Metabolomic Analysis in Heart Failure

Ryutaro Ikegami, MD; Ippei Shimizu, MD, PhD; Yohko Yoshida, MD, PhD; Tohru Minamino, MD, PhD

It is thought that at least 6,500 low-molecular-weight metabolites exist in humans, and these metabolites have various important roles in biological systems in addition to proteins and genes. Comprehensive assessment of endogenous metabolites is called metabolomics, and recent advances in this field have enabled us to understand the critical role of previously unknown metabolites or metabolic pathways in the cardiovascular system. In this review, we will focus on heart failure and how metabolomic analysis has contributed to improving our understanding of the pathogenesis of this critical condition.

Key Words: Amino acids; Fatty acids; Glucose; Ketones; Metabolome

It is thought that at least 6,500 low-molecular-weight metabolites exist in humans, and these metabolites have various important roles in biological systems in addition to proteins and genes. Comprehensive assessment of endogenous metabolites is called metabolomics, and recent advances in this field have enabled us to understand the critical role of previously unknown metabolites or metabolic pathways in the maintenance of homeostasis under both physiological and stress conditions. Techniques of metabolomic analysis have been elegantly reported and reviewed elsewhere, so the details will not be repeated here. Instead, we will describe the metabolites/metabolic pathways that have been characterized using these techniques and analyzed to improve understanding of various physiological and pathological processes in the field of cardiology. In particular, we will focus on heart failure (HF) and how metabolomic analysis has contributed for improving our understanding of the pathogenesis of this critical condition.

Cardiac Metabolism

The number of patients with HF continues to increase and this condition has become a major healthcare issue in many countries. Because the prognosis of severe HF is poor, there are many unmet medical needs for these patients. The pathogenesis of HF is complex and a simple approach cannot describe the whole picture, but cardiac metabolism is one of the most critical factors. The heart is a unique organ destined to work continuously as a “pump” supplying blood to the body. To meet this requirement, the myocardium utilizes a massive amount of ATP. Complete turnover of the myocardial ATP pool is reported to occur within 10s, resulting in the cycling of approximately 6kg of ATP daily, and the heart displays metabolic flexibility to meet this extremely high demand for energy. Under normal conditions, more than 95% of the ATP consumed in the heart is generated by oxidative phosphorylation, while glycolysis is responsible for approximately 5% and the tricarboxylic acid (TCA) cycle for the remainder. Metabolism of fatty acids generates 70–90% of the ATP required by the heart, with the rest being produced by oxidation of glucose, lactate, ketone bodies, and amino acids. It is well known that utilization of fatty acids is reduced in the failing heart and there is a metabolic shift to generation of ATP from glucose. Such metabolic remodeling is considered to be reasonable because HF is associated with hypoxia and ATP generation per oxygen atom is more efficient when glucose is consumed, compared with fatty acids. In patients with advanced HF, the heart is unable to utilize either metabolite and thus “runs out of fuel.” It is reported that the ATP level is approximately 30% lower in failing human hearts compared with non-failing hearts. In addition to this classical premise about the metabolic profile of the failing heart, recent advances in the field of metabolomics have indicated that several metabolites and/or metabolic pathways have a role in HF, as described next.

Lipids

Under physiological conditions, the heart mainly generates ATP from fatty acids, but this process declines with progression of HF. Under left ventricular (LV) pressure overload, excessive lipolysis occurs in visceral fat because of increased adrenergic signaling, and this leads to high circulating levels of free fatty acids (FFAs). The serum...
level of non-esterified fatty acids (=FFA) is also increased in patients with HF. However, fatty acid uptake is reduced in several models of HF. In addition, a decrease in fatty acid oxidation has been reported in the compensated phase of LV hypertrophy, or during the late phase in other models of cardiac dysfunction. Cardiac uptake of FFA is reduced in patients with idiopathic dilated cardiomyopathy, but their FFA oxidation rate is similar to that in healthy controls. Interestingly, both FFA uptake and oxidation increase more when cardiac function declines further and systemic insulin resistance develops. Acylcarnitines are derivatives of fatty acyl-CoAs that reflect changes in mitochondrial fatty acid oxidation. Increases in short-chain, medium-chain, and long-chain acylcarnitines indicate activation of fatty acid oxidation, whereas a decrease in these metabolites suggests defective mitochondrial uptake and oxidation of fatty acids. Reduction in either the short-chain/medium-chain acylcarnitine ratio or the medium-chain/long-chain acylcarnitine ratio is generally thought to reflect defective mitochondrial β-oxidation, whereas an increase in these ratios implies activation of fatty acid oxidation. Circulating levels of long-chain acylcarnitine are increased in patients who have HF with a preserved ejection fraction (HfPeF) compared with individuals without HF, and are further increased in HF with a reduced EF (HfEF). Circulating levels of long-chain acylcarnitines are also increased in patients with end-stage HF compared with those who have chronic HF with systolic cardiac dysfunction, and this increase predicts the risk of hospitalization or death. Interestingly, in contrast to the increased level of circulating acylcarnitine, acylcarnitines are reported to be reduced in the failing heart itself, possibly caused by increased utilization, altered carnitine metabolism, or decreased acylcarnitine synthesis.

Phospholipids are molecules that generally have 2 hydrophobic fatty acid tails and a hydrophilic head, and form the major component of cell membranes. It is reported that degradation of phospholipids is accelerated in the ischemic myocardium, and another study identified alterations of cardiac phospholipid metabolism during pressure overload. Sphingolipids are lipids containing sphingoid bases that are known to mediate various signaling processes. Levels of several sphingolipids are reported to be reduced in models of HF caused by LV pressure overload or myocardial infarction (MI). Sphingosine-1-phosphate (S1P) is a bioactive cardiotrophic sphingolipid that is more abundant in plasma than in the tissues and is mainly associated with high-density lipoprotein (50–60%) or albumin (30–40%). S1P/SIP receptor 1 signaling was recently reported to contribute to Ca2+ homeostasis by maintaining the Na+/H+ exchanger 1 (NHE-1) in cardiomyocytes, thus having a cardioprotective effect in a myocardial ischemia-reperfusion model. The same authors also reported that cardiomyocyte-specific deletion of S1P receptor 1 resulted in progressive cardiomyopathy characterized by both systolic and diastolic dysfunction. In patients with ischemic heart disease and HF, severe systolic dysfunction (EF <40% vs. >40%) and advanced HF stage (New York Heart Association class III/IV vs. I/II) are associated with low plasma levels of S1P. Recently, patients with HF on metoprolol (β1-adrenergic receptor blocker) were shown to have high levels of circulating S1P and it was reported that metoprolol suppresses catecholamine-induced downregulation of S1P receptor 1 and promotes S1P production, indicating that the beneficial effects of β-blockers are partly mediated via activation of S1P/SIP receptor1 signaling. S1P binds to S1P receptor 1 and is thought to exert a cardioprotective effect through multiple pathways, including Akt signaling. Although there is some controversy as to whether S1P/SIP receptor 1 signaling is cardioprotective, the balance of evidence suggests that activation of S1P signaling may be a potential therapeutic target for HF.

Ceramide is another sphingolipid that has been extensively studied in the cardiovascular field. Ceramide is converted to sphingosine, which can be metabolized by isoenzymes of sphingosine kinase to yield S1P. Interestingly, although S1P is generally thought to be cardioprotective as just described, ceramide is an apoptotic metabolite that mediates lipotoxic responses in cardiomyocytes. The circulating level of ceramide is reported to be increased in patients with ischemic heart disease, and ceramide is thought to have a role in promoting atherosclerosis and plaque instability. In another study, the plasma level of ceramide showed a positive correlation with the severity of HF and with HF death. A diet rich in saturated fatty acids increases cardiomyocyte apoptosis, possibly caused by accumulation of ceramide in cardiac tissue. It was reported that reduction of the cardiac tissue level of ceramide had a cardioprotective effect in a mouse model of cardiomyocyte death. In addition, ceramide was shown to accumulate in the myocardium of patients with advanced HF, and those authors concluded that increased de novo ceramide synthesis promotes pathological progression in the failing heart. Ceramide has been shown to induce apoptosis by decreasing the mitochondrial membrane potential and cytochrome-c release through elevation of the cytosolic Ca2+ level. These studies indicate that maintenance of physiological lipid metabolism is critically important for preventing the progression of HF.

Metabolism of Glycolysis and the TCA Cycle

It is generally thought that fatty acids and glucose negatively regulate each other, with utilization of one substrate directly inhibiting use of the other, and this is known as the "Randle cycle". Progression of HF promotes utilization of glucose instead of fatty acids and in the advanced stage of HF the heart becomes unable to efficiently utilize either substrate. The absolute rate of glycolysis is approximately 10-fold higher than glucose oxidation. Pyruvate is produced by glycolysis and is catabolized by pyruvate dehydrogenase (PDH) to become acetyl-CoA and fuel the TCA cycle. In patients with HF, the myocardium is hypoxic because of the reduction in cardiac output and/or hypoperfusion, resulting in an increase of hypoxia inducible factor-1α (HIF-1α), which inhibits PDH and promotes cardiac glycolysis without activation of glucose oxidation. Accordingly, circulating levels of pyruvate and lactate are reported to be increased in HF and are a prognostic indicator for death. In rats with compensated cardiac hypertrophy generated by abdominal aortic constriction, cardiac glycolysis was increased without glucose oxidation. In a rat model of LV pressure overload, glucose oxidation was initially increased during the compensated phase of cardiac hypertrophy and then declined in the decompensated phase of HF. In a murine model of LV pressure overload, glucose oxidation, glycolysis, and lactate oxidation were reduced at 6 weeks after the surgery. However, glucose oxidation was unchanged in a rat model of MI with systolic dysfunction. Among the glycolytic intermediates, one
study found that only pyruvate was reduced in hearts under LV pressure overload. In hamsters with dilated cardiomyopathy, it was reported that glucose-6-phosphate, fructose-6-phosphate, fructose-1, 6-bisphosphate, malate, iso-citrate, and succinate were comparable between the cardiomyopathy group and control group at 4 weeks of age, but were all reduced in the cardiomyopathy group at 16 weeks of age. It is currently accepted that the alterations in glucose utilization vary according to the animal model, pathology, and stage of HF. In patients with severe HF, cardiac levels of glucose, glucose-1-phosphate, glucose-6-phosphate, lactate, citrate, α-ketoglutarate, succinyl-CoA, succinate, and fumarate are reduced, but glucose and lactate increase to the levels in the non-failing heart after implantation of a LV assist device. It was also reported that acetyl-CoA was increased and succinyl-CoA was reduced in cardiac tissue samples from end-stage HF patients undergoing heart transplantation compared with cardiac tissue from brain-dead donors with no history of HF. Considering that the changes in metabolites related to glycolysis and the TCA cycle vary among the rodent models, it is important to further characterize the metabolites involved in these pathways in humans.

Recently, it was reported that excitation-contraction coupling has a close relation with glycolysis in cardiomyocytes. The cardiac ryanodine receptor (Ryr2) is located in the sarcoplasmic/endoplasmic reticulum membrane and releases Ca from intracellular stores during excitation-contraction coupling. Dysfunction of Ryr2 occurs in HF and this leads to impairment of cardiomyocyte contractility. It was reported that heterozygous depletion of Ryr2 resulted in suppression of glucose oxidation, but that fatty acid oxidation, lactate oxidation, and glycolysis were not inhibited. In a murine Ryr2 knock-out model, systolic cardiac function was not affected but the heart rate was reduced, indicating that Ryr2 has a role in stimulating glucose oxidation in vivo. Fructose is a major dietary sugar, and a recent metabolic profiling study showed that fructose metabolism is elevated in the failing heart, which contributes to progression of cardiac hypertrophy. It is well accepted that the myocardium becomes hypoxic with cardiac hypertrophy and HIF-1α increases under such conditions. This leads to upregulated expression of splice factor 3b subunit 1, which mediates splice switching of ketohexokinase-A to ketohexokinase-C and promotes fructolysis to induce cardiac hypertrophy by upregulation of protein and lipid biosynthesis.

Amino Acids

Amino acids are essential for protein synthesis and are also utilized as substrates to generate energy. Under physiological conditions, it is thought that the heart has minimal reliance on amino acids for ATP generation and these molecules are primarily used for anabolic purposes. For example, oxidation of leucine only accounts for 3–5% of cardiac oxygen consumption in the isolated rat heart. However, amino acid metabolism may become important for cardiac energy production under hypoxic conditions. Glutamate, glutamine, and branched-chain amino acids (BCAAs) can serve as anaplerotic substrates for refueling the TCA cycle. In addition, valine and isoleucine can be catabolized to generate succinyl-CoA, and catabolism of leucine and isoleucine generates ketone bodies. Both succinyl-CoA and ketone bodies provide fuel for the TCA cycle, so catabolism of amino acids may have a beneficial effect on cardiac metabolism. It is reported that levels of several amino acids, including BCAAs such as valine, leucine and isoleucine, were increased in a rat model of HF. It is also reported that cardiac tissue levels of BCAAs are increased by both LV pressure overload and MI. Furthermore, examination of plasma metabolomic profiles showed that patients with HF who respond to cardiac resynchronization therapy have higher baseline levels of BCAAs (including valine, leucine, isoleucine, and phenylalanine) and lower glutamate levels than non-responders.

Although BCAAs appear to have therapeutic potential against HF, there is also evidence of a pathological role in cardiac metabolic dysfunction. It was recently reported that Krüppel-like factor 15 suppressed BCAA catabolism in a murine HF model, with impairment of BCAA catabolism reducing cardiac function and promoting HF in the setting of LV pressure overload, together with elevated superoxide production and cardiac metabolic remodeling. It is known that an increase in branched-chain α-keto acids directly reduces mitochondrial respiration by suppression of complex I activity, resulting in elevation of superoxide production, and pharmacological enhancement of BCAA catabolism ameliorates cardiac dysfunction in an LV pressure overload model.

It is reported that total free amino acids are increased in the failing right ventricle. Patients with HF are reported to have elevated circulating levels of phenylalanine and spermidine, as well as lower histidine levels. Measurement of these molecules may have a similar diagnostic value to B-type natriuretic peptide, and their levels were normalized in the patients who recovered. Moreover, patients with severe HF have reduced LV levels of methionine, phenylalanine, tyrosine, histidine, threonine, homoserine, glutamine, and alanine. Although the role of amino acid metabolism in HF is yet to be defined, studies have indicated that maintenance of amino acid homeostasis is crucial to suppressing the pathological progression of HF.

Other Metabolites

Ketones

Ketone bodies are generally produced in the liver and enter the circulation to be mainly utilized by the heart, kidneys, and brain, especially under fasting conditions. Ketone bodies are a source of acetyl-CoA and thus have the potential to maintain mitochondrial respiration in cardiac tissue. Oxidation of β-hydroxybutyrate provides less energy than fatty acid oxidation. Complete oxidation of 1 molecule of palmitate generates 105 molecules of ATP and consumes 46 atoms (23 molecules) of oxygen (ATP produced/O2 consumed [P/O] ratio of 2.33), whereas complete oxidation of 1 molecule of glucose generates 31 molecules of ATP and consumes 12 atoms (6 molecules) of oxygen (P/O ratio of 2.58). The P/O ratio of ketone bodies is 2.50 for ATP production compared with 2.33 for palmitate and 2.58 for glucose, so their efficiency for ATP production per oxygen atom is higher than that of fatty acids and lower than that of glucose. Under physiological conditions, FFAs (60–70%) and glucose (30%) are dominant metabolites utilized to generate ATP, but the contribution of ketone bodies to energy production increases when circulating levels of these metabolites become higher. Cellular uptake of ketone bodies depends on their blood concentration and this becomes significant under starvation conditions. Oxidation
levels of ketone bodies is increased in the failing hearts of both rodents and humans.\textsuperscript{13,56} Levels of β-hydroxybutyrate, a ketone body, are increased in the circulation of patients with HF, but are reduced in cardiac tissue.\textsuperscript{15} It is still unclear whether increased utilization of ketone bodies is a maladaptive or adaptive response. However, results from recent clinical trials have suggested that higher circulating levels of ketone bodies may suppress progression of HF. It is known that ketone body levels are elevated in patients taking sodium-glucose cotransporter 2 inhibitors.\textsuperscript{57} In the EMPA-REG outcome study, diabetic patients treated with the sodium-glucose cotransporter 2 inhibitor empagliflozin showed a 35% relative reduction in the risk of hospitalization for HF.\textsuperscript{58} and elevation of ketone bodies was suggested as one of the possible cardioprotective mechanisms. Studies have shown that the failing heart can utilize ketone bodies effectively, and ketone bodies may ameliorate cardiac dysfunction by improving cardiac efficiency.\textsuperscript{59} According to a recent report, cardiac utilization of ketone bodies is increased in patients with HF, and interestingly is suppressed in the skeletal muscles of the same individuals. Fatty acids, glucose, and ketone bodies are metabolized to acetyl-CoA, so increased metabolism of ketone bodies could suppress metabolism of the other energy sources and promote metabolic dysfunction. In a murine model of LV pressure overload, the hypertrophied and failing heart shifted to utilization of ketone bodies for ATP production at the expense of fatty acid oxidation.\textsuperscript{56,60,61} An increase in ketone body respiration might enhance mitochondrial protein acetylation and suppress mitochondrial activity. Despite these potential undesirable effects on the maintenance of energy homeostasis, studies performed in humans and rodents indicate a beneficial influence of enhanced ketone body metabolism in HF. Thus, further investigation is needed to delineate the exact mechanisms involved.

**Trimethylamine-N-Oxide**

Trimethylamine-N-oxide (TMAO) is generated from choline by the gut flora. It is reported that TMAO and choline are associated with advanced cardiac dysfunction and a worse prognosis in patients with systolic HF independent of other cardiac and renal biomarkers.\textsuperscript{62} The circulating TMAO level is elevated in patients with stable HF and predicted 5-year mortality.\textsuperscript{63} In addition, elevation of TMAO level is a risk factor for development of HF in both diabetic and non-diabetic individuals.\textsuperscript{64} In a murine LV pressure overload model, a diet enriched in TMAO or choline was shown to promote cardiac systolic dysfunction, together with severe cardiac fibrosis,\textsuperscript{65} indicating a causative role of TMAO in promoting HF pathology. In addition, it is reported that TMAO contributes to progression of atherosclerotic plaque by reducing reverse cholesterol transport.\textsuperscript{66} TMAO may enhance the pathological activity of endothelial cells, macrophages, and platelets, thus inducing organ dysfunction in the heart, kidneys, and vessels.\textsuperscript{67} Although further studies are needed to elucidate
the mechanisms through which TMAO promotes cellular dysfunction, suppression of TMAO may potentially become a next-generation therapy for atherosclerosis, HF, and kidney disease.

**Bile Acids**

Primary bile acids are synthesized by the liver and are excreted in the bile of mammals and other vertebrates. Through interactions with the colonic flora, the primary bile acids are converted to secondary bile acids. Studies have indicated that bile acids can act as signaling molecules, including a role in regulation of the cardiovascular system. In patients with HF, levels of primary bile acids are reduced and some secondary bile acids are increased, with this pattern being linked to shorter survival. Further studies are needed to assess the role of bile acids in HF, especially in relation to changes in the gut flora, and to determine whether modulation of this system could have therapeutic value.

**Conclusions and Future Directions**

Metabolomic analysis has become an essential tool for understanding the pathophysiology of cardiovascular disorders, and has contributed to the discovery of previously unappreciated metabolites or metabolic pathways involved in the progression or suppression of HF (Figures 1, 2). Metabolomic technology has also enabled us to find metabolites that may be candidate biomarkers for predicting cardiovascular events in the clinical setting. Development of technologies that allow analysis of metabolites at a single cell level (especially using cells from in vivo samples) is also an interesting field of exploration. There is no doubt that metabolomics will continue to make a huge contribution to understanding the pathology of HF, and may lead to breakthroughs in next-generation therapies for this disorder.

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