Evaluation of taurine content on skeletal muscle of exercised rats using MALDI-TOF MS imaging analysis

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Abstract The amount of taurine and taurine derivatives in the body is affected by various diseases and physiological events, such as exercise. However, there is little information about possible changes in taurine distribution within tissue. In the present study, we examined whether matrix-assisted laser desorption/ionization-imaging mass spectrometry (MALDI-IMS) can be used to determine the effect of exercise on the distribution and content of taurine and acetyltaurine, a taurine derivative present in skeletal muscle. Using 9-aminoacridine to detect the amino acids in tissue samples, the content of taurine and acetyltaurine in homogenates of skeletal muscle was measured by MALDI-IMS. While the intrinsic levels of taurine in skeletal muscle tissue were adequate to be detected by MALDI-IMS, that of acetyltaurine was not observed. Following 120-min of treadmill running (20m/min), taurine content of soleus and plantaris muscles significantly declined. In the gastrocnemius muscle, taurine content is higher in slow-twitch fiber than fast-twitch fiber. However, the taurine content was not significantly changed by treadmill running in both fast- and slow-twitch fiber of the gastrocnemius muscle. In conclusion, MALDI-IMS using 9-aminoacridine as a matrix could detect the distribution of taurine in skeletal muscle before and after exercise.

Keywords : taurine, exercise, skeletal muscle, MALDI-IMS

Background

Taurine is a beta-amino acid found in high concentration in mammalian tissues, especially in skeletal muscle⁽¹⁾. The amino acid helps tissue homeostasis through a variety of biological actions, including regulating cellular osmolality, inorganic ion movement, energy metabolism, attenuating oxidative stress, etc^(2,3). In muscle, it is required for normal contractile function and exercise performance, as evidenced by the reduction in exercise time during enforced treadmill-running or swimming tests of taurine depleted mice^(4,5). Conversely, taurine supplementation supports exercise performance and recovery⁽⁶⁻⁸⁾.

Numerous metabolic studies have demonstrated that taurine content is altered by stress and disease⁽⁹⁻¹²⁾, suggesting that taurine may serve as a biomarker of abnormal muscle conditions. Nonetheless, exercise increases urinary and/or blood taurine concentration^(13,14). Endurance exercises, such as marathons, cause an increase in both

plasma and urinary taurine concentration, while most other amino acids decrease after exercise⁽¹⁴⁾. An increase in blood taurine is partially caused by the release from skeletal muscle, since taurine is released by contracting muscle⁽¹⁴⁾. Indeed, muscle taurine content is decreased by long-term treadmill running in rats⁽¹³⁾. However, the biological significance of the specific reduction in taurine after endurance exercise remains to be elucidated.

Skeletal muscle is composed of fast- and slow-twitch muscle fibers. Whereas fast-twitch fiber mainly generates ATP from glycolysis, the major source of ATP in slow-twitch fiber is oxidative phosphorylation. Most muscle tissue contains a mixture of the two types of fiber, but it is difficult to separate the fibers in order to analyze metabolites. Meanwhile, taurine associates with mitochondrial functions. Taurine may buffer the mitochondrial pH which contributes optimal activity of Acyl-CoA dehydrogenase⁽¹⁵⁾. Moreover, taurine modifies the wobble positions of a few mitochondrial transfer RNAs, which is important for the translation of proteins encoded in mitochondrial DNA^(16,17). Interestingly, taurine content is higher in slow-

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twitch muscle fiber than fast-twitch fiber¹³). Therefore, we hypothesized that differences must exist in the handling of taurine by the two types of fibers.

Acetyltaurine, which is formed by a reaction between taurine and acetyl-CoA and/or acetate, is detectable in skeletal muscle as well as liver^{18,19}. According to Miyazaki et al., the muscle content of acetyltaurine is increased after treadmill running in rats¹⁹. This reaction may remove acetate from skeletal muscle during exercise, as both serum and urinary concentrations of acetate increase after exercise²⁰. The increase in acetyltaurine during exercise might be correlated to the change in taurine content, although this remains to be determined.

Traditionally, the measurement of tissue taurine has mainly been performed by using HPLC following homogenization of whole tissue^{21,22}, a procedure that can barely detect a change in taurine content in a specific region of the tissue. On the other hand, matrix-assisted laser desorption/ionization-imaging mass spectrometry (MALDI-IMS) ionizes and detects plural metabolites directly from tissue sections, thereby providing a visual map of each metabolite on the surface of the tissue section²³. While previous MALDI-MS-based metabolomics analysis revealed the different localization of several metabolites, such as taurine, phospholipids, ATP and carnitine, between slow-twitch and fast twitch parts of muscle, etc.²⁴⁻²⁷, there are no studies which focus on taurine and its derivatives by the MALDI-IMS method. Understanding the changes in the local taurine content of each muscle fiber may contribute to revealing the biological significance and molecular mechanism of a given reduction in muscle taurine. In the present study, we examined whether MALDI-IMS is a useful technique to detect the kaleidoscope of taurine content images on the surface of skeletal muscle sections, which more clearly establishes the distribution of taurine within the muscle.

Methods

Animals and exercise. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Animal experimental procedures were approved by the Animal Experimental Committee of the University of Tsukuba (approval number: 17-415). Male Wister rats (8-week-old, Japan SLC) were fed a standard chow (MF, Oriental Yeast, Japan), had access to water ad libitum, and were maintained on a 12-h light/dark cycle. Rats were habituated to treadmill exercise (FVRO.4E9S-6, Fuji Medical Science Co. Ltd., Chiba, Japan) for 5 days by gradual increases in running speed and duration up to 20 m/min for 10 min. Then, rats were fasted for 3h, and put on a treadmill for 15 min. The rats were forced to either run at 20m/min for 120 min ($n = 3$), or remain sedentary, the latter being the control ($n = 3$). The rats were anesthetized by pentobarbital (i.p. 64.8 mg/kg BW [body weight]), and then, skeletal muscles were

collected from the lower leg. The collected muscles were the soleus, which is a slow-twitch fiber dominant muscle, the plantaris, which is a fast-twitch fiber dominant muscle, and the gastrocnemius, which contains a mixture of slow-twitch and fast-twitch fibers. The muscles were immediately orientated cross-sectionally at the belly (approximately 5 mm thickness) on a cork block, mounted in optimal cutting temperature (OCT) compound (Tissue-Tek; Miles Inc., Elkhart, IN, USA) and finally frozen in liquid nitrogen and stored at -80°C . The remaining sections of muscle were maintained at -80°C until analysis following homogenization of samples.

Muscle sections. Frozen tissues were sectioned into 10 μm thick slices on conductive indium-tin-oxide (ITO)-coated glass slides (Bruker Daltonics) at -20°C .

MALDI-MS. MALDI-MS and IMS were performed using an ultrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Germany). To measure taurine and acetyltaurine using MALDI-MS, a diluted muscle homogenate (100mg tissue/mL water) was mixed with an equal amount of each compound (0.1~1 mM in water) and 3 times volume of 9-aminoacridine (9-AA, 10 mg/mL 70% ethanol). One μL of each sample was dropped onto an ITO-coated glass slide and dried. According to a previous report, we used 9-AA as a matrix for negative-mode detection²⁸.

To measure tissue metabolites using MALDI-IMS analysis, a 9-AA solution (7 mg/mL 70% ethanol) was sprayed on muscle sections with an air-brush (Procon Boy FWA platinum, Mr. Hobby, Japan). Data were acquired in the negative ion mode for taurine and acetyltaurine. The operating parameters of the mass spectrometer were set according to the manufacturer's protocol. Raster scanning was performed at 200 μm pitch. Signals between m/z 50 to 1000 were collected and analyzed by FlexImaging software (Bruker Daltonics). MS/MS scan was performed with LIFT mode. The images from signal intensities were obtained using the FlexImaging software. Data processing, normalization and quantification were performed using SCiLS Lab software (Bruker Daltonics). To compare the taurine level in the muscle section, the peak intensities for taurine (m/z 124.0) were normalized by the intensities for 9-AA (m/z 193.0).

Immunohistochemistry. For detection of slow-twitch muscle fibers, muscle sections were immunostained by using anti-slow skeletal myosin heavy chain antibody (1:200, NOQ7.5.4D; Sigma-Aldrich, St. Louis, MO, USA) and Alexa Fluor 488-conjugated second antibody (1:400; Life Technologies, Carlsbad, CA, USA) with Can Get Signal Immunostain according to manufacturer's protocol (Toyobo, Osaka, Osaka). Images were acquired using a fluorescent stereo microscope (Leica, Germany).

Statistics. Each value was expressed as mean $\pm$ standard deviation. Student's t-test and repeated-measures 2-way ANOVA performed with EZR software were used to determine statistical significance between groups and differences were considered statistically significant when the calculated P value was less than 0.05.

Results and discussion

To determine whether taurine and acetyltaurine can be detected in muscle using MALDI-IMS, a drop of mixture containing either taurine or acetyltaurine combined with muscle homogenate and 9-AA was subjected to MALDI-

IMS (Fig. 1). The mass spectrum for [taurine-H]⁻ (m/z 124) was obtained, and the average signal intensities in a drop area were examined in a dose-dependent manner (Fig. 1B). Similarly, the mass spectrum for [acetyltaurine-H]⁻ (m/z 166) was obtained from a drop of a mixture of acetyltaurine, muscle homogenate and 9-AA (Fig. 1C, D).

Next, we examined whether the intrinsic taurine and acetyltaurine levels of 9-AA-sprayed skeletal muscle sections were detectable using the MALDI-MS-imaging method. The average mass spectra obtained from a soleus muscle section is shown in Fig. 2A. The highest intensity signal was at m/z 124, with isotopic signals detectable at m/z 125 and 126). The signal was identified as [taurine-

Fig. 1 Detection of taurine and acetyltaurine on ITO-glass slides using MALDI-MS.

(A,B) Average mass spectra obtained from taurine-tissue homogenate-9-AA mixture (A) and MS images of m/z 124.0 ([taurine-H]⁻). (C,D) Average mass spectra obtained from acetyltaurine-tissue homogenate-9-AA mixture (C) and MS images of m/z 166.0 ([acetyltaurine-H]⁻). C) Representative average mass spectra obtained from acetyltaurine-tissue homogenate-9-AA mixture.

Fig. 2 Detection of taurine and acetyltaurine in soleus muscle sections.

(A) Representative average MS spectra obtained from soleus muscle sections and MS-image of taurine. (B) MS/MS spectra of m/z 124.0 ([taurine-H]⁺) on taurine standard and muscle section on ITO-slide.

H⁺] by MS/MS analysis (Fig. 2B). In the skeletal muscle sections, the intensity of the signal at m/z 124 was the highest among all the intrinsic metabolites, at least over the range of m/z 50 ~ 1000. This may be related to the high concentration of taurine in muscle, as well as the sensitivity of the MALDI-IMS technique.

The average mass spectrum signal at m/z 166, which is a putative signal for acetyltaurine ([acetyltaurine-H]⁺), was detected in the skeletal muscle section. However, the MS/MS data was not detectable due to its low signal intensity. Therefore, the concentration of acetyltaurine is too low to detect using the 9-AA sprayed section method.

Next, we examined the change in muscle taurine content of rats before and after 120 min of exercise on a

treadmill at 20m/min. We have previously reported that treadmill running with a similar intensity (25m/min) causes a reduction in muscle taurine content. Based on the intensity of the mass spectra signal at m/z 124 of control and exercising muscle, running on the treadmill was associated with a net decrease in taurine content of both slow-twitch dominant muscle (soleus muscle) (Fig. 3A, B), and fast-twitch dominant muscle (plantaris muscle) (Fig. 4B, C). The intensity of the signal at m/z 124 clearly indicated a net decrease in taurine content following a 120-min run, and regional-specific changes were observed.

We also analyzed the taurine content of gastrocnemius muscle using MALDI-IMS. Because gastrocnemius muscle contains a mixture of slow-twitch and fast-

twitch muscle fibers, and the taurine content of the two types of fibers may differ, we chose to identify the slow-twitch and fast-twitch muscle fibers based on the content of slow-type and fast-type myosin in the gastrocnemius muscle section, respectively (Fig. 5A). Comparison of the average mass spectra of the gastrocnemius muscle between control and exercised rats revealed that taurine content was affected by both muscle fiber type and exercise [muscle type (slow- vs fast-twitch parts); $p < 0.05$, exercise (rest vs run); $p < 0.05$, interaction; $p > 0.05$ by

repeated measures two-way ANOVA]. Distribution of taurine in the slow-twitch fiber-rich area was significantly higher than in the fast-twitch-rich area (Fig. 5A, B). Moreover, the 120-min run tends to decrease in taurine content in slow-twitch parts ($p = 0.099$, student's t-test), but does not change in fast-twitch parts.

Previous studies showed that the decrease of taurine content by exercise was higher in the slow-twitch fiber dominant soleus muscle (approximately -44%) than in the slow- and fast-twitch fiber mixed gastrocnemius muscle (approximately -12%)^{11,13}. Our data indicate that slow-twitch fiber is more susceptible to exercise than fast-twitch fiber concerning taurine decline. Additionally, since standard deviation of taurine content in the slow-twitch part of gastrocnemius muscle was observed, it may cause the failure to detect a significant difference between control and 120-min run samples.

While we revealed the distribution of taurine in skeletal muscle and its reduction by moderate-intensity exercise, we could not elucidate the other metabolites. Since various metabolites are conjugated with taurine, it is possible that the production of taurine-conjugated metabolites in muscle contribute to the reduction of taurine during the endurance exercise. Additionally, since acetyltaurine is increased in the muscle after marathon and long-term treadmill running^{19,20}, we tried to measure acetyltaurine in the present study. However, the intrinsic acetyltaurine in skeletal muscle was not detectable by MALDI-IMS, indicating the amount of muscle acetyltaurine content is very small compared to taurine. Therefore, most of the reduction in taurine may be due to being released into the blood. The released taurine may play an important role

Fig. 3 Taurine content of rested and exercised soleus muscles. (A) Representative MS images of taurine content of soleus muscle sections of rested and exercised (run) rats. (B) Average taurine intensity obtained from MS spectra intensity ($n = 3$).

Fig. 4 Taurine content of plantaris muscles of rested and exercised rats. (A) Representative MS spectra obtained from rested and exercised plantaris muscle sections. (B) Representative MS images of taurine on plantaris muscle sections of rested and exercised (run) rats. (C) Average taurine intensity calculated from MS spectra of rested and exercised plantaris muscle ($n = 3$).

Fig. 5 Taurine content of gastrocnemius muscle from rested and exercised rats.

(A) Representative immunohistochemical image of gastrocnemius stained with anti-slow skeletal muscle myosin antibody (upper) and MS images of taurine obtained from gastrocnemius muscle sections from rested and exercised (run) rats. (B) Average taurine intensity of gastrocnemius muscle from rested and exercised rats calculated from average MS spectra ($n = 5-6$).

in the body during exercise. Taurine also conjugates the other carboxylic acids, such as bile acids, in other tissues. To measure the small amount of taurine derivatives, including acetyltaurine, in tissue, further technical improvements, such as lowering signal-to-noise ratio and increasing sensitivity, may be necessary.

In summary, we demonstrated that a change in muscle taurine content occurs in response to treadmill exercise, a change that is readily measurable using the MALDI-IMS method. Moreover, MALDI-IMS can detect the distribution of taurine in skeletal muscle sections. Therefore, this method is useful in analyzing changes in the distribution of taurine in skeletal muscle during muscle stimulation and disease. Moreover, future studies, such as the combination of this method with immunohistochemical analysis for the proteins responsible for taurine transport and/or signal transduction, can reveal the biological significances and molecular mechanism of exercise-induced taurine release.

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Conflict of Interests

The authors declare that they have no conflict of interests.

Author contributions

TM, HO, SM and TI conceptualized the study design and protocol, and determined the study institutions. RK and TI collected and assembled the data. RK and TI carried out the analysis and interpretation of data. RK, SWS and TI drafted the manuscript. All authors have critically reviewed, revised and approved the manuscript.

Informed consent

This article does not contain any studies with human participants requiring informed consent.

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