Development of an LC-IMS method for profiling novel N-acyl amides in fecal samples

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MOLECULAR SYSTEMS BIOLOGY

Introduction

Lipids are vital in multiple physiological functions, such as cell signaling and structure[1]. Lipid profiles are sensitive to changes in the environment, immune system or diet. Fecal lipid analysis offers information on gut physiology, and lipid levels are known to be associated with microbial production[2]. N-acyl amides (NAAs) are a large and diverse class of lipids comprising an acyl tail and an amine group via an amide bond[3]. It is hypothesized that these molecules could facilitate various physiological functions, and revealing these mechanisms may offer better mechanistic understanding[4,5].

Methods

This study used synthetic standard mixtures of 1,426 NAAs[3] to develop a qualitative LC-IMS method for profiling them in fecal samples. This study used Bruker TimsTOF flex coupled with a Shimadzu UHPLC. Reverse-phase LC was performed on a Phenomenex Kinetex 1.7 µm 100 x 2.1 mm XB-C18 100Å column. The mobile phase consisted of 0.1 % formic acid (FA) in water (phase A) and 0.1 % FA in MeOH:ACN (5:1, v/v) (phase B). The gradient started with 20 % B and was increased to 85 % in 10.5 minutes, followed by a 1-min increase to 100 % and held for 3.5 minutes. Fecal samples from in-vitro colon simulation were collected from four glass vessels mimicking the human colon from the proximal to the distal part. The system was inoculated with fecal samples from three healthy Finnish volunteers[6].

Results

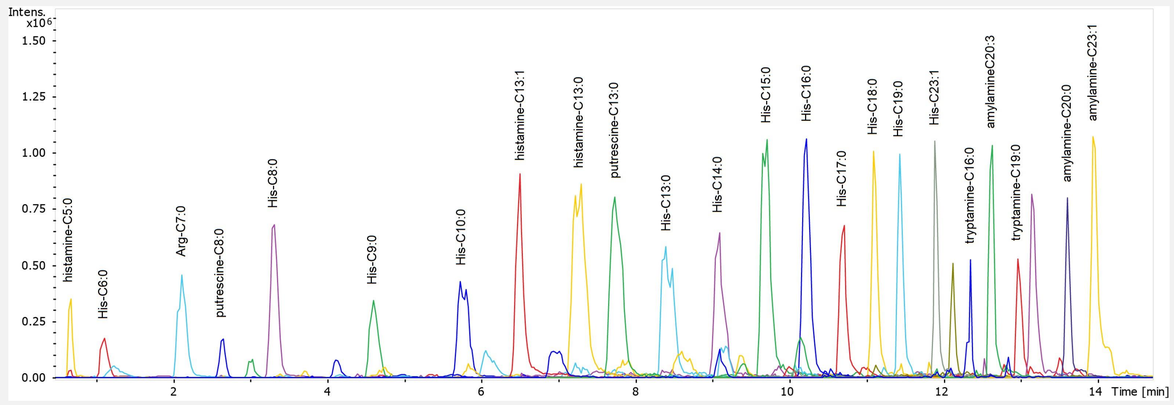
As a result of method development, an in-house library consisting of MS2 spectra, RTs and CCSs values of 910 NAAs was generated. In total, 64 % of possible conjugates were detected. The best coverage was reached for arginine, histidine, histamine and tryptamine conjugates. Extracted ion chromatograms for several of the detected compounds in the standard mixtures are shown in Figure A.

Preliminary results of simulated fecal samples (Figure B) indicate that conjugates are probably metabolized by gut microbes. For example, the abundance of an odd chain C5:0-histamine increases towards the distal part of the simulated colon (Figure C). However, data analysis is still in progress, and the effect of fecal microbes on the NAA profile has yet to be investigated.

Conclusions

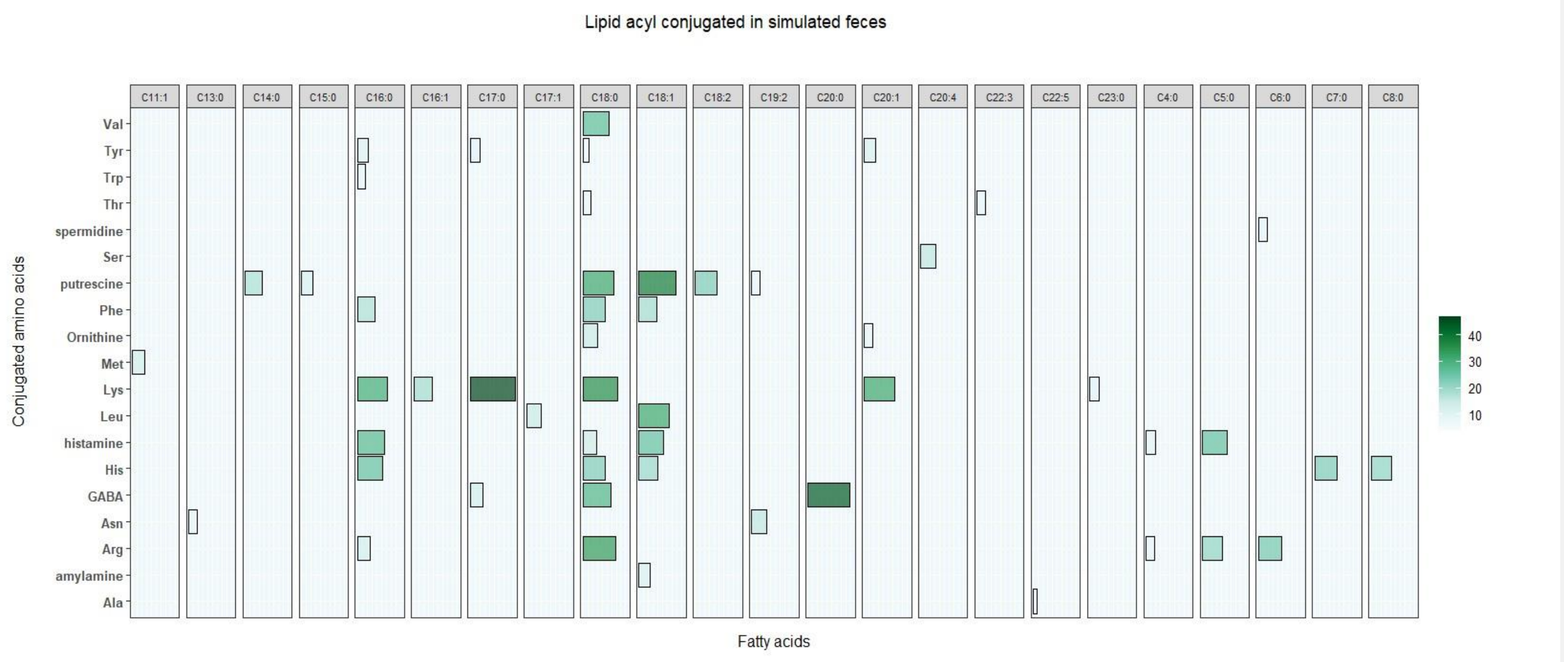
NAAs are present in biological systems, and some of them facilitate various physiological functions, including immune responses. How many acyl amides exist in nature and how they could affect biological functions is unknown. Our experiment showed that these compounds can be detected in biological matrices, but further method development is still desired to increase the number of detectable conjugates.

A



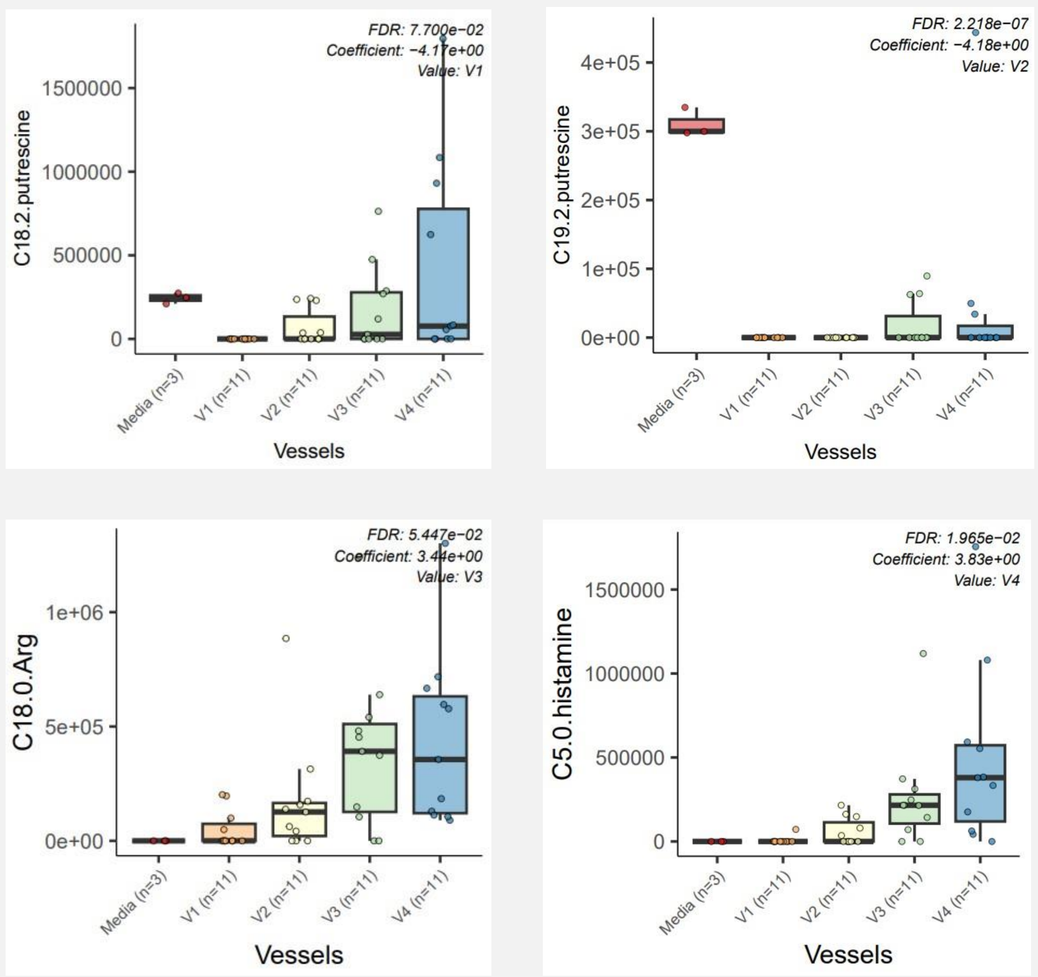
Extracted ion chromatograms of few N-acyl amide compounds in synthetic standard mixtures.

B



The heatmap represents the number of each NAA conjugate detected in the simulated colon chyme slurry. In total, 52 conjugates were detected at least once in the simulation.

C



Variation of four NAAs in colon simulation vessels.

References

1. M. C. I. de Sá and M. G. M. Castor, “Therapeutic Use of Palmitoylethanolamide as an Anti-Inflammatory and Immunomodulator,” Future Pharmacol., vol. 3, no. 4, Art. no. 4, Dec. 2023, doi: 10.3390/futurepharmacol3040058.massa.
2. M. Di Paola et al., “Oleoylethanolamide treatment affects gut microbiota composition and the expression of intestinal cytokines in Peyer’s patches of mice,” Sci. Rep., vol. 8, no. 1, Art. no. 1, Oct. 2018, doi: 10.1038/s41598-018-32925-x
3. E. C. Gentry et al., “Reverse metabolomics for the discovery of chemical structures from humans,” Nature, Dec. 2023, doi: 10.1038/s41586-023-06906-8
4. N. Battista, M. Bari, and T. Bisogno, “N-Acyl Amino Acids: Metabolism, Molecular Targets, and Role in Biological Processes,” Biomolecules, vol. 9, no. 12, p. 822, Dec. 2019, doi: 10.3390/biom9120822
5. X. Chen, Y. Yin, M. Luo, Z. Zhou, Y. Cai, and Z.-J. Zhu, “Trapped ion mobility spectrometry-mass spectrometry improves the coverage and accuracy of four-dimensional untargeted lipidomics,” Anal. Chim. Acta, vol. 1210, p. 339886, Jun. 2022, doi: 10.1016/j.aca.2022.339886
6. M. Kråkström et al., “Dynamics of the Lipidome in a Colon Simulator,” Metabolites, vol. 13, no. 3, p. 355, Feb. 2023, doi: 10.3390/metabo13030355.

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