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Targeted quantification of lipid mediators in skeletal muscles using restricted access media-based trap-and-elute liquid chromatography-mass spectrometry

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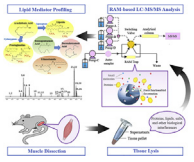
HIGHLIGHTS

- Streamlined RAM-trap pretreatment and LC-MS/MS quantification simultaneously.
- Time savings and higher throughput.
- Rapid and sensitive quantification of lipid mediators in skeletal muscles.
- Biological or chemical matrix interferences are eliminated efficiently.
- Advancing the knowledge of lipid signaling in musculoskeletal health and disease.

GRAPHICAL ABSTRACT



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ABSTRACT

Lipid mediators (LMs) are a class of bioactive metabolites of the essential polyunsaturated fatty acids (PUFA), which are involved in many physiological processes. Their quantification in biological samples is critical for understanding their functions in lifestyle and chronic diseases, such as diabetes, as well allergies, cancers, and in aging processes. We developed a rapid, and sensitive LC-MS/MS method to quantify the concentrations of 14 lipid mediators of interest in mouse skeletal muscle tissue without time-consuming liquid-liquid or solid-phase extractions. A restricted-access media (RAM) based trap was used prior to LC-MS as cleanup process to prevent the analytical column from clogging and deterioration. The system enabled automatic removal of residual proteins and other biological interferences presented

Abbreviations: AA, arachidonic acid; AEA, arachidonoyl ethanolamide; ALA, α-linoleic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; 17,18-DiHETE, (±)17,18-dihydroxy-eicosa-5,8,11,14-tetraenoic acid; DL temperature, desolvation line temperature; EA, ethanolamide; EPA, eicosapentaenoic acid; ESI, electrospray Ionization; IS, Internal standard; GC-MS, gas chromatography–mass spectrometry; GC-MS/MS, gas chromatography–tandem mass spectrometry; HDL, high-density lipoprotein; 8-HDoHE, 8-hydroxy docosahexaenoic acid; 5-HETE-d_8, 5S-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic-5,6,8,9,11,12,14,15-d8 acid; 12-HETE-d_8, 12S-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic-5,6,8,9,11,12,14,15-d_8 acid; 15-HETE-d_8, 15S-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic-5,6,8,9,11,12,14,15-d8 acid; 9-HODE, 9(S)-hydroxyoctadecadienoic acid; 13-HODE, 13(S)-hydroxyoctadecadienoic acid; 9-HOTrE, 9S-hydroxy-10E,12Z,15Z-octadecatrienoic acid; HPLC, high-performance liquid chromatography; 6-keto-$PGF_{1\alpha}$, 6-keto prostaglandin $F_{1\alpha}$; LA, linoleic acid; LC, liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LLE, liquid–liquid extraction; LM, lipid mediators; LOD, limit of detection; LOQ, limit of quantification; LOX, lipoxygenase; LTB_4-d_4, leukotriene B_4-d_4; LTC_4-d_5, leukotriene C_4-d5; LTs, leukotrienes; 5S,14R-LXB_4, 5S,14R,15S-trihydroxy-6E,8Z,10E,12E-eicosatetraenoic acid; MRM, multiple reaction monitoring; MS, mass spectrometry; *m/z*, mass-to-charge ratio; OEA, oleoyl ethanolamide; PGA_2, prostaglandin A_2; PGD_2-d_4, prostaglandin D_2–d_4; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGs, prostaglandins; PUFA, polyunsaturated fatty acid; QC, quality control; RAM, restricted–access media; RSD, relative standard deviation; SPE, solid-phase extraction; STD, standard; TT, transverse-tubules; UPLC, ultra-performance liquid chromatography.

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Matrix effects

in the tissue extracts; the target analytes were retained in the trap and then eluted to an analytical column for separation. Matrix evaluation tests demonstrated that the use of the combined RAM trap and chromatographic separation efficiently eliminated the biological or chemical matrix interferences typically encountered in bioanalytical analysis. Using 14 LM standards and 12 corresponding deuterated compounds as internal standards, the five-point calibration curves, established over the concentration range of 0.031–320 ng mL^{-1}, demonstrated good linearity of $r^2 > 0.9903$ (0.9903–0.9983). The lower detection limits obtained were 0.016, 0.031, 0.062, and 0.31 ng mL^{-1} (0.5, 1, 2, and 10 pg on column), respectively, depending on the specific compounds. Good accuracy (87.1–114.5%) and precision (<13.4%) of the method were observed for low, medium, and high concentration quality control samples. The method was applied to measure the amount of 14 target LMs in mouse skeletal muscle tissues. All 14 analytes in this study were successfully detected and quantified in the gastrocnemius muscle samples, which provided crucial information for both age and gender-related aspects of LMs signaling in skeletal muscles previously unknown. This method could be applied to advance the understanding of skeletal muscle pathophysiology to study the role of LMs in health and disease. Furthermore, we will expand the application of this methodology to humans and other tissues/matrices in the near future.

1. Introduction

Aging is associated with a progressive decline in structure and function of skeletal muscle. About 0.5–1% of muscle mass is lost per year in the individuals older than 30 years, and the rate of decline is dramatically accelerated in the elder persons over 65 years [1]. In addition, the loss of muscle mass is always combined with reduced muscle strength and endurance, thereby associated with adverse health outcomes such as physical disability, muscle injuries, poor life quality, and increased risk of death [1–3]. Furthermore, the reduction in muscle strength outpaces the loss the muscle mass, in that, there is a much larger decrease in force/strength compared to the amount of tissue lost, suggesting that the quality of muscle reduces with aging.

As a result of aging, dysregulation of a set of cellular processes, such as low-grade inflammation, increased oxidative stress, reduced capacity of muscle regeneration, and altered lipid metabolism contribute to this age-related muscle atrophy and muscle weakness, also called sarcopenia [4–6]. However, the primary molecular defects that lead to muscle dysfunction with aging are poorly understood. Even less understood is the mismatch between muscle mass and muscle strength during aging. Research designed to reveal the biomarkers of sarcopenia and other age-related skeletal muscle disorders is essential for diagnosis of these diseases, thus promoting the development of effective treatments to improve health outcomes for older individuals.

Lipid mediators (LMs) are a class of bioactive metabolites of the essential polyunsaturated fatty acids (PUFA) which are involved in many physiological processes. They are generated locally through specific biosynthetic enzymes/receptors in response to extracellular stimuli, and play an important role through their signaling pathways on the regulation of pathophysiological states such as inflammation, metabolic syndrome, and cancer [7–9]. Thus, many LMs have been investigated as biomarkers and for drug development. LMs are structurally classified into three categories: 1) arachidonic acid (AA, ω-6 PUFA)-derived eicosanoids, including prostaglandins (PGs) and leukotrienes (LTs); 2) lysophospholipids and their derivatives; and 3) ω-3 PUFA derivatives, such as docosahexaenoic acid (DHA), α-linolenic acid (ALA), and eicosapentaenoic acid (EPA) [7].

To date, evidence from several studies suggests that lipid mediators may regulate skeletal muscle mass and function and potentially protect against muscle wasting in response to various pathological conditions. Prostaglandin E_2 (PGE_2), one of five major PGs produced from AA via the cyclooxygenase 1/2 (COX1/2) pathways, can accelerate skeletal muscle myogenic differentiation by promoting myoblast proliferation and blocking EP4 receptor results in increased production of intracellular reactive oxygen species (ROS) in myoblasts [8,10]. Besides PGE_2, other major PGs also exhibited the regulatory roles on muscle cell growth and development. For example, an increased proliferation and reduced differentiation in L6 rat skeletal myoblasts can be observed by prostaglandin D_2 (PGD_2) [11], and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) can stimulate skeletal muscle growth by augmenting the size of myotube [12]. Additionally, LMs might also play a particular role in the mismatch between muscle mass and force/strength because of their potential to influence different aspects of muscle function, including the excitation-contraction coupling process. AA-derived lipid mediators eukotrienes and lipoxins have been reported for their regulating in whole-body cholesterol homeostasis and high-density lipoprotein (HDL) function in mammals [13], while cholesterol is enriched in the transverse-tubules (TT) of skeletal muscles and is essential for the formation and maintenance of these membrane structures [14–16] and maintenance of contractile force [17,18]. Proper cholesterol content in the cell membrane is essential for optimal store-operated calcium entry (SOCE) in a variety of different cell types [19,20], which we demonstrated to reduce with aging in skeletal muscles and contribute to sarcopenia and aging-muscle weakness [21,22]. Moreover, numerous preclinical or clinical data have indicated the improved skeletal muscle health by ω-3 PUFA supplements (e.g. DHA or EPA), particularly in the elderly population [23–25]. However, the effects of ω-3 LMs on muscle growth have not yet been fully identified, even though recently these bioactive lipids (resolvins, protectins, etc.) attracted considerable attention due to their potent cell-protective and anti-inflammatory activity [26–28]. Therefore, quantitative analysis of LMs in skeletal muscle is necessary to elucidate the molecular mechanisms underlying the dissimilar lipid metabolism pathways in skeletal muscle from young and older individuals, and may provide a better understanding in the cause of and potential treatments for sarcopenia. Furthermore, this new technique has broader implications for the utilization of LMs in other fields.

A wide variety of analytical methods has been reported for LMs determination. The most commonly used detection methods are mass spectrometry (MS) coupled to either gas chromatography (GC) or liquid chromatography (LC) [29–31]. In comparison with other immunological methods such as ELISA, application of MS offers a fast, highly sensitive, and specific method for high throughput quantification. GC-MS was for a long time the preferred analytical technique for LM determination; however, in the past