

Amino acids as metabolic substrates during cardiac ischemia

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Abstract

The heart is well known as a metabolic omnivore in that it is capable of consuming fatty acids, glucose, ketone bodies, pyruvate, lactate, amino acids and even its own constituent proteins, in order of decreasing preference. The energy from these substrates supports not only mechanical contraction, but also the various transmembrane pumps and transporters required for ionic homeostasis, electrical activity, metabolism and catabolism. Cardiac ischemia – for example, due to compromise of the coronary vasculature or end-stage heart failure – will alter both electrical and metabolic activity. While the effects of myocardial ischemia on electrical propagation and stability have been studied in depth, the effects of ischemia on metabolic substrate preference has not been fully appreciated: oxygen deprivation during ischemia will significantly alter the relative ability of the heart to utilize each of these substrates. Although changes in cardiac metabolism are understood to be an underlying component in almost all cardiac myopathies, the potential contribution of amino acids in maintaining cardiac electrical conductance and stability during ischemia is underappreciated. Despite clear evidence that amino acids exert cardioprotective effects in ischemia and other cardiac disorders, their role in the metabolism of the ischemic heart has yet to be fully elucidated. This review synthesizes the current literature of the metabolic contribution of amino acids during ischemia by analyzing relevant historical and recent research.

Keywords: amino acids, ischemia, cardiac, metabolism, cardioprotection

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Introduction

The heart is a highly active organ that consumes 10% of the body's total oxygen uptake and produces upwards of 35 kg of ATP every day.¹ This high rate of energy flux is required to accomplish the monumental task of pumping over 6500 L of blood per day at a relatively constant pressure and flow rate in the average human heart. This extraordinary amount of work requires a constant supply of metabolic substrates and oxygen (Figure 1).

An understanding of metabolism is essential for any study of the heart. Metabolism is the fundamental system that governs the entire organ's behavior and it ties together all fields of study, including molecular physiology, electrophysiology, toxicology and clinical cardiology. All cardiac behaviors are highly ATP-dependent and, without ATP, the heart will cease to function in a matter of minutes. In many types of heart disease and dysfunction, metabolism is the first area affected, which can then lead to channelopathies, ion imbalance, decreased contractile function, increased free radical production and cardiac death.

Amino acids play a central role in cardiac metabolism, but their cardioprotective roles as a source of acetyl-CoA and as a substrate for anaplerotic reactions during cardiac ischemia, their ability to contribute to NADH and FADH₂ production after reoxygenation and the conversion of glutamine and glutamate to free radical scavengers may not be fully appreciated.

Fortunately, the heart is also quite resilient, being able to maintain contractile function even under ischemic and anoxic conditions. It has been shown that an increase in non-oxidative ATP production in the ischemic and reperfused heart is associated with decreased cell death and increased functional recovery both *in vivo* and in culture, though the mechanism is not entirely clear.^{2–7} In this light, it is important to recognize the heart as a metabolic omnivore; it is capable of utilizing glucose, lactate, fatty acids, ketone bodies and certain amino acids as metabolic substrates.^{3,8–12}

This ability is especially important under conditions of prolonged stress or ischemia. Deprived of fatty acid

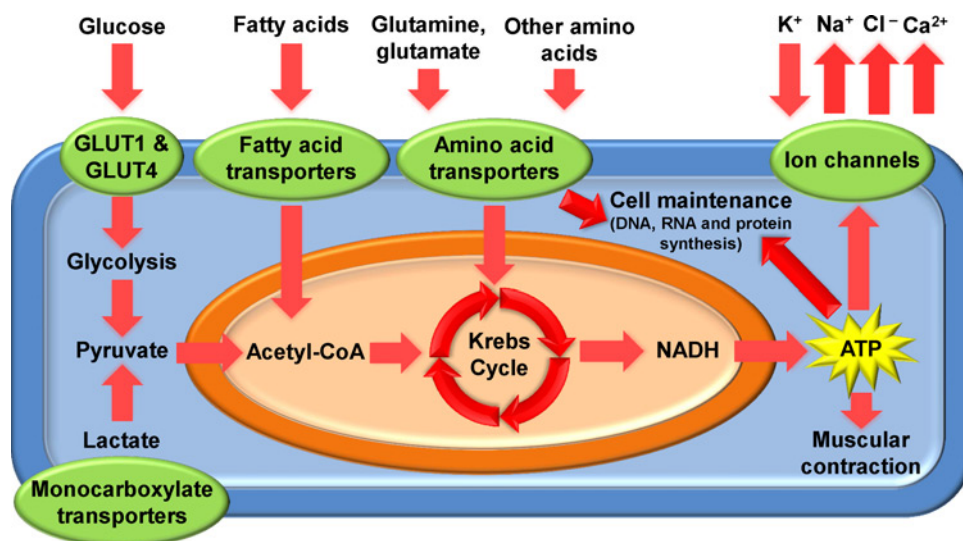


Figure 1 Metabolite supply and function in the heart. The heart is capable of oxidizing a wide variety of substrates to supply its vast energy needs. (A color version of this figure is available in the online journal)

oxidation by the loss of their oxygen supply, cardiomyocytes must derive energy from other molecules, including amino acids. It is well known that the heart's primary metabolic substrates under normal conditions are fatty acids and lactate, but under conditions unfavorable for oxidation, i.e. ischemia, anoxia and many types of cardiomyopathy, fatty acid oxidation is inhibited and the heart preferentially performs glycolysis and substrate-level phosphorylation.^{13,14}

Amino acids are now becoming more widely appreciated as cardioprotective substrates, as evidenced by the recent increase in excellent review articles detailing the importance of cardiac amino acid metabolism.^{15,16} Glucose, fatty acids and other substrates require oxygen for full energy yield and produce significant levels of acidic by-products.¹³ Amino acids are of particular interest in this regard due to their potential for non-oxidative metabolism and their low contribution to cellular acidification. It may be possible to prolong cellular function during – and improve recovery after – anoxia by supplementing cells with glutamate and glutamine due to the ease with which these particular amino acids can be converted to α -ketoglutarate, a Krebs cycle intermediate.^{17–20} Other amino acids, such as asparagine and aspartate, may also be important metabolic indicators due to their ability to remove amine groups and excess Krebs cycle intermediates downstream from the conversion to succinate. The ability to produce ATP directly from glutamine and glutamate through substrate-level phosphorylation makes these amino acids important for ischemic and hypertrophied hearts as they begin to suffer loss of function and increasing metabolic damage from free radicals and low pH. It should be noted, however, that glutamate and glutamine can provide only a small amount of ATP through this mechanism and that the reducing environment of the ischemic heart can inhibit these reactions. Each molecule of glutamate or glutamine can produce one GTP and one NADH molecule in their conversion to succinate. This is, however, an anaerobic process,

does not contribute to acidification and may serve to maintain the levels of NADH and Krebs cycle intermediates through an ischemic event, keeping the metabolic machinery primed to begin oxidative phosphorylation as soon as oxygen returns.

Since amino acids are synthesized in a wide variety of pathways and reactions, some amino acids are more readily converted to metabolic intermediates than others. This is especially true in the heart, as the heart is completely unable to metabolize the aromatic amino acids, whereas alanine is synthesized and secreted in abundance under even the most favorable conditions. Other amino acids, like glutamate, glutamine, aspartate, asparagine and the branched chain amino acids (BCAAs), have been shown to be preferentially used as metabolic and anaplerotic substrates in the Krebs cycle during anoxia and ischemia.^{21–23}

Although it is well known that the heart is capable of metabolizing a wide variety of substrates (Figure 2), the heart's substrate preference under anaerobic conditions is not well understood and is very sensitive to the relative concentration of available substrates and relevant hormones. Anoxic and ischemic tissues suffer from several issues of supply and demand: (1) inadequate substrate supply, (2) unmet energetic demands, (3) excess acid and reactive oxygen species (ROS) production and (4) inadequate buffering capacity for these metabolic by-products. Without oxygen, the heart cannot break down fatty acids and ketone bodies into acetyl-CoA. This becomes especially important during acute ischemia, as a lower pH also inhibits glycolysis, forcing the heart to utilize metabolic substrates that do not require oxidation and do not require glycolytic conversion, which contributes to increased acidification. Certain amino acids could serve in this capacity.¹²

It is important to note that while amino acid metabolism may have several significant functions and provides a measure of protection against ischemia and anoxia, it is not capable of sustaining the heart's extraordinary energy demands for extended periods. Amino acids should be

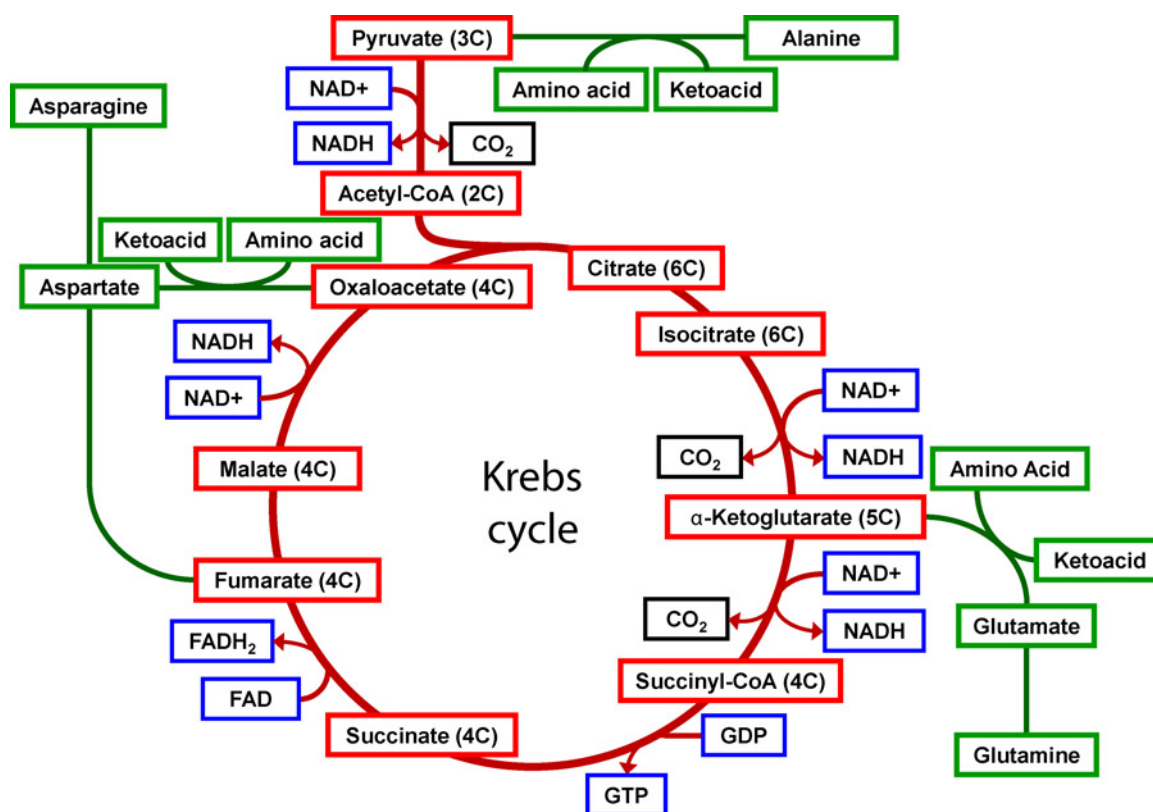


Figure 2 Amino acids and the Krebs cycle. The amino acid conversions are all reversible and hence are not drawn with arrows. In the glutamate, alanine and aspartate transamination reactions, any amino acid can be used as an amine donor, converting it to its respective ketoacid. (A color version of this figure is available in the online journal)

viewed as an adjuvant for standard therapies in that they will extend the length of time between the onset of ischemia and the point of irreversible cardiac damage. In the case of cardiac ischemia, ROS production and metabolic demands will far outstrip the supply of antioxidants and metabolic substrates, but even minor increases in these stores should improve the function and recovery of the tissues over unsupplemented hearts.

Amino acid metabolism in the heart

Amino acids seem to be especially well suited for use as metabolic substrates in the absence of adequate tissue perfusion. Glutamate and glutamine are notable due to their ready conversion to α -ketoglutarate, which not only maintains the levels of Krebs cycle intermediates, but also provides cellular energy through substrate-level phosphorylation during the conversion to succinate. This is an oxygen-free metabolic conversion, with no acidic or reactive by-products. Indeed, changes in amino acid flux have been seen at rest as well as during ischemia, indicating that repeated bouts of acute ischemia induce biochemical changes that enhance the heart's ability to utilize these alternative substrates.²¹ Peuhkurinen *et al.*²⁴ specifically indicates that chronic ischemia results in increased glutamate uptake and alanine release. Nishimura *et al.* demonstrated the heart's ability to utilize valine as an alternative to glucose in order to maintain action potential duration.³

Studies conducted by Schwartz *et al.*²² indicated that BCAAs are metabolized by the myocardium even during normal cardiac function, though this has not been seen in all studies.^{21,23} The heart is unable to produce the aromatic amino acids (phenylalanine, tyrosine and tryptophan), so uptake of other amino acids without commensurate uptake of aromatic amino acids indicates that the amino acids are not being used for protein synthesis.^{1,8-10,25} Indeed, amino acids are still consumed even when there is no net protein synthesis or when protein is being degraded, indicating that the amino acids are being metabolized.²⁴⁻²⁶ Glutamine supplementation leads to rapid glutamine uptake and increased levels of intracellular glutamate due to high levels of glutaminase activity in the heart.¹⁷ Cardiac ischemia and cell damage therefore are expected to be accompanied by altered amino acid flux during and after ischemic episodes.

Amino acids can be readily metabolized into Krebs cycle intermediates; however, not all amino acids are metabolized equally in the heart. There are several different processes by which amino acids can be used to supply cardiomyocytes with energy (Table 1):

- (1) Glutamate and α -ketoglutarate are readily interconvertible via a transamination reaction. This increase in Krebs cycle intermediates is called anaplerosis and contributes to the maintenance of oxidative capacity. The α -ketoglutarate can then be processed through the Krebs cycle to yield NADH and GTP. Glutamine can

Table 1 Amino acids and their role in cardiac metabolism

Amino Acid	Plasma Conc. ($\mu\text{mol/L}$)	Metabolic Role in the Heart
Alanine	230–510	Alanine is interchangeable with pyruvate via transamination.
Arginine	13–64	Arginine plays an important role in nitrogen balance. In the urea cycle, the enzyme arginase hydrolyzes the guanidinium group to yield urea and the L-amino acid ornithine.
Asparagine	45–130	The amide in asparagine is easily hydrolyzed, converting asparagine to aspartate.
Aspartate	0–6	Aspartic acid and oxaloacetate are interconvertible by a simple transamination reaction, just as alanine and pyruvate are interconvertible.
Cysteine	30–65	Cysteine can react with itself to form an oxidized dimer by formation of a disulfide bond. The environment within a cell is too strongly reducing for disulfides to form, but in the extracellular environment, disulfides can form and play a key role in stabilizing many such proteins, such as the digestive enzymes of the small intestine. This may affect amino acid measurements if not kept in a reducing environment.
Glutamate	18–98	Glutamic acid and α -ketoglutarate are interconvertible by transamination. Glutamic acid can therefore enter the Krebs cycle for energy metabolism, and be converted to glutamine, a crucial molecule in nitrogen metabolism. It is also readily converted to proline.
Glutamine	390–650	Glutamine is the amide of glutamic acid, and is uncharged under all biological conditions. It is rapidly taken up in the heart and converted to glutamate.
Glycine	170–330	Glycine is the smallest amino acid, and it acts as a precursor in many synthetic pathways.
Histidine	26–120	Histidine is probably not actively metabolized by the working heart, but little is known about its role in the heart.
Isoleucine	42–100	Isoleucine is one of the three amino acids having branched hydrocarbon side chains. It is usually interchangeable with leucine and occasionally with valine in proteins.
Leucine	66–170	Leucine is one of the three amino acids having branched hydrocarbon side chains. It has one additional methylene group in its side chain compared with valine.
Lysine	150–220	The lysine amino group is highly reactive and often participates in enzymatic reactions.
Methionine	16–30	Methionine as the free amino acid plays several important roles in metabolism. It can react to form S-adenosyl-L-methionine (SAM), which serves as a methyl donor in reactions. Unlike cysteine, the sulfur of methionine is not highly nucleophilic, although it will react with some electrophilic centers. It is generally not a participant in the covalent chemistry that occurs in the active centers of enzymes.
Phenylalanine	41–68	Phenylalanine is quite hydrophobic and even the free amino acid is not very soluble in water. This is one of the amino acids that is not metabolized by the heart, and is therefore a good marker for protein flux.
Proline	110–360	Proline is technically an imino acid; nonetheless, it is called an amino acid. It is unknown what role it may play in cardiac metabolism.
Serine	56–140	Serine differs from alanine in that one of the methylenic hydrogens is replaced by a hydroxyl group.
Taurine	45–130	Taurine is a very important amino acid for the heart. It has been shown to exert cardioprotective effects by reducing the severity and rate of arrhythmias and improving contractile function.
Threonine	92–240	Threonine is another hydroxyl-containing amino acid. It differs from serine by having a methyl substitution in place of one of the hydrogens on the β carbon, and it differs from valine by replacement of a methyl substitution with a hydroxyl group. Note that both the α and β carbons of threonine are optically active, which may affect detection.
Tryptophan		Tryptophan, an essential amino acid, is the largest of the amino acids. The indole functional group absorbs strongly in the near ultraviolet part of the spectrum. This is one of the amino acids that is not metabolized by the heart, and is therefore a good marker for protein flux.
Tyrosine	45–74	While tyrosine is hydrophobic, it is significantly more soluble than phenylalanine. This is one of the amino acids that is not metabolized by the heart, and is therefore a good marker for protein flux.
Valine	150–310	Valine differs from threonine by replacement of the hydroxyl group with a methyl substitution. Valine is often referred to as one of the amino acids with hydrocarbon side chains, or as a branched chain amino acid. Note that valine and threonine are of roughly the same shape and volume. It is difficult even in a high resolution structure of a protein to distinguish valine from threonine.

be converted to glutamate, or glutamate can be amidated in order to transport amine groups out of the cell. Glutamate, however, is also a neurotransmitter and may interfere with normal function of the nervous system, causing unintended side-effects. Glutamine does not suffer from this shortfall and can be supplemented at very high levels relative to glutamate.²⁷

- (2) Asparagine can serve as a means of exporting nitrogen from the cell, or it can be converted to aspartate. Aspartate can be transaminated to oxaloacetate, a crucial regulator of levels of Krebs cycle intermediates. This reaction is critical, as it allows aspartate and asparagine stores to serve as sinks for excess oxaloacetate produced from supplemented α -ketoglutarate precursors.
- (3) Alanine can also be transaminated to become pyruvate. Pyruvate oxidation produces three molecules of NADH and one of GTP. However, alanine is usually transported out of the myocardium to remove amine groups from the working cell.
- (4) Many amino acids can be catabolized by the heart, through many different pathways, and the energy recovery from each will vary.^{23,24,28,29} The BCAAs, for example, can be converted to acetyl-CoA, pyruvate or succinyl-CoA through somewhat lengthy conversion processes, or transaminated with 2-oxoglutarate to form their respective oxoacids and glutamate.²⁴ This makes their involvement in metabolism difficult to quantify. A recent review by Huang *et al.*¹⁵ explored the important role of BCAAs in the failing heart.

In the fully functional heart, amino acid metabolism makes up a very small percentage of cardiac ATP production, but as the heart becomes oxygen-limited, amino acids become more important as a fuel source.^{8,9,20,29} Without oxygen, the fatty acids cannot be broken down into acetyl-CoA.^{13,30} There is also a decrease in fatty acid consumption and an increase in glycolysis in hypertrophied hearts, despite sufficient oxygen.^{12,31,32} Glucose and glycolysis become the primary means of producing ATP, but this yields far less energy per molecule and produces two protons per molecule of glucose consumed.^{9,12,33,34} This energy deficit and acidification increase become especially important during acute ischemia, as a lower pH inhibits glycolysis.^{1,12} The heart is eventually unable to produce sufficient ATP through glycolysis, and will out of necessity turn to anaerobic, non-glycolytic fuels, such as amino acids. It is unlikely that these fuels will be able to meet the cardiac metabolic demand for more than a brief interval, but any increase in function is associated with lower mortality.

Amino acids are of particular interest as metabolites in the ischemic, diseased or hypertrophied heart for many reasons. They are plentiful in the cell, though most are bound in proteins and unavailable for immediate metabolism. This is particularly true during acute ischemia as proteolysis is decreased.^{1,24} The limited availability of myocardial proteins and the potential loss of function and increasing metabolic damage from free radicals and low pH create an important role for the provision of a blood-borne amino acid supply as a potential substrate for ATP formation in ischemic and hypertrophied hearts. It may be possible to improve recovery and maintain function by gaining a more thorough understanding of the role amino acids play in ischemia and hypertrophy.

Cardiac ischemia, typically the result of the partial or complete occlusion of a coronary artery, is manifested as a fall in intracellular levels of ATP, glycogen, glutamate and aspartate and increased lactate production and alanine-to-glutamate ratios. It has been proposed that supplementation with exogenous glutamine, glutamate and aspartate may enhance cardiac metabolism when administered in the reperfusate after removal of coronary blockages.^{17-20,35} Amino acid supplementation contributes to cardioprotection in several ways: (1) anaplerosis of Krebs intermediates, (2) elimination of inhibition of succinate-CoA reductase by oxaloacetate and increased substrate-level phosphorylation, (3) providing high levels of NADH and FADH₂ for rapid oxidation once oxygen returns and (4) the production of reduced glutathione, an antioxidant that can protect the heart from free radical damage.^{17,36}

In a study by Julia *et al.*,²⁹ immature dog hearts were found to be more tolerant of ischemic conditions than mature hearts and also to contain higher intrinsic levels of glutamate. Other studies have shown that elevated levels of intracellular glutamate are at least partially responsible for the heart's resistance to ischemic damage.¹⁷⁻¹⁹ Blockage of glutamate transamination using aminooxyacetic acid (AOA) inhibits glutamate and aspartate utilization and reduces the heart's ability to recover from ischemic episodes. Specifically, the blockage of glutamate

transamination in tissue homogenates resulted in an immediate decrease in lactate, alanine and succinate production, all of which should be produced by tissue homogenates. In the absence of AOA, the hearts were able to withstand 45 min of global ischemia, requiring no external stimuli to resume normal function and showing little or no ill effect from the prolonged insult. Administration of AOA, however, resulted in severe loss of function after the same period. It also resulted in much less lactate, alanine and succinate production, and much lower glutamate conversion.²⁹ These results demonstrate that cardiac function and long-term recovery require glutamate and aspartate transamination, while blockage of transamination results in decreased recovery and increased mortality.²⁹ Cardiac transaminases are so prevalent, in fact, that their presence in the blood was diagnostic for heart damage for many years before troponin and creatine kinase tests.

Glutamate and aspartate have very large muscle/plasma ratios, indicating that they are taken up into cells with high affinity. However, during ischemia the large concentration gradient leads to their release^{24,35} because of the loss of transporter function that normally maintains the gradient. Cardiac cells supplemented with excess glutamate show significantly increased NADH/NAD⁺ ratios and ATP levels *in vitro*. A fall in intracellular glutamate is associated with increased alanine production and decreased ATP concentrations after at least 10 min of ischemia.^{24,36} Glutamate loading also improves the cells' contractile activity and Ca²⁺ homeostasis after chemical hypoxia.^{35,36} Increased intracellular glutamate stores are correlated with improved metabolic recovery, increased cardiac output and elevated glutathione levels after an ischemic episode.¹⁷⁻¹⁹

Ischemia significantly reduces the stores of tissue glutamate, ATP and glutathione while increasing lactate levels.¹⁷ In response to ischemia, the heart will significantly enhance alanine production and glutamate consumption, though these effects become significant only when the partial pressure of oxygen in the tissue falls to 5% of normal levels. While the uptake of glutamate does not directly correlate with alanine production and release, it is a contributor under reduced oxygen conditions.²³ Though glutamate is taken up by the heart, intracellular stores of the amino acid are readily transaminated with aspartate and alanine, allowing a greater storage and flux capacity than might otherwise be expected.²⁴ Glutamate and glutamine may also work as reservoirs to protect against postischemic reduction in cardiac output by maintaining metabolic intermediates.²⁸ Glutamate and glutamine are readily interconvertible in the heart and may serve as a means of controlling levels of Krebs cycle intermediates via conversion of glutamate to α -ketoglutarate.¹⁹

Glutamine supplementation prior to an ischemic event improves cardiac function and reduces free radical damage by improving ATP production and content, oxidative capacity and glutathione content.^{17,19,20} Studies have shown that glutamine supplementation significantly increases glutathione and reduces cardiac damage from surgery, toxins and presumably from transient ischemia as well.^{37,38} Glutamine does not appear to activate heat shock

proteins but it does increase myocardial COX-2 levels, which are associated with decreased infarct sizes and reduced ROS damage during reperfusion and subsequent ischemia.³⁹

Despite the evidence supporting the role of glutamate in the ischemic heart, it may not be clinically feasible to supply intravenous glutamate due to possible neural and cardiac toxicity at high plasma concentrations (20–70 mmol/L).¹⁷ A single dose of oral or intravenous glutamine was shown to improve tissue glutamate levels without the need for high levels of glutamate in the plasma.^{17,18} This is due to the presence of high-affinity transporters for glutamine in the heart, and a high level of glutaminase activity allowing the heart to rapidly convert circulating glutamine into intracellular glutamate stores. The improved cardiac performance from glutamine has been seen with circulating glutamine levels as low as 1.25 mmol/L, a level easily achieved by an oral dose of glutamine.^{17,18} Intracellular glutamate protects against loss of Krebs cycle intermediates and improves postischemic oxidative metabolism, which may help to explain the increased ATP content in the glutamine-treated hearts. Glutamine supplementation increased postischemic phosphocreatine and NAD⁺/NADH content after ischemia and reperfusion while decreasing lactate production. Glutamine's protective effects have been seen even when administered 18 h before ischemia, though the effects are greater when administered 4 h before ischemia.^{17,18} This has important clinical implications as it has allowed cardiac surgeons to easily and reliably administer prophylactic glutamine treatments before surgeries.

Glutamine cardioprotection is also associated with increased levels of UDP-GlcNAc and protein O-GlcNAc, nucleoplasmic proteins which have been shown to protect against ischemic injury and other damaging events.²⁰ Blockage with azaserine and alloxan of the pathways that lead to the formation of these molecules eliminates the protection afforded by glutamine supplementation. Glutamine alone increased ATP levels three-fold after ischemia and reperfusion compared with controls, but the addition of azaserine and alloxan reversed this effect.²⁰

Conflicting reports about whether amino acid supplementation is actually cardioprotective may be attributed to the effects of differing K⁺, Na⁺ and H⁺ gradients, which may vary widely across experimental preparations.^{35,36} Inclusion of aspartate, glutamate and glutamine in animal heart perfusates and cardioplegic solutions improves metabolic and functional recovery and elevates high-energy phosphate levels postischemia, and they have been used experimentally in cardioplegic solutions with good results.^{17,18,27,40} Questions regarding the efficacy of these amino acids may arise from xenotypic differences, protocol incompatibility or the use of free acids versus amino acid salts.³⁵

Another assumption is that perfused amino acids are readily available for intracellular functions, without regard to transporter activity. Glutamate has a very high rate of uptake in isolated cells, and this uptake is enhanced by anoxia. The cells are also able to establish intracellular glutamate stores against very high gradients.⁴¹ Rennie *et al.*¹¹

explored the role of glutamine transporters in both cardiac and skeletal muscle and showed the beneficial effects of glutamine supplementation and possible mechanisms of glutamine uptake stimulation. A more general view of amino acid transporters and their distribution was taken by Malandro and Kilberg.⁴²

Despite an abundance of evidence showing that amino acid supplementation improved myocardial protection during ischemia and surgery, relatively little is known about endogenous amino acid metabolism or the exact mechanism of the cardioprotective effect. While the use of amino acid supplementation for cardioprotection has been explored, there are few studies addressing electrophysiological changes concomitant with the supplementation.

Data from human subjects with exercise-induced ischemia showed that these patients release significantly more alanine during cardiac ischemia and take up more glutamate than non-ischemic subjects.²¹ This has been seen in other model systems as well, indicating that this is a conserved process.²⁴ Changes in amino acid flux were seen not only during acute ischemic episodes, but for many days after the episode, indicating that bouts of acute ischemia induce biochemical changes that enhance the heart's ability to utilize alternative substrates.

Alanine is produced and secreted by the heart under almost all conditions, including hypoxia and ischemia. This has been demonstrated in patients with chronic ischemic heart disease²⁴ and exercise-induced ischemia,²¹ as well as in hypoxic²³ and ischemic³⁵ heart tissues and insulin-clamped working hearts.²² Large amounts of alanine can be found in hypoxic tissues, including the tissues of diving mammals and hypoxic skeletal muscle, especially in chronic ischemic heart disease. Taegtmeier *et al.*²³ showed that alanine production was not significantly enhanced until the concentration of oxygen was decreased to less than 5%. In another study, glutamate supplementation had a negligible effect on papillary muscle alanine production in the presence of glucose in both aerobic and anaerobic conditions, and glutamate consumption was not enhanced until the oxygen concentration of the buffer was reduced to 5%.^{23,36} L-Cycloserine, an inhibitor of alanine aminotransferase, markedly decreased intracellular alanine and increased glutamate levels, even above baseline levels, showing that glutamate is not directly responsible for all of the observed alanine production, although it is a contributor. This also indicates that alanine production is most likely due to glutamate/pyruvate transamination, but acute alanine production may not necessarily reflect the rate of glycolytic pyruvate production, since ischemia could be causing the release of intracellular stores of alanine.^{23,29}

Pyruvate levels in the cell decrease by 50% during ischemia, and lactate levels in the surrounding media increase 27-fold after 20 min of ischemia.²⁴ Another study showed that increasing the levels of pyruvate in the media results in increased alanine production in both aerobic and anaerobic cells, indicating that substrates are the limiting factor in alanine production and supporting the idea that alanine is produced via transamination of pyruvate.²⁹ In glucose-free media, lactate and alanine production is increased

compared with normal media, and inhibition of glycolysis suppresses this production significantly.²³ Ammonia is also produced by the deamination and deamidation of amino acids, and has been measured at 5.5 times normal levels during ischemia.²⁴

Though the addition of amino acids may improve metabolic function, it does not increase protein synthesis until amino acids are present at five times normal physiological concentrations. Glycine, alanine and glutamate showed net myocardial release under non-ischemic insulin-supplemented conditions. The BCAAs leucine and isoleucine were taken up by the heart in a concentration-dependent manner. No other amino acids showed significant uptake or release with or without insulin supplementation.²² It had been previously demonstrated that glutamine and asparagine were released and leucine and isoleucine were consumed in humans, though Schwartz *et al.* improved glutamine detection methodology and revealed a higher rate of glutamine uptake than had been seen in earlier studies. Despite these findings, BCAA supplementation has shown negligible uptake or improved performance in subsequent studies of ischemia.^{23,24,35}

Any increased uptake of BCAAs likely results in a corresponding increase in their metabolism, since net protein synthesis is negligible over the course of an ischemic episode, and cellular concentrations would have been 2–4 times higher than the arterial concentration were they not metabolized. However, conversion to ketoacids or oxidation necessitates removal of amine groups from the BCAAs with no commensurate increase in alanine or glutamate, so it is unclear exactly how they are metabolized. Since these amino acids have several pathways through which they can be converted, the nitrogen may be lost through any number of metabolites that were not measured.²²

Amino acids as protein precursors

In the healthy heart, the aromatic amino acids (phenylalanine, tryptophan and tyrosine) are not metabolized.²⁶ This makes these amino acids useful markers for protein turnover experiments, as any aromatic amino acid released from the heart must have come from proteolysis, and any aromatic amino acids taken up by the heart are almost certainly being incorporated into nascent protein.²⁵ Protein turnover, however, is a balance between degradation and synthesis, so a zero net aromatic amino acid flux does not necessarily indicate a lack of protein turnover, but rather a balance between proteolysis and protein synthesis.

Studies of phenylalanine incorporation have shown that elevated levels of amino acids lead to greater protein synthesis.²⁶ The human heart contains $284 \pm 17 \mu\text{mol}$ of phenylalanine per gram, and Morgan *et al.* saw that $1.24 \mu\text{mol}$ were incorporated in 90 min, indicating 14.3 d for complete protein turnover *in vitro*. *In vivo* turnover may be faster, since incorporation rates are lower *in vitro* than *in vivo*. The elevated rates of incorporation were not due to increased ATP levels, as an aggregation of ribosomes was seen, but there was no commensurate rise in the ATP/p-creatine ratio.²⁶

Another corollary between amino acid metabolism and ischemia is that long-term ischemia/damage is expected to result in increased proteolysis and contribute to the decline in long-term cardiac function after ischemia, though it should be noted that proteolysis decreases during acute ischemic episodes to 12% of normal.²⁴ In the presence of puromycin (a protein synthesis inhibitor), tyrosine was produced by cells under aerobic and anoxic conditions equally in the presence of 5 mmol/L glucose. Alanine production was not affected by these conditions. Under anoxic conditions without puromycin, twice as much tyrosine was produced as under aerobic conditions, indicating an increase in the rate of proteolytic aromatic amino acid production.²³ This means that protein degradation during oxygen deprivation should be manifested as an increase in the rate of aromatic amino acid release from the myocardium.

In an interesting study by Razeghi *et al.*, a heterotopic anastomized heart model was used to simulate cardiac atrophy. The heart was removed from one rat and placed in another rat to create a cardiac bypass to decrease the load on the main heart, resulting in atrophy. Results showed proteolysis and protein synthesis occurring simultaneously in atrophic re-modeling, rapidly in the first two days, peaking between two and four days and slowing considerably by 28 d. This demonstrates that re-modeling requires activation of both degradative and synthetic pathways, but that the difference between the two would be manifested in an altered flux of the aromatic amino acids. These results can be extended to chronic ischemia, hypertrophy and several other conditions as there is increased protein synthesis, but also increased protein turnover, in long-term cardiac diseases.⁴³

Detection methods

Amino acid monitoring in the heart has historically been done by measuring the difference in amino acid concentrations between coronary arterial input and coronary venous output across the whole heart. Many pioneering studies in cardiac physiology used this method and were able to establish the foundations of cardiac metabolism. That said, A-V difference measurements are insufficiently precise for modern studies, and many methods are now in use for precisely quantifying the amino acid flux in the myocardium, as well as identifying the metabolic fates of the molecules.

The most accurate and widely used amino acid monitoring technique is the radiolabeled tracer study.^{19,41,44} These labels can be detected with positron emission tomography for imaging amino acid accumulation in the tissues as well as quantifying media depletion in a culture setting. Similarly, nuclear magnetic resonance studies detect the labels and can track the labeled carbon or nitrogen through its many conversions to determine how the amino acid was metabolized.⁴⁵ Mass spectrometry is also a common method of quantifying amino acid flux, as it is highly sensitive to even small changes in concentration and is capable of very rapid processing of large numbers of samples.⁴⁶ It has the additional benefit of being highly adaptable to

preseparation protocols like gas chromatography and ion mobility-mass spectrometry.^{47–51}

Unfortunately, once amino acids enter the Krebs cycle, carbon labeling becomes much more difficult to track, as the carbons are fungible. The appearance of labeled CO₂ is highly indicative of amino acid oxidation, but it does not necessarily mean that the entire amino acid has been oxidized or that the conversion was direct, since there are many paths that feed into and out of the Krebs cycle. Nitrogen labeling is reliable insofar as oxidized amino acids must release NH₃, but there are many ammonia acceptors within the cell. Often the amine is transferred to pyruvate to form alanine or α -ketoglutarate to form glutamate and glutamine, but this is not always the case.⁴⁴

Interventions

Heart failure is caused by an imbalance in supply and demand, and often the deficit is in oxygen supply. Interventions that shift metabolism away from fatty acids to substrates with greater ATP produced/O₂ consumed ratios will have antianginal effects. Current medical treatments focus primarily on increasing cardiac output and decreasing afterload, but few treatments attempt to rectify the underlying metabolic dysfunction of the failing or ischemic heart. Medications focus on lowering blood pressure, fluid retention and cardiac work to relieve symptoms of acute and chronic heart failure while the accompanying alterations in cardiac metabolism are left untreated. A few drugs for treatment of the metabolic disease state – for example, trimetazidine – have been developed recently to treat the underlying metabolic disease state instead of just the symptoms and have shown promise.^{52,53} We need to continue learning more about cardiac metabolism if we wish to effectively treat the underlying metabolic derangement. To turn the tide in the battle against heart disease, we must understand in detail how changes in metabolic substrate affect electrophysiology, and we need to find new ways to optimize the heart's fuel usage and minimize the damage incurred by transient ischemia.

Conclusion

The fact that amino acids are not only protein precursors but can also be metabolites, neurotransmitters and antioxidants makes them ideal candidates for determination of underlying cellular functioning in the age of metabolomics. It is already clear that uptake and release of certain amino acids can give a great deal of information about intracellular processes. Even without looking inside the cell, it is recognized that if tyrosine, phenylalanine or tryptophan show net uptake by cardiomyocytes over a period of days or weeks, then they are being incorporated into new proteins. This would indicate not simply protein turnover, but an increase in the total protein pool. Such information can be used to determine the effects of pharmacological agents or other interventions that perturb normal cell function.

Computational models of cardiac myocyte excitation are beginning to include contraction and energy

metabolism.^{54–56} However, we believe that these models need also to include amino acid pathways and fluxes to accurately model the metabolic events that accompany both chronic and acute ischemia and other diseased states of the heart. Such models would also benefit from the inclusion of models of intracellular pH regulation, as it is associated with ischemia and affects many metabolic reactions.

The versatility of amino acids makes them ubiquitous across all cells and tissues. If alterations in amino acid flux can be linked to abnormal cell behavior, tools to monitor amino acid flux could serve to evaluate tissue viability. If, for example, a donor heart could be transported with an amino acid detector, its health could be monitored in transit, and transplant surgeons would have a better understanding of its viability and more information about its expected longevity once it is implanted. If the heart displays characteristics associated with starvation or stress, then special interventions may be needed to improve its condition *before* it is implanted. This scenario is still not possible, but with advances in metabolic monitoring, it becomes a very real possibility in the not-too-distant future.

In summary, we have reviewed the import of the role of amino acid metabolism in the normal and failing heart. Our review of the literature would suggest that additional research is required to understand how amino acid supplementation affects not only the metabolic but also the mechanical and electrical performance of the heart. Comprehensive metabolic profiles of healthy, ischemic and postischemic hearts are needed to fully understand the complex processes involved in ischemia- and reperfusion-induced damage. We hope that this review clearly illustrates the need to unite the disparate fields of electrophysiology and metabolism to understand the complex interplay between action potential and metabolic flux.

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