Visualization of lipids in skeletal muscles by mass spectrometry imaging

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Abstract
Lipids are the major components of cells and, in addition to being involved in cell signaling, play a major role in the skeletal muscles. Lipids comprise a large number of molecular species with their different fatty acid compositions, and it is hard to visualize their spatial localization to identify muscle-fiber-specific lipid dynamics. In this short review, we present a molecular imaging technology, matrix-assisted laser desorption/ionization mass spectrometry imaging, capable of imaging small metabolites including lipids. This technology has the potential to reveal the lipid dynamics that occur during muscle contraction and/or disease status.

Keywords: lipid, mass spectrometry imaging, training, skeletal muscle

Overview

Lipids are the major components of cell membranes and are involved in cell signaling, survival, and apoptosis 1). Lipids comprise a complex range of molecules such as fatty acids, glycerolipids, and sphingolipids. Each type includes a wide variety of lipid molecular species with different fatty acid compositions. Lipids in skeletal muscle play a fundamental role as an energy source both in normal muscle metabolism and in disease states 2). An excess accumulation of lipid in skeletal muscle is associated with several chronic metabolic disorders, including obesity, insulin resistance, and type 2 diabetes 3). Several studies have demonstrated strong associations between high triacylglycerol (TAG) content and enhanced skeletal muscle insulin resistance in obesity. Nonetheless, highly insulin-sensitive, endurance-trained athletes also exhibit high TAG content similar to that observed in insulin-resistant obese patients. To account for this discrepancy, known as the “athlete’s paradox”, a number of studies of lipids have been performed to demonstrate the significance of the “quality of lipids”, not “quantity of lipids.”

Phospholipids are a major lipid species that consist of the molecular layer of the cell membrane. The phospholipid monolayer surrounds the neutral core of the lipid droplet in muscle; thus, lipid accumulation is attributed to phospholipids. The remodeling of the fatty acid content occurs frequently by phospholipase and acyl transferase, and the remodeled fatty acid contents influence cell permeability and receptor stability on the cell membrane. A recent analysis revealed that phospholipid distribution differs among cell types, leading to different types of signal transduction within cells 4).

For comprehensive lipid analyses, we use a “lipidomics” approach, which provides further insights into the complex metabolic networks of biological systems. Mainly, lipidomics approaches have been demonstrated by the use of liquid chromatography (LC) coupled with mass spectrometry (MS) to fully characterize lipid molecular species 5-8). However, this technique includes extraction and purification steps, leading to the loss of lipid distribution throughout biological tissue.

Skeletal muscles are heterogeneous in nature, and are composed of a mixture of muscle fibers that can be distinguished based on their different physiological and biochemical characteristics. The main fibers can be divided into two types: fast-twitch and slow-twitch fibers. These two types are also known as glycolytic and oxidative fibers, respectively, for their metabolic status. These two types of fibers are localized heterogeneously, and it is hard to discriminate and obtain extracts specific to each fiber. Basically, the lipidomics approach utilizing LC-MS has demonstrated the use of extensor digitorum longus (EDL) and soleus as the representative tissues of glycolytic and oxidative fibers, respectively. However, soleus tissue possesses both types of fiber in equal amounts.

Recently, to describe the wide variety of lipid qualities of each type of fiber using spatial information, matrix-assisted laser desorption/ionization-MS imaging (MALDI-MSI), which is an MS-based imaging technique, has been used. MALDI-MSI could visualize the distribution of biomolecules without the need for extraction, purification, separation, or labeling of the molecules (Fig. 1). MALDI-MSI is applicable for the visualization of a wide range of biomolecules such as lipids 9,10), glycolipids 11,12), and proteins 13), as well as nutrients 14,15). This imaging modality is expected to be a suitable tool for investigating the pathologic conditions of different muscle groups. The spatial
resolution is relatively high (5-50 μm), which is sufficient to visualize the fiber-specific molecular distribution. In this review, we show the application of MALDI-MSI to skeletal muscle for screening biomarkers and detecting the molecular changes of lipids and metabolites based on physiological status.

Application of MALDI-MSI for lipids

We applied MALDI-MSI to mouse skeletal muscle to demonstrate the change in lipid quality that occurs due to acute contraction. In this study, we successfully observed the reduction of diacylglycerol and TAG, which are generally associated with muscle contraction. Interestingly, we found the accumulation of some saturated and mono-unsaturated fatty acids and poly-unsaturated fatty acids (PUFA) containing phosphatidylcholine (PC) in contracted muscles. Moreover, energy metabolic activity, which is defined as “energy charge” calculated by the molecular valance of ATP, ADP, and AMP, can be evaluated in each local spot in cells and tissues.

Next, we utilized other rodent models to identify and characterize lipid biomarkers specific to chronic exercise and/or a high-fat-diet model in order to reveal what types of lipids are involved in insulin sensitivity/resistance in skeletal muscle. To detect and identify characteristic lipids, we focused on two types of rat models, namely, rats exposed to chronic exercise training (Training) and those fed a high-fat diet (HFD). In this report, we used three different lipidomics approaches, namely thin-layer chromatography (TLC), MALDI-MSI, and the combination of these two approaches (the TLC-Blot-MALDI-MSI method), because it is hard to identify minor components or low ionization efficiency molecules using MALDI-MSI. As a result, linoleic-acid-containing PC and sphingomyelin- and docosahexanoic-acid-(DHA)-containing PC were characterized as Training-induced lipids. On the contrary, arachidonic acid (AA)-containing PC, phosphatidyethanolamines, and phosphatidylinositol were characterized as HFD-induced lipids. This is the first report to reveal compositional changes in phospholipid molecular species in chronic-exercise and high-fat-diet-induced insulin-resistant models. Importantly, our data in this paper showed the change in EDL glycolytic fiber, whereas the lipid change was completely different in the soleus tissue (Fig. 2). These data implied that the lipid dynamics are completely different between glycolytic and oxidative fibers. Senoo N et al. later applied a lipidomics approach to PGC-1α-mediated changes in phospholipids. Their data are consistent with our previous data showing that DHA-containing phospholipids increased in glycolytic fibers through training. These changes were not observed in oxidative fibers. The mechanism of and reason for the increase of DHA-containing lipids are still unclear; however, protectin DX, which is a DHA-derived lipid mediator, could alleviate insulin resistance by activating the myokine-liver glucoregulatory axis. White PJ et al. demonstrated that protectin DX stimulated the release of the prototypic myokine (IL-6) from skeletal muscle, and also activated AMP-activated protein kinase, which stimulates the glucose and lipid metabolism in skeletal muscle. Based on these findings, some of the health benefits of exercise training may be explained by the alteration of skeletal muscle phospholipid profiles.

An interesting report relating to the lipid metabolic profiles of skeletal muscle was performed by Yan H et al. They demonstrated that gut microbiota can transfer muscle fiber characteristics which can change the lipid
metabolism in skeletal muscles from glycolytic to oxidative fiber. Additionally, dietary fat influences the expression of contractile and metabolic genes in muscle. Mizunoya W et al. administered a 15% fat diet derived from different fat sources to their subjects. Diets composed of soybean oil (n-6 PUFA-rich), fish oil (n-3 PUFA-rich), or lard (low in PUFA) were administered. Myosin heavy chain isoforms were used as biomarkers to delineate the skeletal muscle fiber types. Compared with soybean oil intake, fish oil intake led to significantly lower levels of glycolytic fibers in the EDL muscle. Concomitantly, mitochondrial uncoupling protein 3, pyruvate dehydrogenase kinase 4, and porin mRNA showed upregulated levels in the EDL muscle of fish-oil-fed rats compared to those observed in soybean-oil-fed and lard-fed rats, implying an activation of oxidative metabolism.

Application of MALDI-MSI for small metabolites

MALDI-MSI is an analytical technique that detects the mass-to-charge ratio \((m/z)\) of ionized molecules and visualizes the distribution of ions\(^{22}\). All molecules which could ionize have the potential to be visualized by this technique. Furuichi Y et al. tried to visualize carnitine and its molecular species, acylcarnitine\(^{23}\). Carnitine is well recognized as a key regulator of long-chain fatty acyl group translocation into the mitochondria. In addition, carnitine, as acetylcarnitine, acts as an acceptor of excess acetyl-CoA, a potent inhibitor of pyruvate dehydrogenase. We accurately quantified the acetylcarnitine content and determined its localization in skeletal muscles using MALDI-MSI. We detected an increase in acetylcarnitine content after muscle contraction. Importantly, this increase was not detected using traditional biochemical assays of homogenized muscles. We also demonstrated that the acetylation of carnitine during muscle contraction was concomitant with glycogen depletion.

From a different point of view, to identify the molecular markers of the two different types of fiber, glycolytic and oxidative, MALDI-MSI has been used for metabolomics screening\(^{24}\). The researchers in this previous study selected EDL and soleus tissues for the screening of molecular markers of glycolytic fiber and oxidative fiber, respectively. Multivariate data analysis such as principal component analysis after MALDI-MSI was performed to extract significant features. Different metabolic fingerprints were observed for glycolytic and oxidative fibers. As a result, higher abundances of anserine and acylcarnitines were observed in the glycolytic fibers, whereas taurine and several nucleotides were found to be localized in the oxidative fibers.

Recently, Sato E et al. revealed that the metabolic alterations caused by indoxyl sulfate induce uremic sarcopenia in chronic kidney disease\(^{25}\). In this study, MALDI-MSI visualized indoxyl sulfate that was accumulated in the muscle tissue of a mouse model of chronic kidney disease. Comprehensive metabolomics revealed that indoxyl sulfate induces metabolic alterations such as the upregula-
tion of glycolysis and the acceleration of the antioxidative stress response, which resulted in down-regulation of the TCA (tricarboxylic acid) cycle and led to mitochondrial dysfunction and an ATP shortage in muscle cells. These studies suggested that MALDI-MSI might be a powerful tool for metabolite screening.

Methodological improvement for high-sensitivity and high-resolution imaging

Not only MALDI, but also other ionization methods, including secondary ion MS (SIMS) and desorption electrospray ionization, have been able to be combined with MSI. TOF-SIMS, in particular, has been used in biological applications for high-resolution imaging (nm level). Jiang H et al. demonstrated NanoSIMS imaging, which makes it possible to visualize neutral lipids in cytosolic lipid droplets in intestinal enterocytes, chylomicrones at the basolateral surface of enterocytes, and lipid droplets in cardiomyocytes and adipocytes. In addition, Liu CH et al. have shown a new approach to calcium ion imaging using TOF-SIMS. These approaches might be useful for visualizing intra-myocellular lipids in the future.

Conclusion

MALDI-MSI is becoming an essential tool for the molecular imaging of biological samples. Many great advances have been made in MALDI-MSI technology which have allowed it to visualize lipids in various types of biological samples, although there is still room for improvement in sample preparation, ionization, and instrumentation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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