Metabolomic profiling analysis reveals chamber-dependent metabolite patterns in the mouse heart

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THE MAMMALIAN HEART is divided into four chambers, which are specialized to adapt to different physiological and pathological conditions such as varying degrees of pressure and volume. This specialization is characterized by regulating different sets of genes during early development through adulthood (3, 33). Each chamber has chamber-specific functions and structures as well as pathologies, such as left ventricular (LV) or right ventricular (RV) hypertrophy and atrial fibrillation (7, 19, 33, 35, 39, 41). The main function of the ventricle is to pump blood to the whole body or the lung, and, therefore, its muscle wall is thicker than that of the atria. To adapt to varying workloads of the heart, energy production and use should be closely related to chamber-specific function and structure. The cardiomyocyte is known to mainly use fatty acid oxidation (FAO) to produce ATP under normal conditions (2, 27, 38). A growing body of evidence has demonstrated that under pathological conditions such as heart failure, FAO is impaired and glycolysis and glucose oxidation are increased (2, 21). Although most of the studies regarding the metabolic changes in cardiac muscles have been conducted in an affected cardiac chamber, chamber-specific differences of metabolism remain unclear. Therefore, the value of such studies is limited due to a lack of understanding regarding fundamental myocardial cell metabolism in each chamber.

Recent innovative mass spectrometry (MS)-based methods for comprehensive metabolic profiling have enabled us to analyze the changes in metabolic fuel selection in a variety of cellular and animal models (13, 31, 38). During this study, we used capillary electrophoresis (CE) time-of-flight MS (CE-TOFMS) to comprehensively measure metabolites from mouse atria and ventricles. The basic data of metabolites in each chamber should provide important new insights into cardiovascular metabolism involved in the development, function, and pathophysiology of the cardiovascular system.

MATERIALS AND METHODS

Animals. All animal care and study protocols were approved by the Animal Ethics Committees of Waseda University, and the investigation conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996). We purchased male C57BL6 mice at the age of 6–7 wk from Sankyo Lab. Animals were studied to ensure uniformity among experimental subjects and to minimize variations due to age, sex, and other factors, such as diet habits, that might affect cardiac function. Mice were fed normal chow and water ad libitum. Animals were individually housed in Plexiglas cages and were kept in a room with controlled temperature (22 ± 3°C) and lighting (lights on from 8:00 AM to 8:00 PM). The bedding of the cages consisted of wood shavings.

Heart isolation. Mice were euthanized right after noon because the time was regarded as a resting cycle with less intake of chaw and water. Heart tissues were immediately isolated after cervical dislocation under anesthesia by isoflurane. Hearts were separated into three parts (both atria, the LV, and the RV) and immediately frozen with liquid nitrogen. It should be noted that the interventricular septum was carefully excluded from the ventricular samples to avoid any fusion effect expected from the adjacent lesion. Because the amount of atrial tissue was relatively small, we mixed left and right atrial tissues from four mice to detect metabolites as one atrial sample by CE-MS.

Metabolome analysis. We analyzed metabolites of the heart samples as previously described (20). Briefly, frozen heart tissues (the atria: ~35 mg, the RV: ~31 mg, and the LV: ~52 mg) were immediately plunged into methanol containing internal standards (300 μM each of methionine sulfone for cations and MES for anions) and homogenized to inactivate enzymes. Deionized water was then added, chloroform...
was added, and the solution was thoroughly mixed. The solution was centrifuged, and the upper aqueous layer was centrifugally filtered through a Millipore 5-kDa cutoff filter (Millipore, Billerica, MA) to remove proteins. The filtrate was lyophilized and dissolved in 50 µl Milli-Q water containing reference compounds (200 µM each of 3-aminopyrrolidine and trimmed) before CE-TOFMS.

**Quantitative RT-PCR.** Total RNA was extracted from the atria and ventricles using TRIzol reagent (Invitrogen, Carlsbad, CA), as recommended by the manufacturer. cDNAs were synthesized with a High-Capacity cDNA Reverse Transcription Kit (ABI), and RT-PCR was performed using a StepOne Real-Time PCR System with Fast SYBR Green Master Mix (ABI) as recommended by the manufacturer. Sequences of PCR primers were designed based on the mouse nucleotide sequences in PrimerBank (http://pga.mgh.harvard.edu/primerbank/). We used the following primers: monocarboxylate transporter 1 (MCT1; Primer Bank ID: 6677995a1), carnitine palmitoyltransferase (CPT)-1b (Primer Bank ID: 6753512a1), CPT-2 (Primer Bank ID: 6753514a1), pyruvate dehydrogenase (PDH) kinase (PDK)1 (Primer Bank ID: 27369966a1), PDK2 (Primer Bank ID: 19526816a1), PDK3 (Primer Bank ID: 21704122a1), and PDK4 (Primer Bank ID: 7305375a1). In addition, we compared the relative expression levels of isoform mRNAs, such as MCT, PDK, lactate dehydrogenase (LDH), and CPT, by threshold cycle (Ct) values.

**Western blot analysis.** Total proteins extracted from the atria and ventricles were used for Western blot analyses using antibodies against AMP-activated protein kinase (AMPK)-α, phosphorylated AMPK (Thr172) (Cell Signaling Technology, Beverly, MA), PDH-E1-α, phosphorylated PDH (Ser293) (Abcam, Cambridge, MA), PDK1 (Cell Signaling Technology), PDK2 (Assay Biotechnology, Sunnyvale, CA), PDK3 (Abgent, San Diego, CA), and PDK4 (Abcam), as previously described (13, 17). Briefly, tissues were homogenized in ice-cold buffer [containing (in mM) 10 imidazole (pH 7.0), 300 sucrose, 5 sodium pyrophosphate decahydrate, and 1 DTT] with phosphatase inhibitors (Complete Mini, Roche, Basel, Switzerland) and phosphatase inhibitors (PhosSTOP, Roche). Protein content was determined using the Bradford assay (Bio-Rad, Hercules, CA), and BSA (0.1–1 mg/ml) was used as a standard. Protein samples (1.5 µg) were separated in the same gel by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore). When the molecular size of target proteins was different, polyvinylidene difluoride membranes were cut in accordance with their size. When the molecular size of target proteins was similar, we reused the same membrane for a different antibody after washing the membrane with stripping buffer [containing (in mM) 62.5 Tris (pH 6.8), 100 2-mercaptoethanol, and 2% SDS]. After application of a secondary antibody, quantification of the target signals was performed using the LAS-3000.

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![Fig. 1. Heat map analysis of the amount of metabolites among chambers. A and B: differences in the amount of cationic metabolites (A) and anionic metabolites (B). The amount of metabolites increased from green to red. Gray indicates that no signal was detected. n = 2 ~ 5. RV, right ventricle; LV, left ventricle.](http://ajpheart.physiology.org/)

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imaging system (Fujifilm, Tokyo, Japan). For reuse, the membrane was washed with stripping buffer at 55°C for 10 min and then washed three times with 0.1% Tris-buffered saline-Tween 20 buffer.

**CPT assay.** CPT-2 enzyme activities were measured using the spectrophotometric method. Frozen tissues were homogenized in homogenization buffer [containing (in mM) 250 sucrose, 1 EDTA, and 3 Tris·HCl (pH 7.2)] with protease inhibitors (Complete Mini, Roche). We mixed atrial tissues from four mice into one sample to measure the activity. The homogenate was centrifuged at 500 g for 10 min at 4°C. The supernatant was collected in new tubes and centrifuged at 9,000 g for 10 min at 4°C. The pellet was then washed with homogenization buffer and centrifuged at 9,000 g for 10 min at 4°C twice. The washed pellet was resuspended in 30 μl of 1.25 mM L-carnitine and read at 412 nm at 30°C using a spectrophotometer. Frozen tissues were homogenized in a homogenization buffer and centrifuged at 9,000 g for 10 min at 4°C. The supernatant was collected in new tubes and centrifuged at 9,000 g for 10 min at 4°C. The washed pellet was resuspended in 30 μl of homogenization buffer as the sample. To determine total CPT-2 activity, the sample was assayed in 970 μl reaction buffer [containing (in mM) 58 Tris·HCl (pH 8.0), 1.25 EDTA, 0.25 5,5′-dithiobis-(2-nitrobenzoic acid), 0.04 palmitoyl-CoA, and 0.1% Triton X-100] and 20 μl sample. Reactions were initiated by the addition of 10 μl of 1.25 mM l-carnitine and read at 412 nm at 30°C using a spectrophotometer. The homogenate was centrifuged at 500 g for 10 min at 4°C, and the supernatant was removed for assay. We mixed 10 μl sample and 190 μl working reagent containing MTT solution, NAD solution, PMS solution, and substrate buffer and read optical density at 565 nm and again after 25 min on a plate reader. The activity was normalized by each protein concentration.

**Statistical analysis.** Group data are expressed as means ± SE. Statistical analysis was performed among multiple groups by one-way ANOVA followed by post comparisons with the Newman-Keuls test. An unpaired two-tailed Student’s t-test was used for the Ct value comparison. P values of <0.05 were considered significant.

**RESULTS**

**Metabolic activities are higher in the ventricles than in the atria.** The amounts of metabolites from glycolysis, the tricarboxylic acid (TCA) cycle, nucleotides, and amino acids were comprehensively measured in mouse hearts by CE-TOFMS. We found that overall metabolic profiles were similar between the RV and LV. On the other hand, the atria exhibited a distinct metabolic pattern from those of the ventricles (Fig. 1).

Importantly, the high-energy phosphate pool (the total concentration of ATP, ADP, and AMP) was higher in both ventricles than in the atria (P < 0.001, the RV or LV vs. atria; Fig. 2A). ATP content was the highest in the LV among the samples (P < 0.01 vs. the atria and P < 0.05 vs. the RV; Fig. 2B). ADP content was also highest in the LV (P < 0.001 vs. the atria and P < 0.05 vs. the RV; Fig. 2C), whereas there were no differences in AMP content among these chambers (Fig. 2D). Accordingly, the ratio of ATP to ADP plus AMP was

![Fig. 2. Amounts and ratios of energetic molecules. A–E: amount and ratio of energy phosphate. The amounts of total phosphatases and ADP were significantly higher in both ventricles (A and C). Those of ATP and the ratio of ATP to ADP plus AMP were highest in the LV (B and E). There were no significant differences in AMP among the chambers (D). The amount of NAD+ plus NADH was significantly higher in both ventricles (F), whereas there were no significant differences in the ratio (G). The amount of FAD was significantly higher in both ventricles (H). Values are expressed as means ± SE; n = 4 ~ 5. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with the atrium (At); †P < 0.05 compared with the LV.](http://ajpheart.physiology.org/ by 10.220.33.4 on March 31, 2017)
In addition, the total amount of electron transfer coenzymes (NADH and NADP⁺) was higher in both ventricles than in the atria (P < 0.001; Fig. 2E). The ratio of NADH to NADP⁺ was lower in both ventricles than in the atria, although it did not reach statistical significance (Fig. 2G), indicating that metabolites through the aerobic pathway such as oxidative phosphorylation are higher in the ventricles than in the atria. Moreover, we found that FAD, a redox cofactor involved in carrying high-energy electrons used for oxidative phosphorylation, was higher in the ventricles than in the atria (Fig. 2H). FADH₂, a reduced form of FAD, was not measurable in the present study. On the other hand, there was no chamber-specific difference in ribulose 5-phosphate and NADPH, although NADP⁺ was higher in the ventricles than in the atria (Fig. 3).

The amount of metabolites in the TCA cycle was higher in the ventricles than in the atria. We found that the content of acetyl CoA, a key molecule in aerobic metabolism for energy production, was higher in the ventricles than in the atria, although there were no differences between both ventricles (Fig. 4). Furthermore, the total content of metabolites involved in the TCA cycle was higher in both ventricles than in the atria (P < 0.001; Fig. 4). Among the substrates in the TCA cycle, succinate displayed the most significant difference between the ventricles and atria (Fig. 4). On the other hand, fumarate and malate were higher in the RV than in the LV (Fig. 4). The present CE-TOFMS analysis could not detect other TCA cycle-related metabolites and therefore these are not shown in Fig. 4.

In contrast, the total content of metabolites involved in glycolysis was not different among the chambers (Fig. 4), although there were several chamber-specific differences in each metabolite (Fig. 4). Interestingly, lactate content was approximately twofold higher in the ventricles than in the atria (P < 0.001), whereas there were no differences between both ventricles (Fig. 4).

Amino acid metabolism in the ventricles was different from that in the atria. Profiles of amino acid metabolism were also different between the ventricles and atria. Among the 20 amino acids that serve as the building blocks of proteins, approximately half of them (alanine, asparagine, arginine, glutamine, histidine, lysine, phenylalanine, serine, and threonine) were significantly higher in the ventricles than in the atria (Fig. 5). Among these nine amino acids, four essential amino acids (histidine, lysine, phenylalanine, and threonine) were included. On the other hand, three amino acids (aspartate, glycine, and proline) were significantly lower in the ventricles than in the atria. In addition, amino acids related to urea metabolism, such as creatine, citrulline, and ornithine, were higher in the ventricles than in the atria. We also found that S-adenosylhomocysteine was lower in the ventricles than in the atria.

Key enzymes of energy production by FAO in the heart. The comprehensive metabolomic profiles suggested that the ventricles use FAO to produce energy in mitochondria more than the atria. We then found that the phosphorylation level of AMPK protein, a central player in cellular energy homeostasis, was higher in the ventricles than in the atria, although it did not reach statistical significance (Fig. 6). Expression levels of AMPK protein itself were not different among the three chambers (Fig. 6).

To investigate whether the capacity of mitochondria respiration was different among the chambers, we analyzed the expression levels of CPT-1a, CPT-1b, and CPT-2, which are

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**Ribulose 5-phosphate**

- **G6P**
- **NADP⁺**
- **NADPH**
- **G6P**
- **NADPH/NADP⁺**
- **GSSG(oxidized)**
- **GSH(reduced)**

![Diagram of metabolic pathways](image)

**Fig. 3.** The pentose phosphate pathway. The ratio of NADPH to NADP⁺ was lower in the ventricle. Since the amount of ribulose 5-phosphate was not different among the chambers, the pathway is unlikely to contribute to the higher energy production in the ventricles. G6P, glucose 6-phosphate. n = 4 – 5. **P < 0.01 and ***P < 0.001 compared with the At; ††P < 0.01 compared with the LV.
compared with the At; † †
P
their Ct values (Fig. 7) when the relative abundance of CPT mRNAs was estimated by C

Fig. 7 significantly higher in both ventricles than in the atria (Fig. 7), whereas expression levels of CPT-1b mRNA were significantly higher in both ventricles than in the atria. The amount of pyruvate was not available. Five substrates involved in TCA cycle were detected in the present study. There were no significant differences in the amounts of citrate and isocitrate. Succinate and malate were higher in the ventricles, and fumarate was higher in the RV. F6P, fructose 6-phosphate; F1,6P, fructose 1,6-bisphosphate; 3PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate. Values are expressed as means ± S; n = 4 ~ 5. **P < 0.01 and ***P < 0.001 compared with the At; †P < 0.05 and ††P < 0.01 compared with the LV.

As shown in Fig. 4, the amount of lactate was higher in both ventricles than in the atria (Fig. 8A), whereas the expression levels of MCT4 mRNA were lower in the ventricles than in the atria (Fig. 8B). The Ct value between the ventricles was significantly higher in the ventricles than in the atria (Fig. 8C). We also found that the expression levels of LDH isoform (LDHa and LDHb) mRNAs were significantly higher in the ventricles than in the atria (Fig. 8D). The Ct value between MCT1 and MCT4 mRNAs indicated that the abundance of MCT1 was much greater than that of MCT4 in the atria and ventricles (Fig. 8C).

Utility of lactate and pyruvate in the atria and ventricles. As shown in Fig. 4, the amount of lactate was higher in the ventricles than in the atria. Lactate is transported across the plasma membrane by a family of MCTs. Among the 14 identified MCT isoforms, MCT1 and MCT4 are the key transporters in cardiac muscle: MCT1 facilitates lactate uptake, whereas MCT4 extrudes lactate from the cytosol (4, 16). We found that the expression levels of MCT1 mRNA were significantly higher in the ventricles than in the atria (Fig. 8A), whereas the expression levels of MCT4 mRNA were lower in the ventricles than in the atria (Fig. 8B). The Ct value between MCT1 and MCT4 mRNAs indicated that the abundance of MCT1 was much greater than that of MCT4 in the atria and ventricles (Fig. 8C).

We also found that the expression levels of LDH isoform (LDHa and LDHb) mRNAs were significantly higher in the ventricles than in the atria (Fig. 8D). The Ct value between MCT1 and MCT4 mRNAs indicated that the abundance of MCT1 was much greater than that of MCT4 in the atria and ventricles (Fig. 8C).
Fig. 5. Amounts of amino acid in each property. SAM\(^+\), S-adenosylmethionine; SAH, S-adenosyl-L-homocysteine. Values are expressed as means ± SE; \(n=4\) - 5. *\(p<0.05\), **\(p<0.01\), and ***\(p<0.001\) compared with the At; †\(p<0.05\), ††\(p<0.01\), and †††\(p<0.001\) compared with the LV.

Fig. 6. Expression and phosphorylation levels of AMP-activated protein kinase (AMPK) protein. There were no significant differences in expression levels of total AMPK and phosphorylated (p-)AMPK, although the phosphorylation level tended to be higher in the ventricles. Protein expression was normalized by \(\beta\)-actin. Values are expressed as means ± SE; \(n=4\) in each group.
lated by PDKs, it becomes inactive. We found that the phosphorylated level of PDH protein was lower in the RV than in the LV and atria (Fig. 9). The expression level of PDP1 mRNA was significantly higher than that of PDP2 mRNA (Fig. 10 A). Moreover, the expression level of PDP1 mRNA was lower in the LV than in the atria and RV, but the expression level of PDP2 mRNA was higher in the ventricles (Fig. 10 B). In addition, we found that the expression level of PDK4 was the highest of the four isoforms among the chambers and that PDK4 mRNA expression was significantly higher in the ventricles than in the atria (Fig. 10 C). Each PDK isoform was differently expressed in the ventricles and atria (Fig. 10 D). Expression levels of PDK2 and PDK4 mRNA were significantly higher in the ventricles than in the atria, although there were no differences between both ventricles. Interestingly, the expression level of PDK3 mRNA was significantly higher in the atria than in the ventricles. There were no significant differences in PDK1 mRNA level among the three chambers. Furthermore, we analyzed the protein expression level of PDK isoforms (Fig. 11). Levels of PDK2 and PDK4 proteins were higher in the LV than in the atria and RV. There were no significant differences in PDK1 and PDK3 protein levels among the three chambers, although the expression level of PDK3 mRNA was significantly higher in the atria than in the ventricles.

**DISCUSSION**

CE-TOFMS enabled us to comprehensively analyze metabolites in each cardiac chamber. The main aim of the present study was to especially clarify the different character of metabolism between the atrium and ventricles under a physiological condition. A considerable number of studies have already demonstrated the metabolomics of the heart under physiological and/or pathological conditions (2, 10, 38). However, to our best knowledge, this is the first report demonstrating chamber-specific metabolic profiles in the mouse heart under a physiological condition. Importantly, we found that the metabolic profiles of the ventricles were different from those of the atria, whereas the overall metabolic profiles were similar between the RV and LV. The chamber-specific metabolic profiles displayed a similar pattern with chamber-specific gene expression profiles that were found in previous studies (3, 12, 33). Accordingly, these studies have demonstrated that the number of genes that differentiated between the two ventricles is much smaller than those differentiating between the atria and ventricles. It also indicates that the genes related to metabolic and energy-deriving processes are highly expressed in the ventricles compared with the atria. It should be noted that the present study has a limitation in that we used a mixed atrial specimen because of the small amount of tissues to examine their expression levels.
Fig. 8. The mRNA expression levels of monocarboxylate transporters (MCTs) and lactate dehydrogenases (LDHs) and LDH activity. Expression levels of MCT1 mRNA were significantly higher in both ventricles (A). In contrast, expression levels of MCT4 mRNA were significantly lower in both ventricles (B). C: C t value comparison between MCT1 and MCT4 mRNAs. Both LDHa and LDHb mRNA levels were significantly higher in the ventricles, especially the RV (D and E). F: C t value comparison between LDHa and LDHb mRNAs. The expression of target mRNAs was normalized by 18S rRNA. LDH activity was the highest in the atria (G). 1 IU was defined that LDH catalyzes the conversion of 1 μmol lactate to pyruvate per minute at pH 8.2. Values are expressed as means ± SE; n = 5–6 in each group. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with the At; †††P < 0.001 compared with the LV.

Fig. 9. Phosphorylation levels of pyruvate dehydrogenase (PDH). Although there were no significant differences between the atria and LV, the phosphorylation level was the lowest in the RV. Values are expressed as means ± SE; n = 5–7 in each group. †P < 0.05 compared with the LV.
metabolites by CE-TOFMS. However, we have recently developed our technique to analyze a small amount of sample and then examined metabolites in each atrium of the mice at 13 wk of age (unpublished data). Although the number of samples was too few and some metabolites were not detected due to an insufficient sample amount, we found that the metabolite profiles were very similar between the right and left atria. Further studies are required to identify whether there is a characteristic difference between the right and left atria.

The present study suggested that the activities of energy production in the ventricles could be greater than those in the atria, although metabolome analysis only identifies a snapshot quantification of metabolites and does not necessarily represent the “flux” of metabolic pathways. In fact, the contents of total phosphates (ATP, ADP, and AMP), acetyl CoA, and TCA cycle metabolites were higher in the ventricles than in the atria. Furthermore, our data are consistent with previous reports (12, 43) demonstrating that the number of mitochondria is much higher in the heart than in the atrium. Interestingly, succinate was significantly the highest substrate of the ventricles in the higher levels of succinate in the ventricle. We think that these enzymes are more activated in the ventricles than in the atrium, resulting in the higher levels of succinate in the ventricle.

We also found that the profiles of amino acids were different between the atria and ventricles (Fig. 5). Amino acids are involved in a variety of key biochemical and physiological activities. Some of them can be a source of substrate for energy production, such as the TCA cycle (22). Most of the amino acids that can be the source of substrate, such as glutamine, histidine, lysine, arginine, and asparagines, were higher in the ventricles, suggesting that these amino acids can be involved in energy supply in the ventricles, which are likely to have higher energy demands than the atria. Moreover, amino acids are...
important energy sources in impaired hearts, although they play a minor role in energy sources under normal conditions (22, 30, 34). Therefore, they may work as an energy reserve in the ventricles.

Since the cardiomyocyte is known to mainly use FAO to produce ATP under normal conditions (2, 27, 38), the role of CPT, which transports fatty acid from the cytosol into the mitochondria, is important. CPT-1 is located at the mitochondrial outer membrane, and CPT-2 is at the inner membrane (11). In the present study, we found that CPT-2 activity was significantly higher in the RV. Although we did not measure the activity of CPT-1 because CPT-1 activity is lost in a frozen sample, the expression level of CPT-1b mRNA, which was the most abundant isofrom in each chamber, was significantly higher in the LV, assuming that CPT-1 activity is higher in the LV.

AMPK plays an important role in glucose uptake, glycogen content, and fatty acid metabolism. Activation of AMPK accelerates glycolysis and FAO (23). In our data, the phosphorylation levels of AMPK tended to be consistently higher in the ventricles (Fig. 6). Taken together, these data support that FAO is more active in the ventricle than in the atrium.

PDH is a key enzyme that combines the pathways of glycolysis and the TCA cycle. PDH is inactivated in a phosphorylated state by PDK and is activated in a dephosphorylated state by PDP. Importantly, it has also been reported that pharmacological PDK inhibition improves RV hypertrophy and failure (26, 27). We found that PDH is activated more in the RV than in the atria (Fig. 9), suggesting that energy production in the TCA cycle via glycolysis is higher in the RV. Interestingly, it has been reported that the alteration of mitochondria function due to cardiac damage such as chronic hypoxia is delayed in the RV compared with the LV (24). The high activity of PDH in the present study may help to increase resistance to hypoxic damage in the RV. We found that expression levels of PDP1 mRNA were higher than those of PDP2 mRNA in each chamber (Fig. 10A), suggesting that PDP1, a Ca^{2+}-sensitive enzyme, is a major activator of PDH in the heart. This is consistent with a previous study (18) using rat hearts. In the expression levels of PDK isofoms, the expression levels of PDK2 and PDK4 proteins, which are enhanced by fatty acid (1, 32), were higher in the LV than in the atria and RV (Fig. 11). This may reflect the greater activity of FAO in the LV than in the atria and RV. Interestingly, the expression patterns of PDK2, PDK3, and PDK4 proteins were different from those of mRNAs. In particular, there were no significant differences in PDK3 protein levels among the three chambers, although the expression levels of PDK3 mRNA were significantly higher in the atria than in the ventricles. In addition, there was a discordant expression pattern of PDK2 and PDK4 isofoms between mRNA and protein levels. These data suggest that the expression levels of PDK proteins are regulated at the postranscriptional level. For instance, some previous studies (29, 36) have reported that microRNAs regulate the expression of PDKs. The regulatory mechanism of chamber-specific PDK expression should be elucidated in a future study.

The present metabolome analysis demonstrated that lactate is higher in the ventricles. In this regard, we found that LDH activity was higher in the atria than in the ventricles, suggesting that the rate of conversion from lactate to pyruvate is greater in the atria, whereas the expression levels of LDHa and LDHb mRNAs were higher in the ventricles. Since LDHb is known to be a predominant isofrom in the heart, which is consistent with the present study (9, 15) and facilitates the conversion of lactate to pyruvate, we do not explain the discrepancy of the results of LDH activity and its expression level. The conversion from lactate to pyruvate is negatively regulated by NADH, which is higher in the ventricles than in the atria. Therefore, higher NADH may contribute to the lower LDH activity in the ventricles than in the atria. We also found that the expression of MCT1 mRNA was also higher and the expression of MCT4 mRNA was lower in the ventricles. It is known that MCT1 facilitates lactate uptake (5) and that MCT4 extrudes lactate from the cytosol, although MCT4 is rarely expressed in the heart (5, 14). Taken together, lactate is likely to be retained in the ventricles.

The LV chamber is exposed to higher pressure than the RV chamber. Therefore, one may assume that the LV requires more energy demand. Accordingly, ATP and the ratio of ATP to ADP plus AMP were the highest in the LV. Nevertheless, we found that the overall metabolic profiles were similar between the RV and LV, which is consistent with previous studies (3, 12, 33) demonstrating that chamber-specific gene expression profiles were similar between the RV and LV. Further investigation is required to clarify the reason why the RV and LV display similar metabolic profiles.
The present study also has a limitation in that we did not separate cardiomyocytes and noncardiomyocytes in our metabolomic analyses. Therefore, we could not exclude the effect of noncardiomyocytes on our results because the composition of noncardiomyocytes in the atria may differ from that in the ventricles. It is intriguing to investigate that a metabolic pattern is different between cardiomyocytes and noncardiomyocytes, especially under a pathological condition.

In the present study, we found different metabolic patterns among each cardiac chamber. Our data provide a basis for understanding the metabolic differences in pathophysiology in the atria and ventricles.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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REFERENCES


