学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Development of a quantitative analytical method for unsaturated short-chain fatty acid esters of hydroxy fatty acids and their biological applications
（ヒドロキシ脂肪酸の不飽和短鎖脂肪酸エステルの定量分析法の開発とその生物学的応用）

Lipids are highly diverse molecules that are essential for maintaining cell structure, managing metabolism, and facilitating signaling pathways vital for homeostasis. Abnormal lipid metabolism is increasingly linked to conditions such as obesity, inflammation, and neurodegenerative diseases. Advances in analytical techniques, especially liquid chromatography/mass spectrometry (LC/MS), have become the gold standard in lipidomics due to their ability to resolve complex lipid mixtures with high sensitivity. Untargeted LC/MS provides a comprehensive overview of the lipidome, enabling the discovery of novel lipid species and broad metabolic insights. However, targeted LC/MS is crucial for accurate quantification, structural confirmation, and confident biomarker evaluation. Among new lipid types, short-chain fatty acid esters of hydroxy fatty acids (SFAHFAs) are remarkable for their potential to reduce inflammation and regulate metabolism. However, their low levels, structural variety, and lack of commercial standards have posed challenges for mechanistic and quantitative research. This thesis examines the synthesis, quantitative method development, sensitivity enhancements of SFAHFA lipids and biological applications, integrating lipid chemistry with advanced LC/MS anlysis to gain a deeper understanding of their physiological roles.

Chapter 1 provides an overview of lipids and their classification, highlighting their structural and functional diversity in biological systems. It emphasizes the central role of lipid metabolism in health and disease. The chapter introduces the FAHFA family and their subclass, SFAHFAs, as emerging gut-derived signaling lipids potentially formed through host-microbiota interactions. The section further discusses the challenges in their absolute quantification, such as low endogenous abundance and the absence of reference standards and justifies the need for developing robust LC/MS-based analytical workflows for their accurate detection and biological interpretation.

Chapter 2 describes the chemical synthesis and structural characterization of 30 unsaturated SFAHFA standards. These compounds were synthesized from six commercially available unsaturated fatty acids via a two-step reaction: α-hydroxylation followed by short-chain fatty acid acylation. Reaction conditions were carefully optimized to preserve double-bond geometry. The products were characterized by 1H-NMR and high-resolution mass spectrometry (HRMS) techniques, confirming structural integrity and high purity of prepared standards. This chapter establishes the first comprehensive synthetic library of unsaturated SFAHFAs, filling a critical gap in lipidomics research by providing authentic reference compounds for absolute quantification and functional studies.

Chapter 3 focuses on LC/MS method development for unsaturated SFAHFAs using a triple quadrupole mass spectrometer (TSQ) coupled to an ultra-fast liquid chromatography (UFLC). Method optimization involved chromatographic separation on a reversed-phase column and negative electrospray ionization for sensitive detection. The developed targeted LC/MS/MS method demonstrated excellent linearity (R^2 > 0.99), recovery (85–110%), and precision (< 20%), with minimal matrix interference. Validation parameters, including selectivity, accuracy, and stability, confirmed the method's reliability for quantitative analysis. This optimized workflow provides a solid foundation for profiling SFAHFAs in complex biological matrices.

Chapter 4 utilizes the established LC/MS method to explore how SFAHFAs are biologically modulated under different metabolic and cognitive states. In mice, an obesogenic diet led to a reduction in saturated SFAHFAs and a trend toward increased unsaturated species, regardless of exercise. Interestingly, exercise combined with a control diet led to an increase in gut beneficial SFAHFA levels. In contrast, fecal samples from individuals with mild cognitive impairment showed no significant differences compared to healthy controls, indicating possible disease- and matrix-specific regulation of SFAHFAs. These findings demonstrate the translational potential of SFAHFAs as gut-associated lipid mediators and emphasize their dynamic regulation by diet and physiological conditions.

Chapter 5 explores a phenylethylamine (PEA)-based derivatization strategy to enhance the detection sensitivity of SFAHFAs in LC/MS analysis. By targeting the carboxylic functional group, PEA derivatization forms stable amide linkages, significantly improving ionization efficiency in electrospray mode. The derivatized SFAHFAs exhibited nearly 1000-fold higher sensitivity compared to their native forms. Application to fecal samples showed markedly increased peak intensities, confirming enhanced detectability and quantification reliability. This straightforward derivatization protocol offers a powerful approach for detecting low-abundance lipid metabolites in biological systems.

Chapter 6 extends the lipidomic investigation to a broader metabolic context, employing untargeted LC/MS to profile lipid alterations induced by chronic obesogenic diets (OBD) and exercise interventions in murine models. Two-month-old male C57BL/6 J mice were fed a control (CON diet) or OBD for 10 months, then assigned to sedentary (Sed) or exercise (Exe) groups for 2 weeks. A total of 363 lipid species were identified across plasma, tissue, and fecal samples. Multivariate analysis revealed distinct group separation between the control and OBD groups in both Sed and Exe groups. Lysophosphatidylethanolamine (LPE 22:5) was significantly upregulated in the liver, plasma, and left ventricle of the OBD mice in both Sed and Exe groups; in contrast, DHA-containing phosphatidylglycerol (PG (22:6/22:6)) was significantly downregulated. Exercise modestly modulated the lipid profile under OBD, lowering plasma ceramides and partially reversing lipid alterations in feces.

Overall, this thesis integrates synthetic lipid chemistry, advanced LC/MS/MS method development, derivatization strategies, and multi-matrix lipidomic applications to establish a comprehensive framework for the study of SFAHFAs. The findings provide new analytical tools and biological insights into the regulation of these novel lipids under metabolic and neurocognitive conditions. This research not only overcomes long-standing methodological barriers in SFAHFA quantification but also opens new avenues for understanding gut-derived lipid signaling in health and disease.