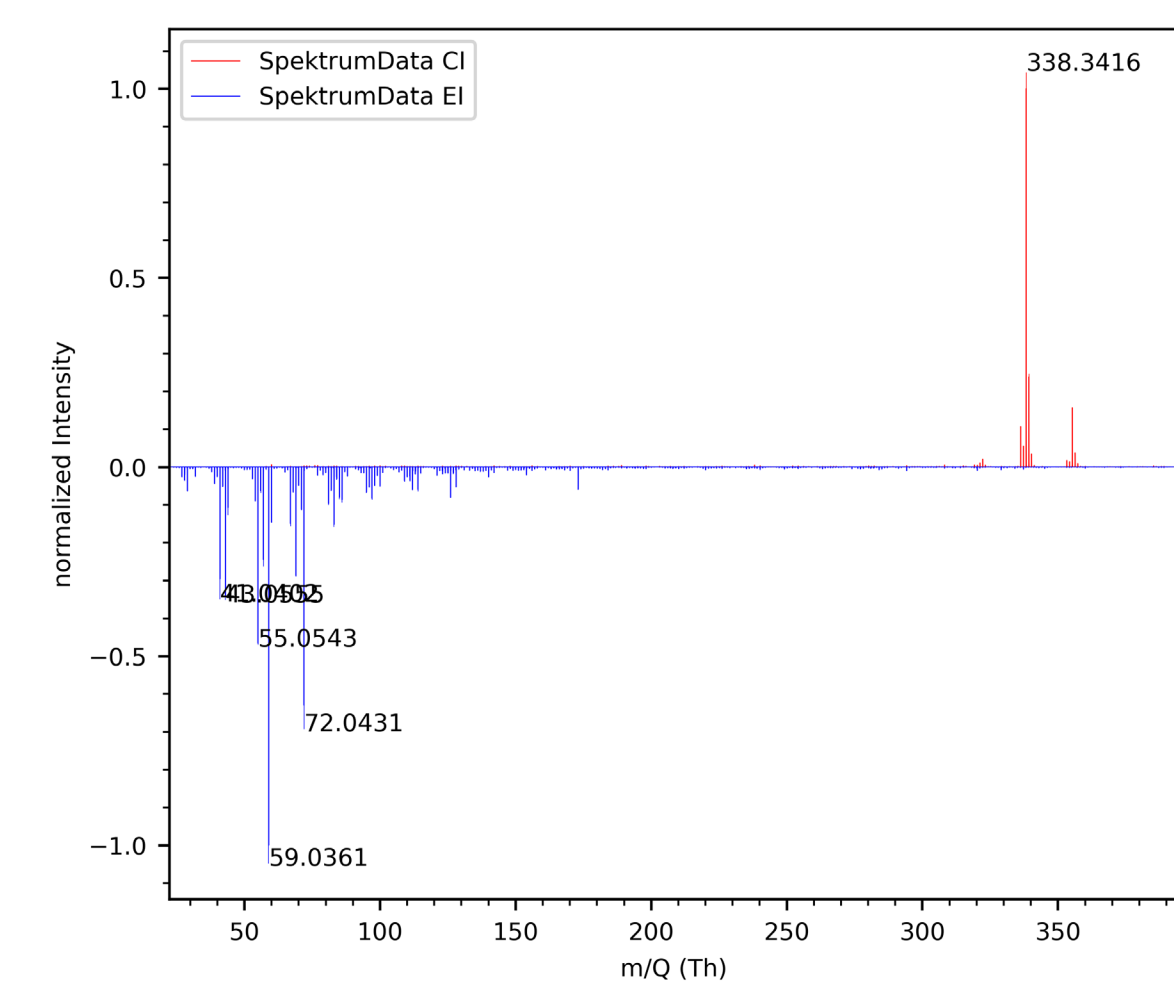


Fig. 1: Aligned CI and EI spectra of the putatively annotated feature 13-docosenamide, (Z)-



[1]Fiehn, O. Metabolomics by Gas Chromatography-Mass Spectrometry: Combined Targeted and Untargeted Profiling. *Current Protocols in Molecular Biology* **114**, 30.4.1–30.4.32 (2016).
[2]Scalise, M.C., Simon, M., Bernhardt, J., et al. Defined Microbiota Modulates Host Metabolome and Skeletal Adaptation to Diet-Induced Obesity. *The FASEB Journal* **40**, e71531. (2026).
[3]Brezeanu, D., Brezeanu, A.-M., Chirila, S., Tica, V. The Role of Lactic Acid in Episiotomy Wound Healing: A Systematic Review. *Healthcare* **13**, **956** (2025).
[4]Hamberger, A., Stenhagen, G. Eruamide as a Modulator of Water Balance: New Function of a Fatty Acid Amide. *Neurochemical Research* **28**, 177–185 (2003).
[5]Molnar, N.M. Eruamide. *Journal of the American Oil Chemists' Society* **51**, 84–87 (1974).
[6]Watson, J., Greenough, E.B., Leet, J.E., et al. Extraction, Identification, and Functional Characterization of a Bioactive Substance from Automated Compound-Handling Plastic Tips. *Journal of Biomolecular Screening* **14**, 566–572 (2009).

Results

- Volcano plot analysis of the CI data comparing wound samples from healthy and obese mice possessing a microbiome revealed five significantly ($\log_2FC > 1$, $p_{raw} < 0.05$) altered features, with four elevated in the healthy control group and one increased in obese mice wound samples (Fig. 2).
- Database search yielded limited annotation confidence; however, one feature with m/z 338.342 (RT 14.66 min) was putatively identified as 13-docosenamide, (Z)- and supported by matching EI spectra (spectrum in Fig. 1).
- These differences were not observed in germ-free mice, suggesting a potential microbiome-associated effect.
- EI data analysis identified five significantly ($\log_2FC > 1$, $p_{raw} < 0.05$) differential features after normalization and scaling, all showing higher abundances in healthy wound samples (Fig. 3).
- Putative annotation of the significant features revealed lactic acid, 13-docosenamide, (Z)-, glycerol, 9-hexadecenoic acid (Z)- and ethyl oleate. Some features may be relevant for the wound healing process (e.g. lactic acid is known to possess regenerative and anti-inflammatory properties [3]) but due to the preliminary nature of this study, biologically relevant conclusions should be drawn with caution.
- 13-Docosenamide, (Z)- was of particular interest, as it was identified as significant in both MS-DIAL and MetaboScape workflows and has previously been associated with stimulating angiogenesis [4]. However, 13-docosenamide, (Z)- is also used as polymer additive [5] and has been shown to dissolve from certain pipetting tips [6]. Therefore, the analyte may originate from exogenous sources and its biological interpretation should be interpreted with caution. Nevertheless, in method and derivatization blanks, the feature was not detected.
- Annotation confidence was improved by combining complementary EI and CI information, highlighting the benefit of orthogonal ionization using the ecTOF platform. However, further experiments are required to increase confidence in the reported putative biomarkers.

Conclusion

- The ecTOF platform increased annotation confidence.
- 13-Docosenamide, (Z)- could be a microbiome-associated metabolite, potentially relevant in the process of wound healing (angiogenesis).
- Due to the limited sample size, the results should be interpreted cautiously.
- All identified features are only based on database matches and should therefore be validated with analytical standards and evaluated further within an appropriate biological context.

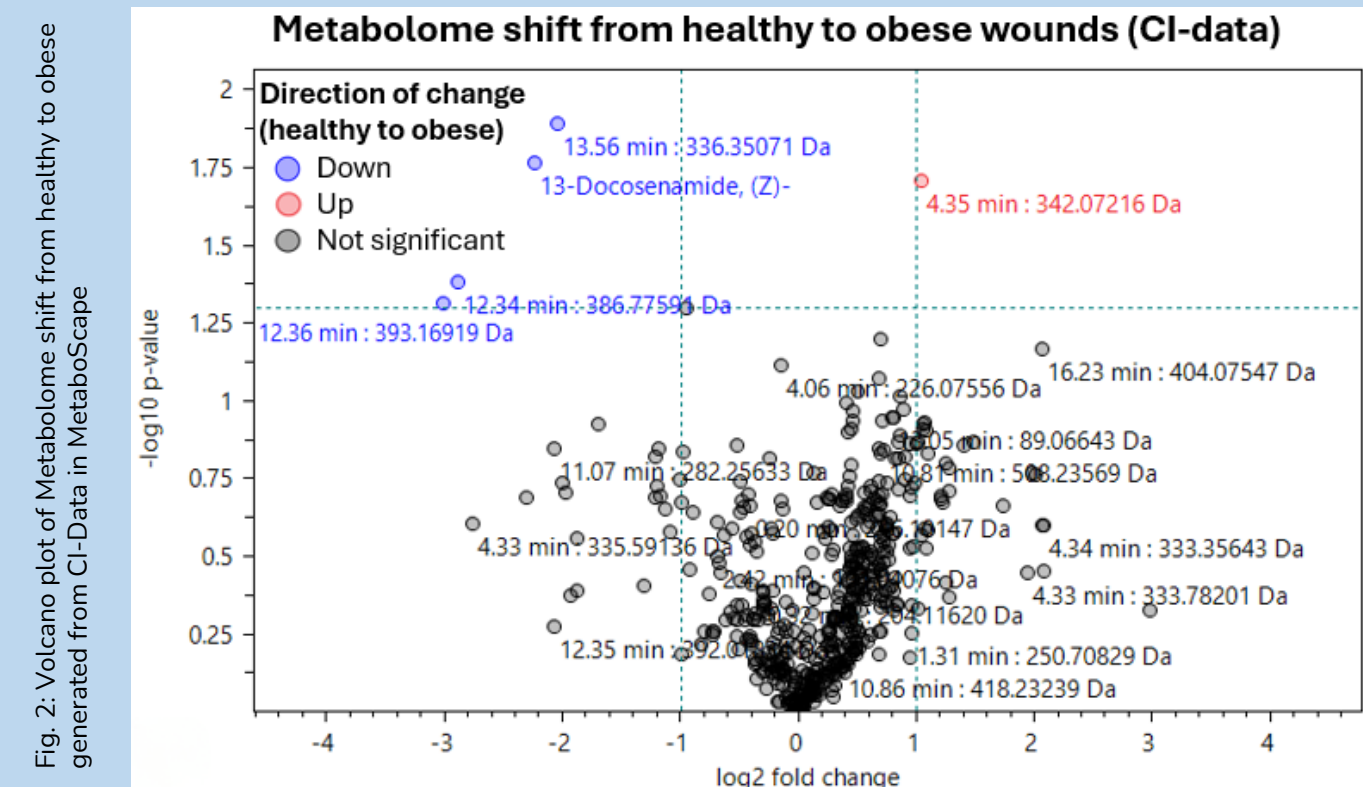


Fig. 2: Volcano plot of Metabolome shift from healthy to obese generated from CI-Data in MetaboScape

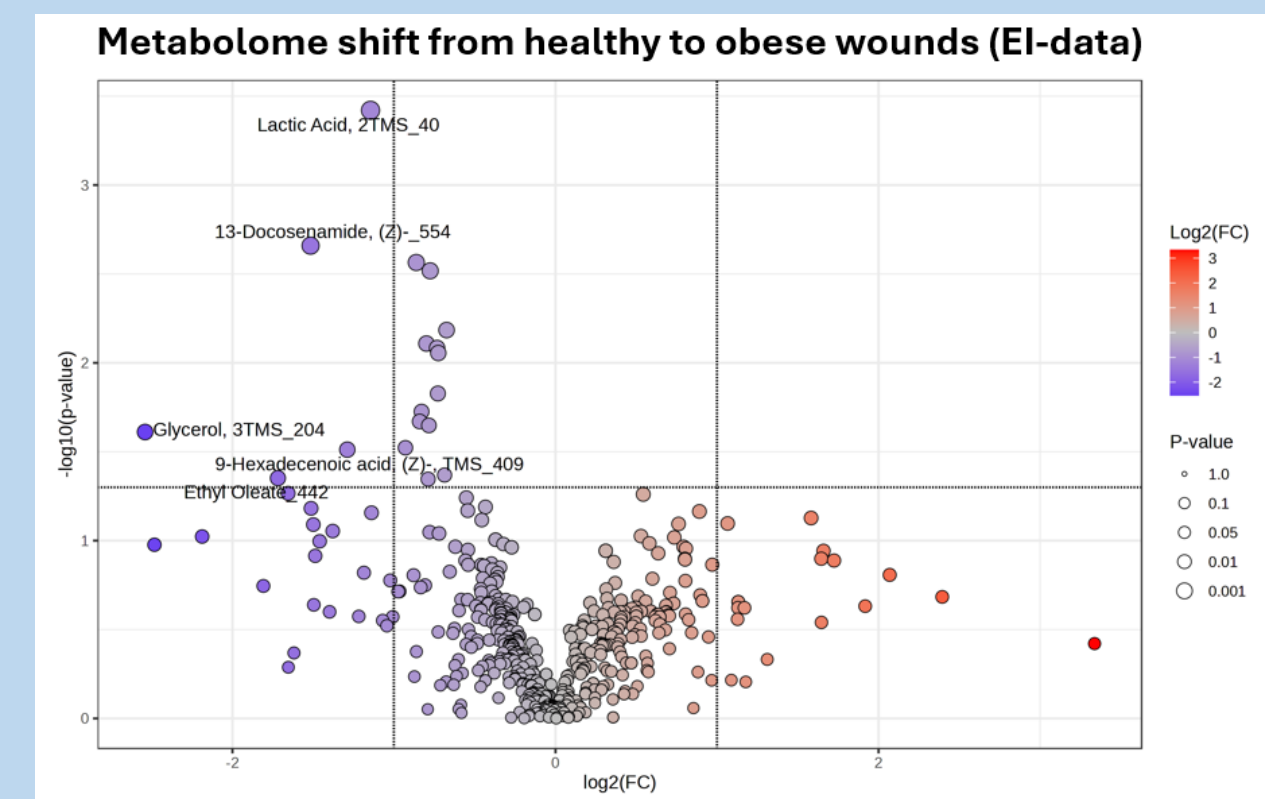


Fig. 3: Volcano plot of Metabolome shift from healthy to obese generated from EI-data in MetaboAnalyst