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学位論文内容の要旨

博士の専攻分野の名称：博士（保健科学）　　氏名：李　泳翰

学位論文題名

Development and application of highly sensitive LC/MS analysis of oxylipins and short-chain fatty acids

(オキシリピンおよび短鎖脂肪酸の高感度LC/MS分析法の開発とその応用)

[Background and objective]

Oxylipins, a family of oxygenated natural products derived from polyunsaturated fatty acids (PUFAs) play crucial roles in various physiological processes. Assessing oxylipin levels in vivo is crucial for understanding their roles in health and disease. Given the presence of numerous isomers, developing efficient and accurate analytical methods for the identification and quantification of oxylipins is important. On the other hand, short-chain fatty acids (SCFAs), produced through microbial fermentation, are also essential metabolites with diverse physiological functions. This study aims first to develop a sensitive and selective analytical method, followed by a systematic evaluation of oxylipin, PUFA, and SCFA concentrations in the plasma of children, exploring associations with factors such as age, sex, and body mass index (BMI).

[Methods]

A high-sensitivity LC-MS/MS analytical method for oxylipins was developed using a 100 mm C18 column, with subsequent method validation. Plasma samples were obtained from 342 children (181 boys and 161 girls) born between 2006 and 2010 in Hokkaido, all collected under non-fasting conditions. Oxylipins and PUFAs were extracted using an ice bath-assisted protocol, wherein the samples were incubated in methanol for 45 minutes at 0℃. During the extraction process, 0.01% butylated hydroxytoluene was added as an antioxidant to prevent oxidative degradation. For extraction of SCFAs, ethyl acetate was employed as the extraction solvent, followed by derivatization using a previously established N,N-dimethylethylenediamine (DMED) derivatization method. The processed samples were then subjected to LC-MS analysis for quantification.

[Results]

A robust LC-MS/MS method was successfully established for the quantification of 64 oxylipins and 4 precursor PUFAs within 36 minutes. The limits of quantification ranged from 0.25 to 50 pg, with most analytes showing recoveries and matrix effects between 85–110% and 90–110%, respectively. Intra- and inter-day precision values were within 15%. In the analysis of plasma samples, no significant correlation between oxylipin levels and age, sex, or BMI. However, among the PUFAs, boys had higher eicosapentaenoic acid and arachidonic acid levels than those of girls, with a significant increase in eicosapentaenoic acid levels in the overweight group compared with those in the underweight group.

The analysis of SCFAs using the DMED derivatization method demonstrated recovery ranging from 83% to 108%. Acetic acid was identified as the most abundant SCFA across all age groups and sexes. The concentrations of butyric acid, valeric acid, and hexanoic acid exhibited an age-dependent increase, reaching their highest levels in 12-year-old children. In contrast, 4-hydroxy valeric acid levels showed a decreasing trend with increasing age groups.

[Conclusions]

This study successfully developed and validated analytical methods, providing reliable technical support for quantitative analysis of oxylipins in biological samples and enriching the current methodological framework for oxylipin metabolism research. Furthermore, the successful quantification of oxylipins, PUFAs, and SCFAs in pediatric plasma lays a solid foundation for future studies on their roles in child growth and development, nutritional interventions, and health promotion, as well as potential exploration of their utility as biomarkers.