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Metabolomics assessment reveals oxidative stress and altered energy production in the heart after ischemic acute kidney injury in mice

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Author contributions

HG, CA, and SF designed the study; HG and CA carried out the experiments; DA, MW, and JH performed the metabolomics portion of the experiment; BMF, SF, DA, JH, MW, and NC performed metabolomics data analysis; RAB quantified cardiac ATP levels and SAW and KRH performed echocardiographic analyses; LKR and CA performed blood pressure measurements. CA performed pH and potassium measurements. All authors participated in data interpretation and manuscript preparation; BMF and SF drafted and prepared the final version of the paper.

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Abstract

Acute kidney injury (AKI) is a systemic disease associated with widespread effects on distant organs, including the heart. Normal cardiac function is dependent on constant ATP generation, and the preferred method of energy production is via oxidative phosphorylation. Following direct ischemic cardiac injury, the cardiac metabolome is characterized by inadequate oxidative phosphorylation, increased oxidative stress, and increased alternate energy utilization. We assessed the impact of ischemic AKI on the metabolomics profile in the heart. Ischemic AKI was induced by 22 minutes of renal pedicle clamping, and 124 metabolites were measured in the heart at 4 hours, 24 hours, and 7 days post-procedure. 41% of measured metabolites were affected, with the most prominent changes observed 24 hours post-AKI. The post-AKI cardiac metabolome was characterized by amino acid depletion, increased oxidative stress, and evidence of alternative energy production, including a shift to anaerobic forms of energy production. These metabolomic effects were associated with significant cardiac ATP depletion and with echocardiographic evidence of diastolic dysfunction. In the kidney, metabolomics analysis revealed shifts suggestive of energy depletion and oxidative stress, which were reflected systemically in the plasma. This is the first study to examine the cardiac metabolome after AKI, and demonstrates that effects of ischemic AKI on the heart are akin to the effects of direct ischemic cardiac injury.

Keywords

organ cross talk; metabolomics; cardiorenal syndrome; acute kidney injury

Introduction.

Acute kidney injury (AKI) is a common complication in hospitalized patients, and independently increases mortality rates.[1,2] Clinical and basic research into the mechanisms by which AKI leads to increased mortality has revealed that AKI is a systemic disease with widespread deleterious effects on distant organs, including the heart [3–5] whereby cardiorenal syndrome type 3 defines the syndrome in which AKI causes acute cardiac dysfunction.[6] AKI is associated with both short and long term adverse effects on cardiac function,[6–9] especially in the presence of other cardiac complications such as heart failure,[7] myocardial infarction,[10,11] post-cardiac surgery,[12–14] and after percutaneous coronary interventions.[15,16].

Compelling data from animal studies demonstrate that AKI directly impairs cardiac function. Two separate research groups have demonstrated that cardiac contractility by echocardiograms is reduced in rodent models of AKI at 48 [17] and 72 [18] hours. Normal cardiac contractility is dependent on constant ATP generation and the preferred energy source is oxidative phosphorylation which occurs in the mitochondria. Thus, it is not surprising that mitochondrial defects (e.g., fracturing) [18] and apoptosis [18,19] are also associated with AKI-mediated heart injury. Indeed, reduced ATP generation due to inadequate mitochondrial oxidative phosphorylation is a fundamental mechanism of impaired cardiac contractility.[20] The hallmarks of inadequate oxidative phosphorylation include oxidative stress and utilization of alternative mechanisms of energy production; metabolomics analyses of the heart in numerous models of cardiac injury - including

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myocardial infarction/ischemia [21,22], myocarditis [23], and heart failure [21] – have revealed these effects. To date, however, metabolomics analysis in AKI-mediated heart injury has not been performed and the extent to which AKI effects energy production and metabolism in the heart is unknown.

In the present study, metabolomics analysis was performed on heart tissue at 4 hours, 24 hours, and 7 days after ischemic AKI in mice to test the hypothesis that the cardiac metabolome would be characterized by impaired oxidative phosphorylation, increased oxidative stress, and evidence of alternative energy production.

Results

Time course of AKI

Serum creatinine and BUN were determined in normal mice and at each time point after sham and AKI. As shown in Figure 1, serum creatinine was significantly increased at 4 and 24 hours post procedure in AKI versus sham and was not significantly different at 7 days; BUN was significantly increased in AKI versus sham at all three time points.

Significant metabolites in the heart via HCA, PCA, PLS-DA

Of the 124 named metabolites measured in the heart, 89 were significantly different among experimental groups based on univariate ANOVA without post-hoc analysis, as shown in Supplemental Table 1. Hierarchical clustering analysis (HCA) and principal component analysis (PCA) were performed for these 89 metabolites to visualize overall trends in the data. The HCA heat map is shown in Figure 2; the PCA observations plots and biplots are shown in Figure 3. Both HCA and PCA reveal that the most notable differences in metabolites has occurred in the 24 hour AKI group.

To further examine overall trends, PLS-DA was performed in order to identify the metabolites that contribute to the greatest variance between experimental groups (Figure 4A,B). The top 20 metabolites with the highest sum of variance between groups, which is referred to as a variable of importance in the projection (VIP) score, are displayed in the correlation plot in Figure 4B. Overall, PLS-DA further reveals the significance of the 24-hour AKI group.

Metabolites and metabolic pathways affected in the heart by surgical stress

Since surgery itself is associated with cardiac stress, we determined which metabolites were affected by surgical stress alone, independent of AKI, by comparing the sham groups with the normal group (by ANOVA) as shown in Supplemental Table 2A. Pathway analysis identifying significant pathways affected by surgical stress is shown in Supplemental Table 2B.

Specific metabolites and metabolic pathways affected in the heart by AKI

Specific metabolites affected by AKI are shown in Table 1; overall, 51 of the 124 measured metabolites (41%) were affected after AKI. The most dramatic metabolic changes occurred at 24 hours with 20 metabolites significantly decreased and 20 metabolites significantly

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increased. Changes at 4 hours were minimal, with 6 metabolites decreased and only one increased. Interestingly, metabolic effects were still noted 7 days post AKI, with a change in 15 of the measured metabolites.

Pathway analysis was performed and significant pathways affected by AKI are presented in Table 2.

Relative changes in individual metabolites, and implications regarding energy production and oxidative stress.

To better clarify the effects of AKI on the heart and visualize relative changes in metabolite levels, autoscaled values for all the significant metabolites affected after AKI are presented in Figures 5 to 8 and grouped into the following major categories: amino acids and amino acid metabolites (Figure 5); anti-oxidants and metabolites involved anti-oxidant pathways (Figure 6); metabolites involved in alternate energy production (Figure 7); and urea cycle and other metabolites (Figure 8).

Amino acid deficiency and implications.—As shown in Figure 5, several amino acids were decreased in the heart at 4 and/or 24 hours after AKI and include alanine, aspartate, glutamate, glycine, lysine, and tyrosine. Amino acid depletion characteristically occurs during cardiac ischemia and other forms of cardiac stress and reflects a number of injury-associated events such as hypoxia, ischemia reperfusion injury, ATP depletion, inadequate oxidative phosphorylation, increased alternative energy production, and increased oxidative stress [24–26]. Normally, amino acid metabolism constitutes a very small percentage of cardiac ATP production; however, when oxidative phosphorylation is inadequate, amino acid metabolism increases and becomes an important source of ATP production [25]. In particular, glucogenic amino acids such as aspartate and glutamate can be converted to glycolysis and TCA cycle intermediates to generate ATP.

The reduction in glutamate and aspartate is particularly notable as these amino acids are reduced in cardiac biopsies in patients after cardiac bypass and reductions are associated with ATP depletion and cardiac ischemia. [24–26] Thus, decreased glutamate, aspartate, and alanine likely reflect ongoing amino acid metabolism. An additional explanation is the use of glutaminolysis and transamination fueling the production of aspartate, which is then used in purine nucleotide metabolism to generate fumarate;[27] fumarate is reduced to succinate via the reverse activity of succinate dehydrogenase (complex II), and succinate accumulation drives reactive oxygen species generation.

Since anaerobic metabolism of glutamate is accompanied by an increase in alanine production, an increase in alanine to glutamate ratio has been shown to correlates with increasing tissue ischemia [24,26]. As shown in Figure 5D, the alanine to glutamate ratio was significantly increased 24 hour post AKI as further evidence of increased cardiac stress and a shift toward anaerobic metabolism. Glycine (Figure 5F) is also depleted in the heart after myocardial infarction in rats [22], and – like glutamate - is necessary for glutathione synthesis. Thus, the depletion of glutamate and glycine is also suggestive of increased oxidative stress and an attempt to restore depleted glutathione stores (discussed further, below).

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Oxidative stress in the heart after AKI.—As shown in Figure 6, metabolite changes associated with imbalanced redox homeostasis were present after AKI, particularly at 24 hours. Increased oxidative stress is a hallmark of cardiac stress and occurs in a wide range of diseases including myocardial infarction [22], myocarditis [23], dilated cardiomyopathy [28], and heart failure [29,30]. The significant reduction in glutathione (GSH) (Figure 6A) 24 hours post-AKI is particularly notable since, like amino acid depletion, depletion of glutathione also occurs during direct cardiac ischemia. Glutathione is a potent anti-oxidant which is crucial to normal cardiac function [29,30] and a key defender against excess reactive oxygen species (ROS) and ensuing cell damage.

Additional markers of redox imbalance after AKI include increased 2-aminoadipate (α-aminoadipate) (Figure 5M), 5-hydroxyisourate (Figure 7K), *S*-allantoin (Figure 7L), [21] threonate (Figure 6F), and ascorbate (Figure 6E) [21] and decreased thioredoxin disulfide (Figure 6G). 2-Aminoadipate is a product of lysine degradation and a marker of protein oxidation; [31,32] its increase may be the result of oxidative stress and protein oxidation as well as lysine degradation. Increased 5-hydroxyisourate and S-allantoin are a result of increased purine metabolism (as judged by the concomitant reduction in the purines adenosine and adenine). Purine catabolism leads to an increase uric acid and downstream products 5-hydroxyisourate and allantoin in ROS-generating reactions[33]; thus, increased S-allantoin is a well-established biomarker of increased oxidative stress [34–37]. Threonate is a metabolite of ascorbate (Vitamin C), and both threonate and ascorbate were increased in the heart after AKI. Ascorbate is increased in murine models of heart failure. [21]

Effects of AKI on alternative energy pathways in the heart—As shown in Figure 7, metabolites involved glycolysis, the TCA cycle, pentose phosphate pathway, and nucleotide metabolism were affected in the heart after AKI and suggest a shift toward alternative energy production (e.g., ATP production via mechanisms alternative to oxidative phosphorylation). As with the metabolite changes described above, these energy shifts are common during cardiac stress from multiple causes such as heart failure, [20,21,38–40] cardiac ATP depletion, [40] myocardial ischemia/infarction, and myocarditis.[23] In particular, excess purine metabolism is associated with decreased ATP and anaerobic ATP production [41]; increased purine metabolism is suggested by the marked decreased in the purines adenine and adenosine accompanied by increases in their end products (5-hydroxyisourate and S-allantoin). Pyrimidine consumption is also present as evidenced by changes associated with 5–6 dihydrothymine, cytidine, and UMP.

Echocardiography

To determine if the metabolic changes in the heart were associated with functional consequences, serial echocardiography was performed at baseline, and at 24 and 72 hours after sham and AKI. As shown in Figure 9, the E/A ratio was slightly decreased at 24 hours and was significantly decreased at 72 hours post-AKI; this result at 72 hours represents significant diastolic dysfunction. Other parameters of cardiac function were assessed and are listed in Table 3.

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ATP levels

ATP depletion is a well described cause of diastolic dysfunction [42] and the metabolic changes strongly suggested the presence of ATP depletion. Therefore, ATP levels were measured in normal mice, sham, and AKI at 4, 24, and 72 hours. As shown in Figure 10, ATP levels were non-significantly decreased at 4 hours of AKI, and were significantly decreased at 24 and 72 hours of AKI; these result are thus consistent with the metabolic observations and echocardiographic findings.

Mean arterial blood pressure (MAP) and weight

Mean arterial blood pressure and weight were determined at baseline, and 24 and 72 hours after sham or AKI in a separate cohort of mice. Plasma creatinine was determined at 24 hours as shown in Figure 11A. As shown in Figure 11B, MAPs were not different between sham and AKI at any time point. As shown in Figure 11C, weights were not different between sham and AKI at any time point (a non-significant trend for a decrease in weight, 72 hours after AKI was present).

Volume overload may be associated with diastolic dysfunction [43]; however, our data indicate that volume overload is not a feature of our AKI model given that AKI did not affect either MAP or weight; indeed, a slight trend for decreased BP was observed after AKI. Thus, changes in MAP or volume status do not plausibly account for the changes in the cardiac metabolome or function.

Serum potassium

Potassium levels were determined in separate cohorts of mice. Plasma potassium (mEq/L) was 4.17±0.6 in normal mice (n=3), 5.86±0.48 24 hours after AKI ($P<0.001$ versus normal, n= 8), and 4.13±0.58 72 hours after AKI ($P<0.001$ versus 24 hour AKI; P=NS versus normal; n=4)(paired t test; 2 separate experiments; mean±SD).

Thus, statistically significant hyperkalemia was observed 24 our after AKI, although it is unlikely that this level of potassium has pathologic significance in the heart given that the level was below 6.0 mEq/L. This is especially true since hyperkalemia is associated with cardiac arrhythmias, but not changes in energy metabolism nor diastolic dysfunction.

Arterial pH

Arterial pH was determined in separate cohorts of mice. As shown in Figure 12, the only significant difference between sham and AKI was at 24 hour with pH values of 7.25±0.03 in sham and 7.12±0.03 in AKI ($P<0.001$, paired t test; 2 separate experiments; mean±SD). 72 hours after AKI, pH levels were similar to levels in sham and normal mice.

While acidosis is known to impair cardiac contractility, significant effects are generally noted with severe acidosis (e.g., pH <7.0) as studied *in vitro*.[44] Interestingly, acidosis is protective against ischemia-reperfusion induced ATP depletion.[44] 72 hours post-AKI, pH has normalized, when ATP depletion and cardiac dysfunction are most severe; thus, it is unlikely that acidosis is responsible for these cardiac effects. Whether the acidosis observed

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at 24 hours contributed to the metabolic changes in the heart at 24 hours is unresolved and merits additional study.

Metabolite analysis in the kidney and plasma

In order to gain insight into the mechanism by which ischemic AKI might lead to the changes in energy metabolism observed in the heart, metabolomics analysis of kidney and plasma was performed on samples obtained from the same cohorts of mice from which heart metabolomics was performed. Based on univariate ANOVA without post hoc analysis, 141 of the 177 (80%) metabolites measured in the kidney and 117 of the 128 (91%) metabolites measured in the plasma were significantly different among experimental groups as shown in Supplemental Table 4 and 5. HCA heat maps are shown in Figures13 and 14 and PCA observation plots and biplots for these significant metabolites are shown in Supplemental Figures 1–4.

In Tables 4–7 all of the metabolites that were significantly decreased or increased in the kidney, plasma, or heart at 4 hours (Tables 4, 5) and at 24 hours (Tables 6, 7) are presented (metabolites that were present in 2 or more sites are in the top section; metabolites present in only one site are in the bottom section and grouped by pathway).

Few metabolites changed similarly among the kidney, plasma, and heart with only 5 metabolites similarly affected in all three sites. With regard to the plasma and heart, 16 metabolites were similarly affected and represent a variety of metabolites/pathways (e.g., amino acid deficiency, oxidative stress/glutathione deficiency). With regard to the kidney and plasma – as would be expected - several metabolites that were increased in the kidney were also increased in the plasma: 7 at 4 hours and 17 at 24 hours.

Although changes in individual metabolites were minimal, the overall changes in metabolites are informative with notably similar effects in both the kidney and heart, as evidenced by amino acid depletion, increased oxidative stress, and alternative energy production that were evident in the kidney at 4 hours, and then manifested in the plasma and heart at 24 hours. For example, in the plasma, only 3 metabolites were decreased at 4 hours, yet at 24 hours, 34 metabolites were decreased including the amino acids alanine, arginine, cysteine, lysine, methionine, phenylalanine, proline, threonine, tyrosine, and valine. With regard to oxidative stress, glutathione is decreased in the kidney at 4 hours, but plasma levels do not decrease until 24 hours. Both amino acid deficiency and deficiency of antioxidant capacity at 24 hours likely contribute to cardiac ATP depletion and increased cardiac oxidative stress.

Additionally, metabolites reflecting shifts in energy production were affected during AKI, with the most notable effects being related to nucleotide catabolism as evidenced by decreased levels of purines and pyrimidines and increased levels of their metabolites in the kidney and plasma. Increased nucleotide metabolism, which also occurred in the heart, is most likely the result of a shift to anaerobic metabolism, and most certainly due to the hypoxic environment within the kidney due to direct ischemia.

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Discussion

In the present study, our data demonstrate that 41% of metabolites measured in the heart were affected at some time point after AKI, with the most dramatic effects occurring 24 hours after AKI. The number and acuity of the changes in metabolites after AKI are notable; by comparison, metabolic profiling after myocardial infarction revealed change in approximately 40% of metabolites in one study [21] and 50% of metabolites in another [22]; in contrast, cardiac stress induced by pressure overload affected only 1.3% of metabolites [21]. Remarkably, the metabolites affected after in the heart after AKI are akin to the changes induced after direct cardiac injury from myocardial ischemia reperfusion as evidenced by amino acid depletion, glutathione depletion, increased oxidative stress, and evidence of anaerobic energy production; these metabolic effects also occurred within the kidney and were similarly reflected in the plasma. Thus, AKI – within the kidney and systemically - is characterized by marked energy substrate depletion, increased oxidative stress, and changes in energy metabolism. Whether depletion of amino acids and other energy substrates are due to increased consumption, decreased production, or both, is not resolved by our study, however, it is plausible that this energy substrate deficient state contributes to reduced ATP generation in the heart after AKI. Indeed, re-introduction of the amino acids glutamate and aspartate (markedly decreased in the heart after AKI) improves ATP production during direct cardiac ischemia, and supplementation of cardioplegic solutions for bypass with aspartate and glutamate has been standard practice for decades.[26,45] Thus, future studies to test the potential benefit of amino acid supplementation during AKI-induced cardiac injury are warranted.

Increased oxidative stress after AKI is particularly evident by the marked reduction of kidney, plasma, and cardiac glutathione at 24 hours. Glutathione is essential for normal cardiac function,[29,30] and glutathione deficiency is an early trigger of apoptotic cell death [46] which has been observed in rodent models of AKI-induced cardiac injury.[18] Depletion of cardiac glutathione occurs in animal models of myocardial infarction and heart failure and also associated with longer term outcomes as cardiac decreased glutathione levels correlates with decreased cardiac function and heart failure progression in humans.[30] Glutamate supplementation is thought to beneficial during hypoxia and bypass surgery by increasing levels of glutathione [25], and could be similarly beneficial during AKI.

In addition to the marked deficiencies in metabolites, AKI was also characterized by the accumulation of numerous metabolites with a significant increase in 42 of 144 measured metabolites at 24 hours. It is possible that one or more of these metabolites may account for the changes in energy metabolism, ATP depletion, and cardiac dysfunction observed. Of particular relevance, 2-oxoglutarate (also known as alpha ketoglutarate), which was increased in the kidney, plasma, and heart 24 hours post-AKI, was recently identified as having an important role in cardiac energy metabolism. Specifically, plasma accumulation of alpha-ketoglutarate affected hepatic tryptophan metabolism to produce kynurenic acid which activates hypoxia inducible factor (HIF) in the heart.[47] Activation of HIF shifts cells away from glycolytic metabolism towards reliance on amino acid metabolism via regulation of pyruvate dehydrogenase kinase. In our data, the significant increase in alpha ketoglutarate is was accompanied by evidence of increased amino acid metabolism in the heart, consistent

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with a HIF-1 effect. During AKI, this shift in metabolism could be maladaptive given the marked reduction in circulating amino acids and thus lack of sufficient amino acid substrate. Thus, it is possible that the cardiac effects of AKI result from synergist effects of both metabolite accumulation and depletion.

Our data in the present study may have considerable implications regarding the potential benefit, or harm, of renal replacement therapy (RRT) during AKI. RRT results in the indiscriminate removal of small and medium sized metabolites and proteins. While it has long been evident that removal of the accumulation of certain metabolites during AKI and end stage renal disease (ESRD) is beneficial, much less attention has been paid to the potential harms of RRT and that numerous metabolites and proteins essential for optimal organ function may be removed during the process. Enthusiasm for the potential benefits of RRT during AKI is evident by the number of past and current clinical trials devoted to the study of increased dose and early initiation.[48] Our data demonstrate that a dramatic number of measured metabolites (42) are increased in the plasma 24 hours after AKI, and it is possible that one or many of these metabolites may adversely affect distant organ function thus supporting the argument that early RRT may be of clinical benefit. Remarkably, however, a large number of metabolites (34) were significantly reduced in the plasma 24 hours after AKI. One wonders whether the additional removal of these, and other substrates essential for normal cardiac function and energy production such as glutamate, aspartate and glycine might result in adverse clinical outcomes.

Since supplementation of these amino acids is beneficial during direct cardiac ischemia, it is plausible to conclude that the removal would be harmful. Another potential beneficial metabolite that would be expected to be removed by RRT includes the major antioxidant glutathione, which was reduced in the plasma, kidney and heart at 24 hours of AKI; given the increase in oxidative stress noted in the kidney and heart after AKI, further and continued removal of glutathione by RRT might be expected to be harmful. Removal of potentially beneficial metabolites may be particularly important to consider when AKI is accompanied by myocardial infarction or congestive heart failure in which an already compromised heart may be especially susceptible to removal of amino acids and other energy substrates. The potential competing benefits and harms of RRT is a plausible explanation for the lack of clinical benefit seen in studies of higher dose of dialysis during AKI, as well as overall lack of improvement in mortality in patients with RRT requiring dialysis over the past several decades.

Limitations.

Metabolite levels reported reflect steady state levels that are the cumulative effect of numerous interconnected pathways. Definitive assessment of substrate sources for key metabolites and implications for metabolic pathways disrupted require additional focused experimentation. Accurate metabolite identity involves the use of appropriate standards, and may be subject to error. Raw datasets are available for review upon request.

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Summary and conclusions.

In the present study, we demonstrate that AKI is associated with dramatic changes in cardiac metabolism, cardiac ATP depletion, and diastolic dysfunction. While the systemic complications of AKI are traditionally considered to be due to the accumulation of metabolites and other circulating mediators, our data demonstrate that numerous essential metabolites were *depleted* after AKI which likely contribute to the deleterious effects of AKI on the heart. We suggest that methods to improve energy metabolism in the heart after AKI may have substantial potential to improve outcomes. Furthermore, our data highlight the complexity of the systemic consequences of AKI and suggest that more precise tools of therapy beyond RRT will be necessary to optimize patient outcomes.

Materials and Methods

Complete descriptions of methods are available in “Supplemental Methods“

Animals

Adult (8–10-week-old), male C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) weighing between 20–25 g were used. Mice were maintained on a standard diet, and water was freely available.

Surgical protocol

Two surgical procedures were performed: (1) sham operation (i.e., laparotomy) and (2) ischemic AKI as previously described. [3,49]

Experimental groups and accounting for mice included in experiments and analysis.

For metabolomics analysis, we began with 10 mice per group for each of the 7 experimental groups: 1) normal (no surgical procedure), 2) 4 hour sham, 3) 4 hour AKI, 4) 24 hour sham, 5) 24 hour AKI, 6) 7 day sham, and 7) 7 day AKI for a total of 70 mice. However, 1 mouse in the 7 day sham group died, and 4 mice in the 7 day AKI group died. Thus, the numbers of animals included in the final data analysis are as follows: 1) normal: n=10, 2) 4 hour sham: n=10, 3) 4 hour AKI: n=10, 4) 24 hour sham: n=10, 5) 24 hour AKI: n=10, 6) 7 day sham: n=9, and 7) 7 day AKI: n=6.

Metabolomics analyses

Frozen heart and kidney samples were milled with a mortar and pestle in the presence of liquid nitrogen, then weighed to the nearest 0.1 mg and extracted in ice-cold lysis/extraction buffer at a concentration of 10 mg/mL. Samples were then agitated at 4°C for 30 min and then centrifuged at 10,000 g for 15 min at 4°C. Protein and lipid pellets were discarded, while supernatants were stored at −80°C until metabolomics analyses via ultra high pressure liquid chromatography coupled to online mass spectrometry (UHPLC-MS) as previously reported [50]. Plasma samples were snap frozen and stored at −80 until UHPLC-MS analysis. Untargeted UHPLC-MS-based metabolomics analysis provided the measurement of annotated metabolites (124 for the heart, 177 for the kidney, and 128 for the plasma). No data points were excluded in the analysis.

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Echocardiography, ATP assay, mean are blood pressure, and potassium and arterial blood pH measurements are described in supplemental methods.

Statistical analysis

To normalize the metabolite data for statistical analysis, the raw peak areas were autoscaled using MetaboAnalyst 3.0 (available at http://www.metaboanalyst.ca) [51] in order to achieve a standard deviation of 1 for each metabolite, thus allowing for homoscedastic statistical tests [52,51]. Univariate ANOVA (significant threshold $p < 0.05$) was performed on autoscaled data in Metaboanalyst 3.0 followed by post-hoc analysis with Bonferonni-Sidak's multiple comparison test (significance threshold for $p < 0.05$). Hierarchical clustering analysis (HCA), principal component analysis (PCA), partial least squares- discriminant analysis (PLS-DA), and pathway enrichment and ranking analysis were performed on significant metabolites after univariate ANOVA. HCA was performed using Morpheus (available at: https://software.broadinstitute.org/morpheus/); PCA and PLS-DA were performed using XLSTAT (available at https://www.xlstat.com/en/); pathway analysis was performed using MetaboAnalyst 3.0. For all analyses, rigorous statistical profiling methods and thresholds were utilized in order to minimize false discovery. For PCA biplots, the metabolites with a minimum sum of squared cosines of 0.5 were considered significant. For pathway analysis, pathway significance was based on the total number of metabolites that map to a pathway and their respective significances; a pathway was considered significant if the number of significant metabolites (i.e. "hits") was ≥ 2 and the Holm adjusted P value was less than 0.05.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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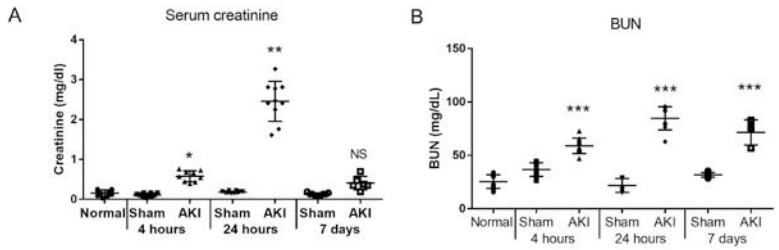

Figure 1. Time course of AKI.

(A) Serum creatinine and (B) BUN were determined in normal controls and at 4 hour, 24 hours, and 7 days after Sham and AKI. (n=6 to 10)).(0ne way ANOVA comparing the mean all groups with Tukey's multiple comparison test. Mean+/−SD. * P<0.001 vs. 4 hr Sham; **P<0.0001 vs. 24 hour sham; NS vs 7 day sham; ***P<0.0001 vs. 4 hr Sham, 24 hour sham, or 7 day Sham respectively).

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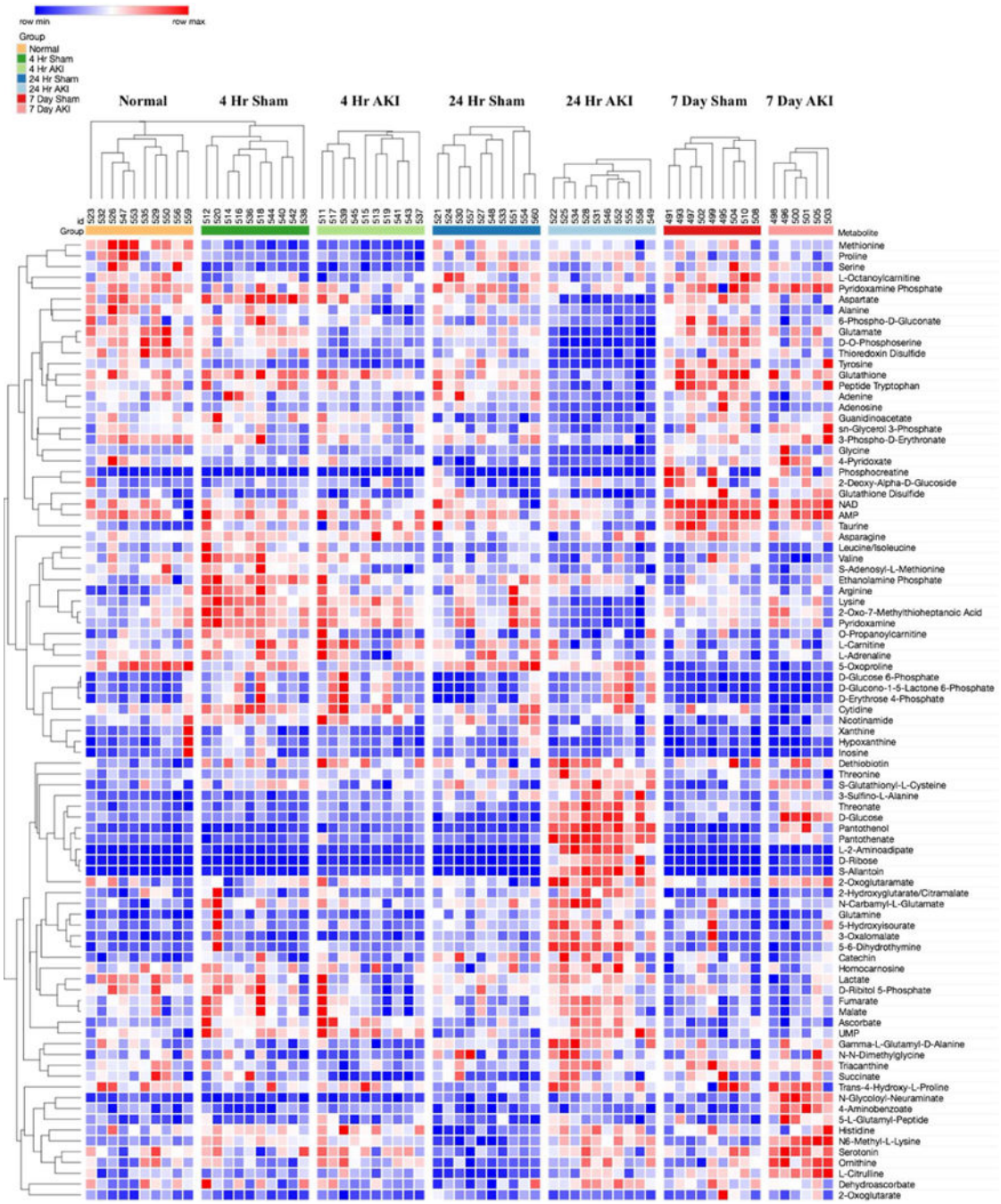

Figure 2. Hierarchical clustering analysis (HCA) of heart metabolites.

HCA, with predefined sample clusters, was performed for the 89 heart metabolites significantly altered (ANOVA) to determine trends across the metabolome (red is increasing, blue is decreasing across each row). The correlation metric used was the Pearson (n-1) correlation with an average linkage method.

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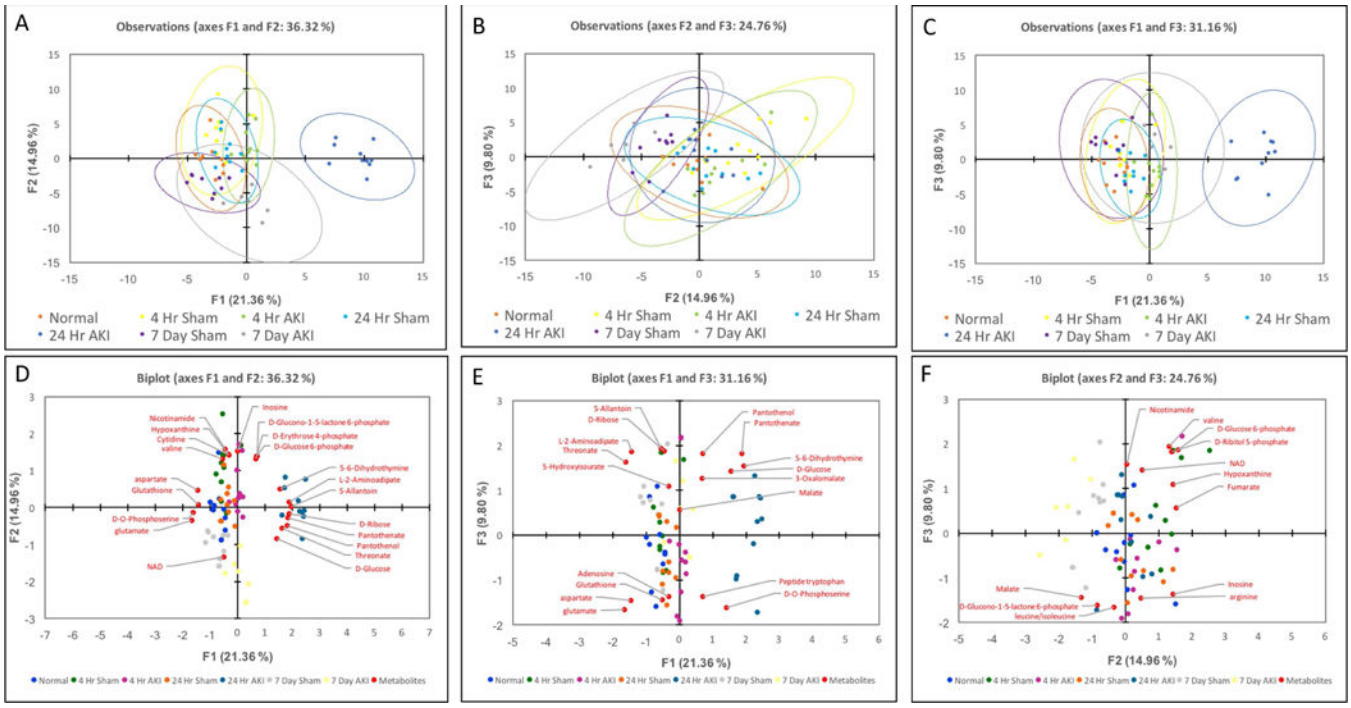

Figure 3. Principal Component Analysis Sample Group Observations Plots (A-C) and Biplots (D-E). Observations plots:

Principal component analysis was performed using the Pearson (n-1) correlation to determine if the 7 groups were distinguishable and identify variances in the data as a whole. Principal component 1 (Axis F1) represented 21.36% of the variation in the data, principal component 2 (Axis F2) represented 14.96% of the variation in the data, and principal component 3 (Axis F3) represented 9.80% of the variation in the data. 24 hour AKI is the most distinguishable group, illustrated by F1. Dots represent individual samples and confidence ellipses were graphed. (A) Observations plot of principal component 1 (F1, 21.36%) and principal component 2 (F2, 14.96%) (B) Observations plot of principal component 2 and principal component 3 (F3, 9.80%) (C) Observations plot of principal component 1 and principal component 3. **Biplots:** Principal component analysis was done to distinguish groups and find metabolites contributing to the group separation. PCA biplots are graphed here and include both observations (samples) and loadings (metabolites) plotted on the same graph. The metabolites shown are those that have a minimum sum of squared cosines of 0.5 between the two principal components graphed. The sum of squared cosines is a measure to find significant variables in the projection. Metabolites far from the origin in the same location of a group contribute to that group's separation. These metabolites are positively correlated with each other and are increased in that group. If the group were mirrored over the axes, metabolites in the same location as the mirrored group are decreased in that group and positively correlated with each other. (A) Biplot of principal component 1 and principal component 2 (B) Biplot of principal component 2 and principal component 3 (C) Biplot of principal component 1 and principal component 3.

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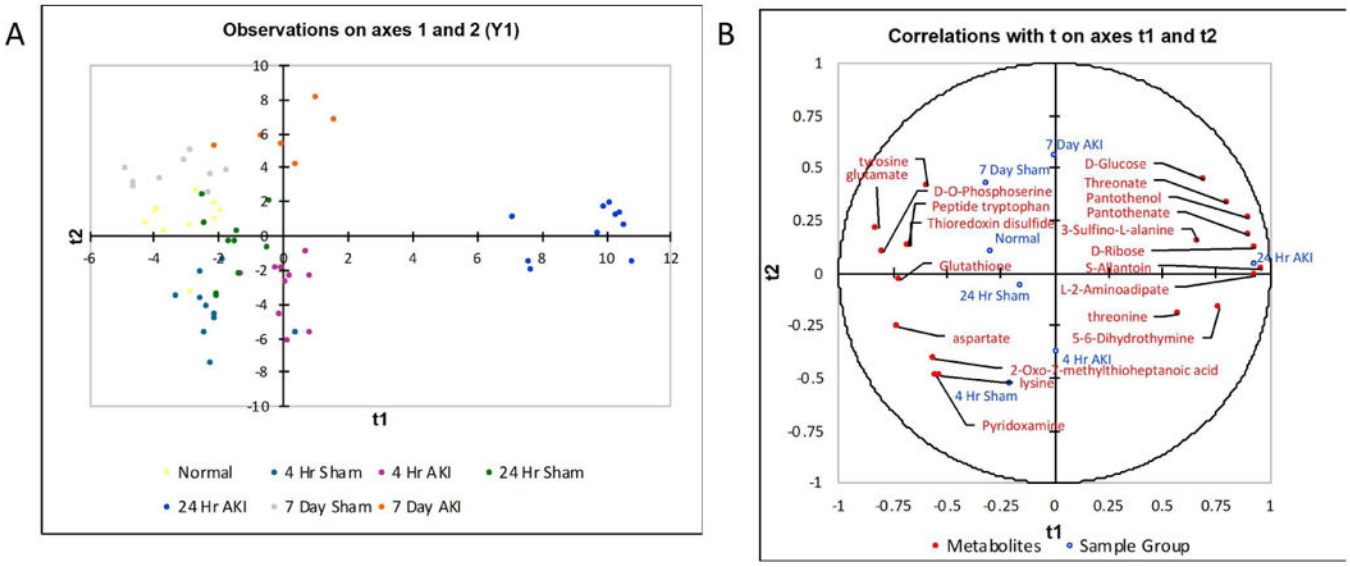

Figure 4. Partial Least Squares Discriminant Analysis (PLS-DA) Sample Group Observations Plot and Correlation Biplot.

(A) PLS-DA was performed to further distinguish sample groups and identify metabolites contributing to the group separation. (B) The top 20 metabolites with the highest sum of variable importance in the projection (VIP) of the two components. In the correlations biplot, the closer the variables are to the unit circle, the higher the contribution in the projection. The group weights are also indicative of the importance of the group in the projection based on their proximity to the unit circle. Group weights and metabolites in proximity to each other are positively correlated. If the group weights were mirrored over the axes, the metabolites in close proximity to the mirrored group weights are negatively correlated.

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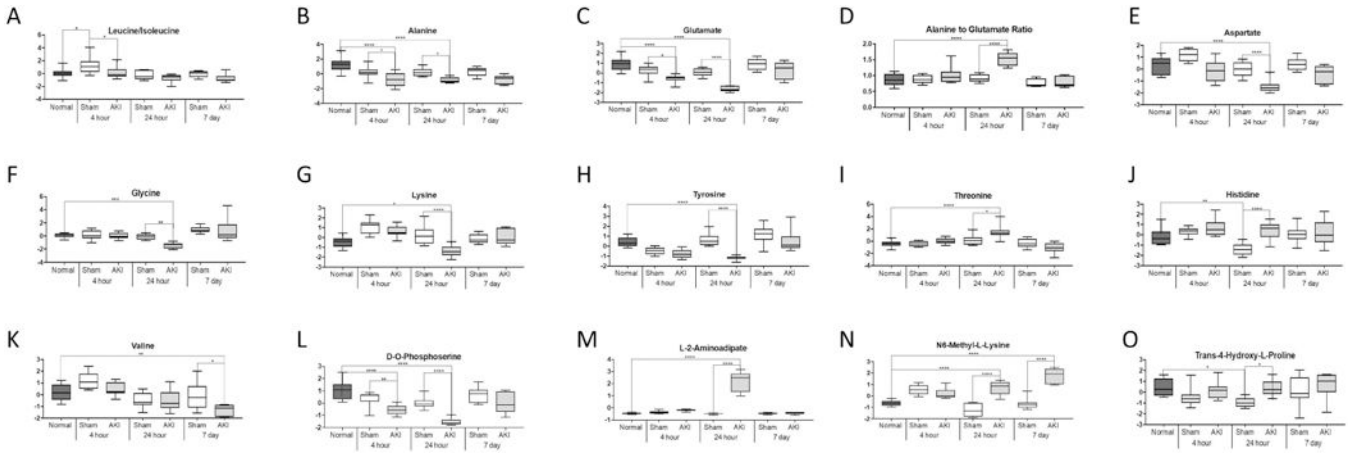

Figure 5. Amino acids and amino acid products affected in the heart after AKI.

10 amino acids (A-C;E-K) and 4 amino acid products (L-O) were affected at 4, 24 hours, and/or 7 days after AKI in the heart. Leucine/isoleucine, alanine, and glutamate (A-C) were decreased 4 hours after AKI relative to sham; glutamate, alanine, glycine, aspartate, lysine and tyrosine (B-G) were decreased in the heart 24 hours after AKI relative to sham; threonine and histidine (H, I) were increased 24 hours after AKI relative to sham; and valine (J) was decreased in the heart 7 days after AKI relative to sham. (D) The alanine to glutamate ratio is marker of increased anerobic metabolism of glutamate; its increase at 24 hours post AKI is indicative of anaerobic metabolism and ATP depletion (Data are normalized values via autoscaling and presented as box and whisker plots which show median and the 25th and 75th percentiles as the ‘boxes’ and the min and max values as the ‘whiskers’. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

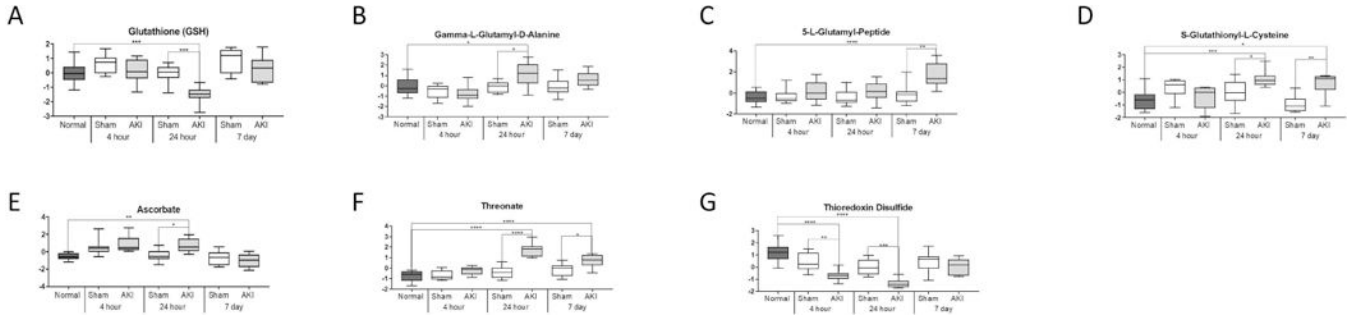

Figure 6. Metabolites involved in oxidative-stress associated pathways in the heart following AKI.

Glutathione (an anti-oxidant) (A), glutathione metabolites (B-D), ascorbate (i.e., Vitamin C, an anti-oxidant) (E), threonate (a Vitamin C metabolite, an anti-oxidant), and thioredoxin disulfide (an anti-oxidant) (G) were affected at 4 hours, 24 hours, and/or 7 days after AKI in the heart. (Data are normalized values via autoscaling and presented as box and whisker plots which show median and the 25th and 75th percentiles as the 'boxes' and the min and max values as the 'whiskers'; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

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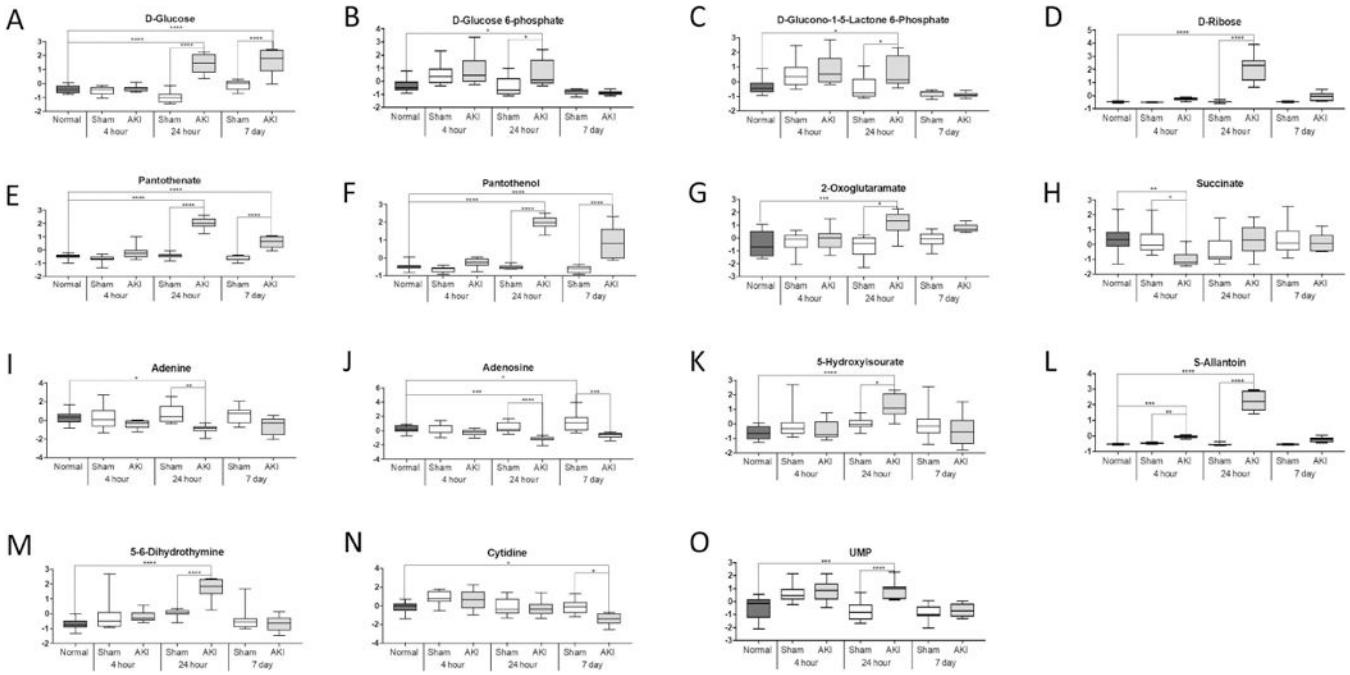

Figure 7. Energy metabolites affected in the heart after AKI.

Metabolite changes implicate glycolysis and the pentose phosphate pathway (A-D); TCA cycle (E-H); purine metabolism ((I-L), and pyrimidine metabolism (M-O) in the heart after AKI. (Data are normalized values via autoscaling and presented as box and whisker plots which show median and the 25th and 75th percentiles as the 'boxes' and the min and max values as the 'whiskers'; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

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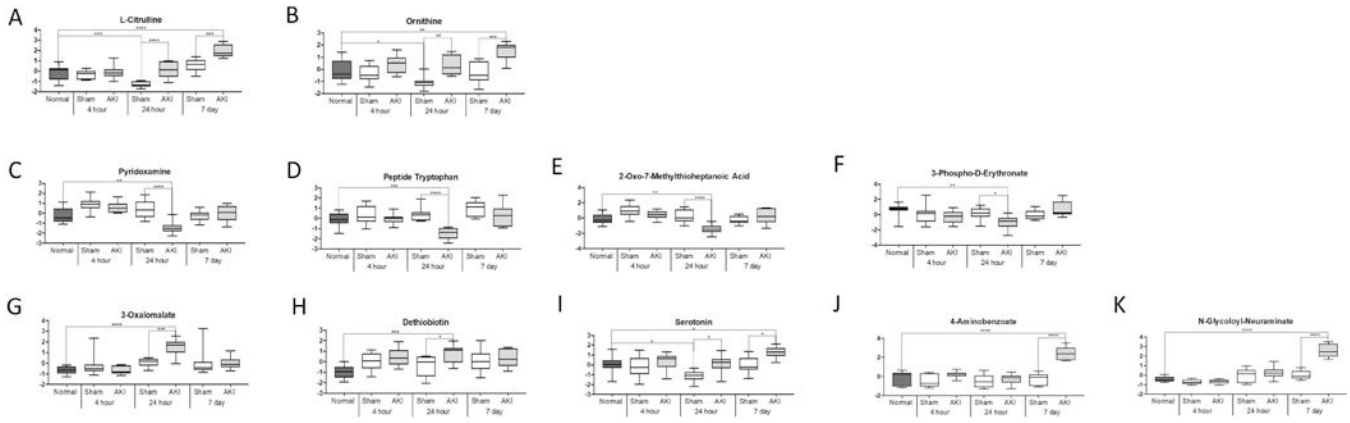

Figure 8. Urea cycle metabolites and other metabolites affected in the heart after AKI.

2 metabolites involved in the urea cycle were affected in the heart after AKI (A-B, citrulline and ornithine). Pyridoxamine (C), peptide tryptophan (D), 2-oxo-7-methylthioheptanoic acid (E), and 3-phospho-D-erythronate (F) were decreased in the heart 24 hours after AKI relative to sham; 3- oxalomalate (G), dethiobiotin (H), and serotonin (I) were increased in the heart 24 hours after AKI relative to sham; serotonin (I), 4-aminobenzoate (J), and *N*-glycoloyl-neuraminate (K) were increased in the heart 7 days after AKI relative to sham (Data are normalized values via autoscaling and presented as box and whisker plots which show median and the 25th and 75th percentiles as the ‘boxes’ and the min and max values as the ‘whiskers’; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

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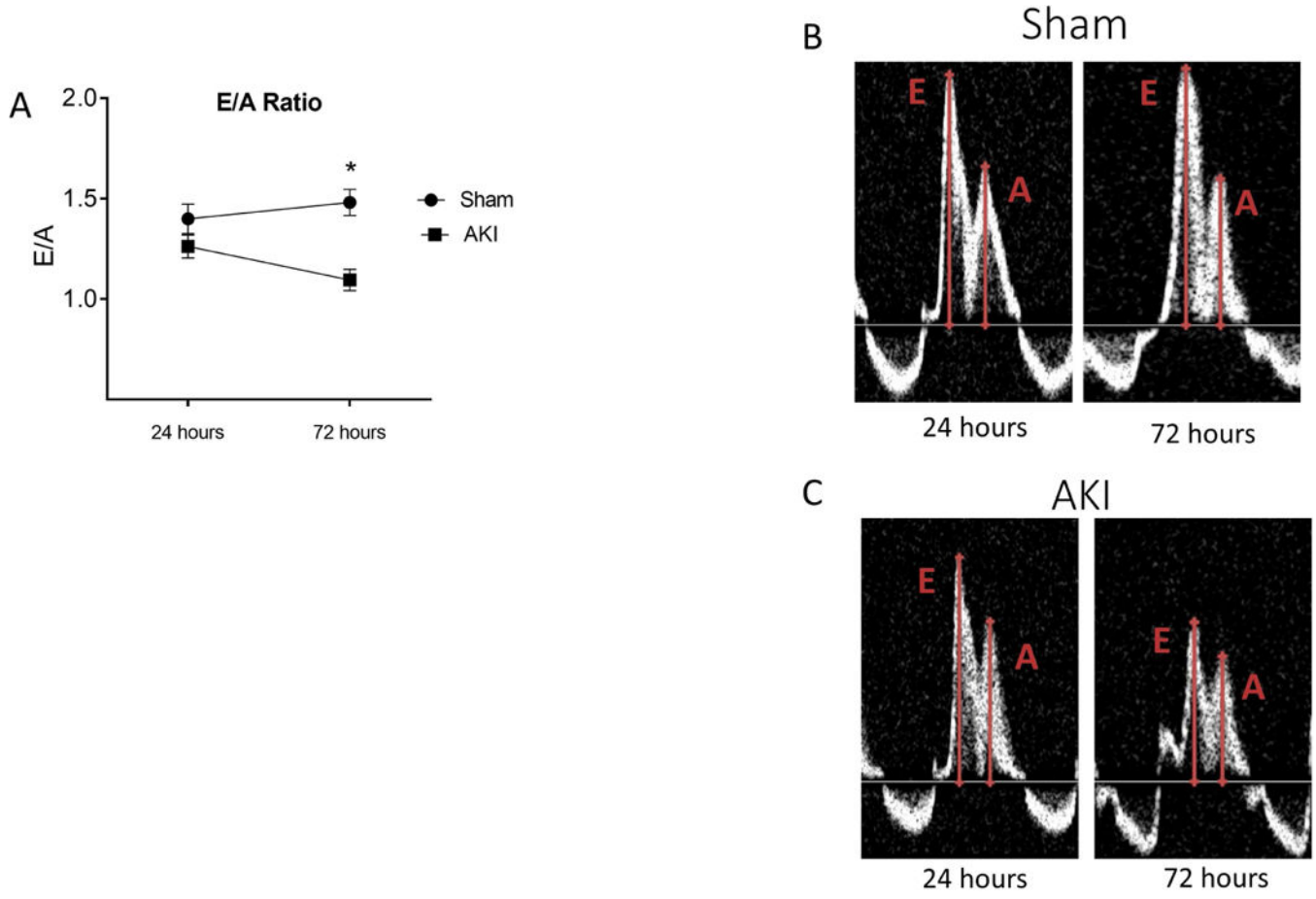

Figure 9. Mice with AKI have diastolic dysfunction.

Echocardiography was performed in mice 24 hour and 72 hours after surgery for Sham and AKI. (A) Doppler measurement of mitral inflow velocity (E/A) in sham and AKI mice at 24 and 72 hours after surgery. *P<0.05 versus Sham; N=3–5) (B) Representative E/A Doppler echocardiography images of sham mice at 24 and 72 hours after surgery. (C) Representative E/A Doppler echocardiography images of AKI mice at 24 and 72 hours after surgery. The ratio of the early filling (E) phase of the left ventricle during diastole and the late filling (A) phase is a common echocardiographic measurement of diastolic function. The AKI mice have a slight decrease in diastolic function at 24 hours and a significant decrease at 72 hours post-AKI as indicated by a significant decrease in the E/A ratio.

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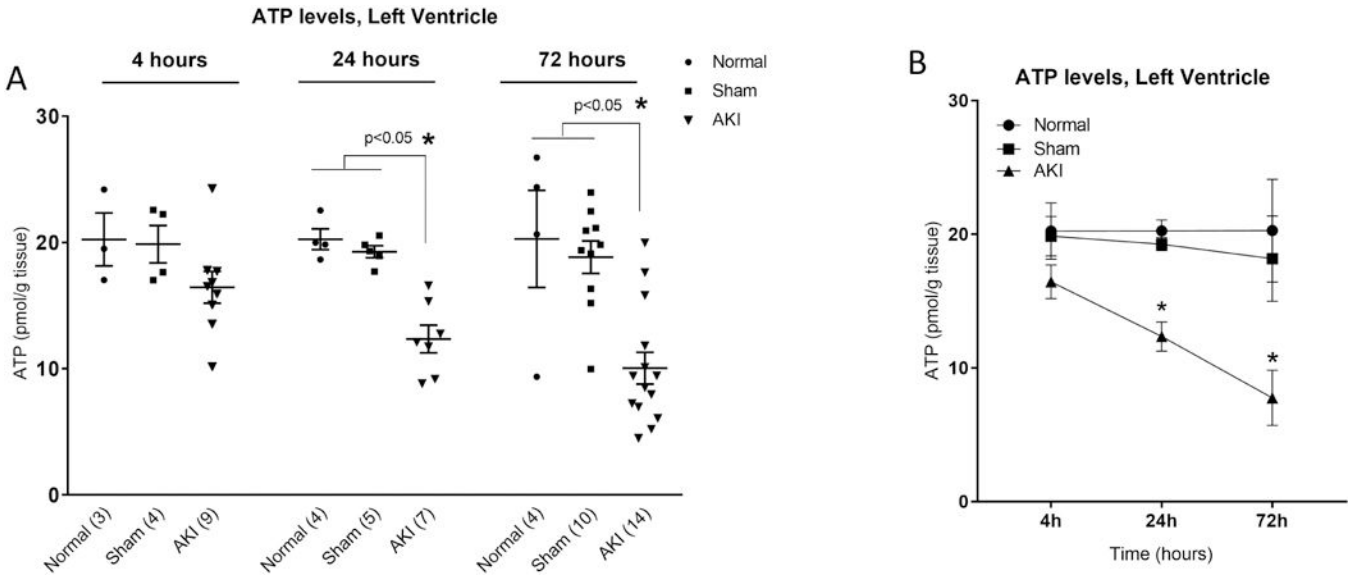

Figure 10. ATP levels in the heart are reduced at 24 hour 72 hours after AKI.

ATP levels were determined in the left ventricle of the heart in normal, sham, and AKI mice at 4, 24, and 72 hours. (A) ATP levels were reduced at 4 hours post AKI compared to normal and sham but did not reach statistical significance and were significantly reduced at 24 and 72 hours of AKI versus normal and sham at the same time point. *P<0.05 versus 4 hour AKI. (N=3–14 as indicated in the figure). (B) Graphic representation of the data in (A) demonstrating the dramatic reduction of ATP levels in the left ventricle during AKI over time. *P<0.05 versus 4 hour AKI

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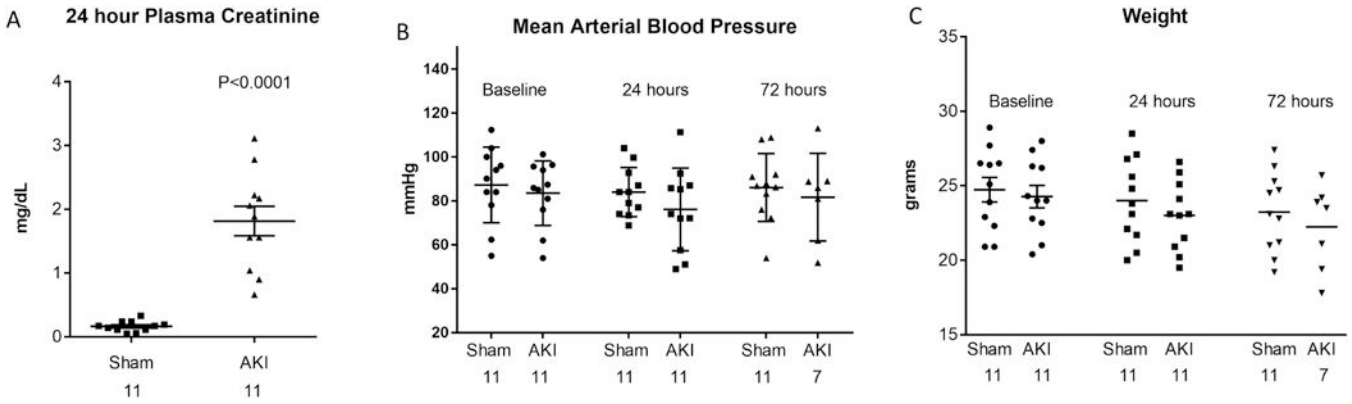

Figure 11. Mean arterial blood pressure and weight after AKI.

Mean arterial blood pressure and weight were determined at baseline, and 24 hour hours and 72 hours after Sham and AKI. Plasma creatinine was determine 24 hours after AKI and was significantly increased (A). Mean arterial blood pressure was not different between Sham and AKI at baseline, 24 hours, or 72 hours (B). Weight was not different between Sham and AKI at baseline (prior to surgery for sham or AKI), 24 hours, or 72 hours; (weight trended to decrease in AKI, but did not reach statistical significance)(C). (2 separate experiments, N is indicated below each experimental group).

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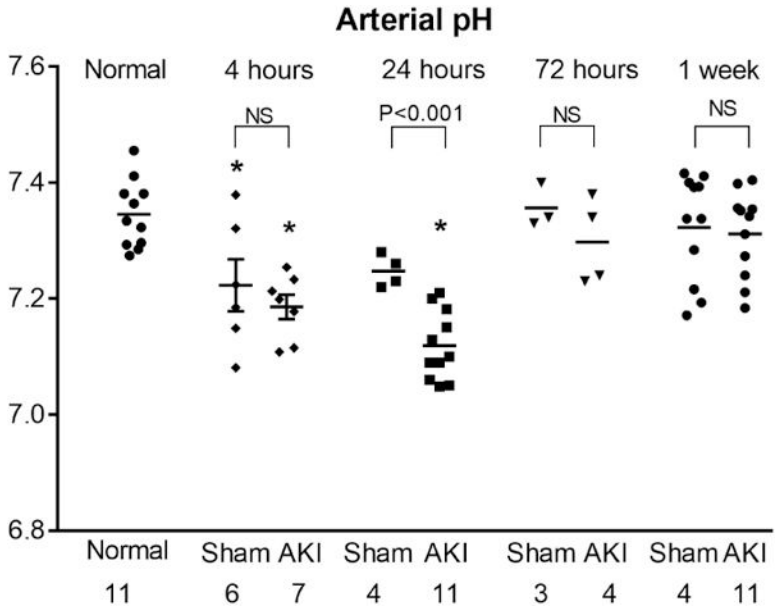

Figure 12. Arterial pH after AKI.

Arterial pH was determined in normal mice, and in mice at 4 hours, 24 hours, 72 hours and one week after Sham and AKI. (4 separate experiments, N is indicated below each experimental group). (*P<0.05)

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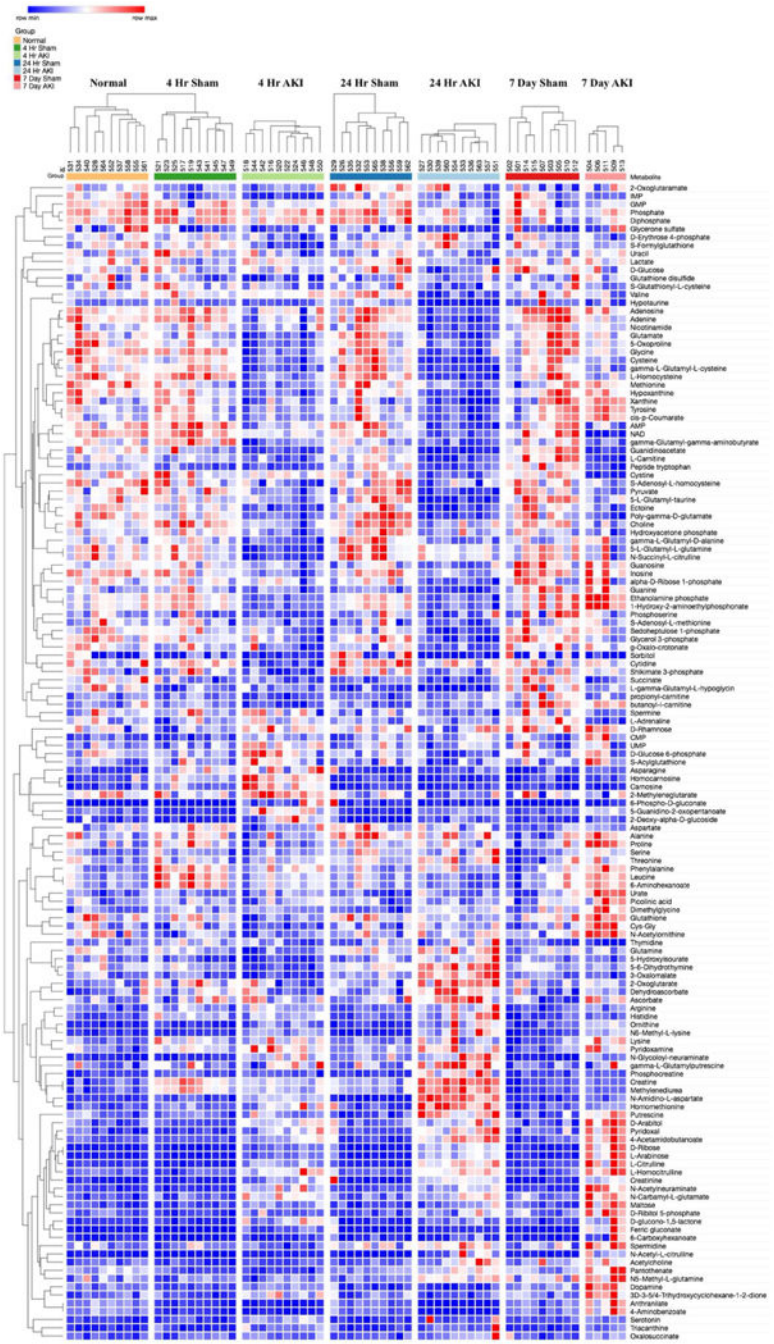

Figure 13. Hierarchical clustering analysis (HCA) of kidney metabolites.

HCA, with predefined sample clusters, was performed for the kidney metabolites significantly altered (ANOVA) to determine trends across the metabolome (red is increasing, blue is decreasing across each row). The correlation metric used was the Pearson (n-1) correlation with an average linkage method.

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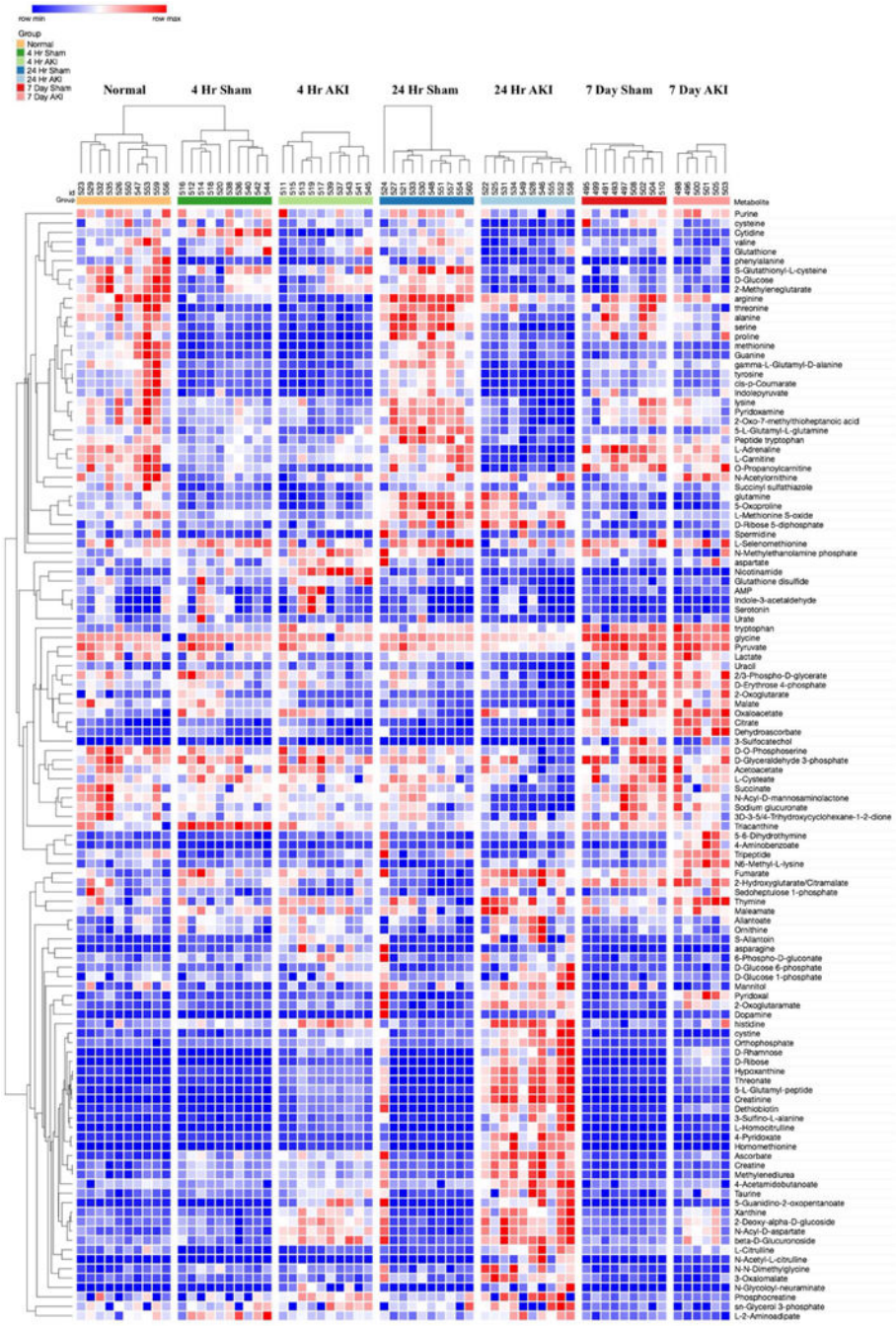

Figure 14. Hierarchical clustering analysis (HCA) of plasma metabolites.

HCA, with predefined sample clusters, was performed for the plasma metabolites significantly altered (ANOVA) to determine trends across the metabolome (red is increasing, blue is decreasing across each row). The correlation metric used was the Pearson (n-1) correlation with an average linkage method.

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Table 1.

Metabolites significantly decreased or increased in the heart at 4, 24, and/or 7 days after AKI.

Metabolites are categorized by major pathway involvement (in parentheses). Metabolites in bold were affected at more than one time point.

Metabolites significantly decreased in the heart after AKI		
4 hours	**24 hours**	**7 days**
Alanine	**Alanine (Amino Acid)**	Valine (Amino Acid)
Glutamate	**Glutamate (Amino Acid)**	Cytidine (Pyrimidine Metabolism)
D-O-Phosphoserine	**D-O-Phosphoserine (Glycine, Serine, And Tdreonine Metabolism)**	
Thioredoxin Disulfide	**Thioredoxin Disulfide (Redox Homeostasis)**	
Succinate (TCA Cycle)	Aspartate (Amino Acid)	
Leucine/Isoleucine (Amino Acid)†	Glycine (Amino Acid)	
	Lysine (Amino Acid)	
	Tyrosine (Amino Acid)	
	Histidine (Amino Acid)	
	Adenine (Purine)	
	Adenosine (Purine Metabolism)	
	2-Oxo-7-Methylthioheptanoic Acid (Carboxylate Metabolism)	
	Glutathione (Glutathione Metabolism)	
	Pyridoxamine (Vitamin B6 Metabolism)	
	L-Citrulline (Urea Cycle)	
	Ornithine (Urea Cycle)	
	Serotonin (Signaling)	
	Trans-4-Hydroxy-L-Proline (Arginine And Proline Metabolism)	
	3-Phospho-D-Erythronate (Other)	
	Peptide Tryptophan (Other)	
	Adenine (Purine)	
	Adenosine (Purine Metabolism)	
	2-Oxo-7-Methylthioheptanoic Acid (Carboxylate Metabolism)	
	Glutathione (Glutathione Metabolism)	
	Glycine (Amino Acid)	

Metabolites significantly increased in the heart after AKI		
4 hours	**24 hours**	**7 days**
S-Allantoin	**S-Allantoin (Purine Metabolism)**	
	Glucose (Glycolysis)	**Glucose (Glycolysis)**
	S-Glutathionyl-L-Cysteine (Glutathione Metabolism)	**S-Glutathionyl-L-Cysteine (Glutathione Metabolism)**
	Threonate (Ascorbate Metabolism)	**Threonate (Ascorbate Metabolism)**
	Pantothenate (Pantothenate And CoA Biosynthesis)	**Pantothenate (Pantothenate And CoA Biosynthesis)**

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Metabolites significantly increased in the heart after AKI		
4 hours	**24 hours**	**7 days**
	Pantothenol (Pantothenate And CoA Biosynthesis)	**Pantothenol (Pantothenate And CoA Biosynthesis)**
	N6-Methyl-L-Lysine (Lysine Metabolism)	**N6-Methyl-L-Lysine (Lysine Metabolism)**
	Threonate (Ascorbate Metabolism)	**Threonate (Ascorbate Metabolism)**
	5-Hydroxyisourate (Purine Metabolism)	Serotonin (Signaling)
	5–6-Dihydrothymine (Pyrimidine Metabolism)	5-L-Glutamyl-Peptide (Glutathione Metabolism)
	UMP (Pyrimidine Metabolism)	N-Glycoloyl-Neuraminate (Aminosugar Metabolism)
	D-Glucose 6-Phosphate (Glycolysis)	Adenosine (Purine Metabolism)
	2-Oxoglutaramate (TCA Cycle)	4-Aminobenzoate (Other)
	D-Glucono-1–5-Lactone 6-Phosphate (Pentose Phosphate Pathway)	L-Citrulline (Urea Cycle)
	D-Ribose (Pentose Phosphate Pathway)	Ornithine (Urea Cycle)
	Ascorbate (Glutathione Metabolism)	
	Gamma-L-Glutamyl-D-Alanine (Glutathione Metabolism)	
	3-Oxalomalate (Glycoxylate And Dicarboxylate Metabolism)	
	Dethiobiotin (Biotin Metabolism)	
	L-2-Aminoadipate (Lysine Metabolism)	

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Table 2.

Metabolic pathways significantly affected in the heart after AKI.

Metabolic pathways significantly affected in t		he heart by AKI
4 hours	**24 hours**	**7 days**
	Pantothenate and CoA biosynthesis	**Pantothenate and CoA biosynthesis**
	Alanine, aspartate and glutamate metabolism	
	Aminoacyl-tRNA biosynthesis	
	Arginine and proline metabolism	
	beta-Alanine metabolism	
	Cyanoamino acid metabolism	
	Glutathione metabolism	
	Glycine, serine and threonine metabolism	
	Histidine metabolism	
	Lysine biosynthesis	
	Lysine degradation	
	Methane metabolism	
	Nitrogen metabolism	
	Pentose phosphate pathway	
	Phenylalanine metabolism	
	Phenylalanine, tyrosine and tryptophan biosynthesis	
	Primary bile acid biosynthesis	
	Purine metabolism	
	Pyrimidine metabolism	
	Selenoamino acid metabolism	

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Table 3.

Echocardiographic findings after acute kidney injury (AKI).

Echocardiography was performed in Normal mice, Sham, and AKI at 24 and 72 hours. Heart rate, ejection fraction, fractional shortening, left ventricular (LV) volume during diastole, LV volume during systole, and E/A were determined. E/A is the ratio of early diastolic filling pressure versus the atrial (late) diastolic filling pressure; the level of reduced E/A 72 hours after AKI is indicative of significant diastolic dysfunction. BPM: beats per minute; LV: left ventricle. *P<0.05 versus Sham; NS for all other comparisons. N=3–5.

24 hours	**Heart Rate (BPM)**	**Ejection Fraction (%)**	**Fractional Shortening (%)**	**LV volume diastole (uL)**	**LV volume systole (uL)**	**E/A**
Normal	562±53	64±2	34±2	60±5	22±3	1.42±0.28
Sham	497±23	62±4	33±3	76±16	29±8	1.40±0.13
AKI	486±13	57 ±4	30±1	81±10	35±7	1.26±0.12
72 hours						
Normal	536±41	62±2	33±1	66±10	25±4	1.42±0.20
Sham	540±85	60±6	32±4	68±13	27±8	1.48±0.13
AKI	495±44	59±3	31±2	56±6	23±3	1.09±0.12*

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Table 4.

Comparison of metabolites that were decreased in the kidney, plasma, and/or heart 4 hours after AKI.

*Top:*Metabolites that were decreased in more than one site 4 hours after AKI are presented in the top section. Only one metabolite, glutamate, was reduced in two or more compartments and was reduced in the kidney and heart. *Bottom*: All of the remaining metabolites that were decreased in either the kidney, plasma or heart 4 hours after AKI are included in the bottom section, and grouped by metabolite type/pathway. In total, 4 hours after AKI, 25 metabolites were decreased in the kidney, 3 were decreased in the plasma, and 6 were decreased in the heart.

Decreased metabolites in the kidney, plasma, and heart at 4 hours			
Kidney	**Plasma**	**Heart**	**Metabolite type or pathway**
Glutamate		Glutamate	Amino acid
Kidney	**Plasma**	**Heart**	**Metabolite type or pathway**
Glycine	Arginine	Alanine	Amino acid
Cysteine		Leucine/Isoleucine	Amino acid
		D-O-Phosphoserine	Serine metabolite
Guanidinoacetate			Arginine and proline metabolism
N-Succinyl-L-citrulline			Arginine and proline metabolism
L-Homocysteine			Cysteine and methionine metabolism
S-Adenosyl-L-homocysteine			Cysteine and methionine metabolism
		Thioredoxin Disulfide	Antioxidant
Glutathione			Glutathione (antioxidant)
5-L-Glutamyl-L-glutamine			Glutathione metabolism
5-Oxoproline			Glutathione metabolism
Gamma-L-glutamyl-D-alanine			Glutathione metabolism
Gamma-L-glutamyl-L-cysteine			Glutathione metabolism
Adenine			Purine
Inosine			Purine metabolism
Hypoxanthine			Purine metabolism
5–6-Dihydrothymine			Pyrimidine metabolism
Cytidine			Pyrimidine metabolism
NAD			Vitamin B3 metabolism, NAD/NADH reactions
Pyruvate			Glycolysis
	Malate	Succinate	TCA cycle
Ethanolamine phosphate			Glycerophospholipid metabolism
	O-Propanoylcarnitine		Carnitine and fatty acid metabolism
Poly-gamma-D-glutamate			Other
S-Formylglutathione			Other
Shikimate 3-phosphate			Other

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Decreased metabolites in the kidney, plasma, and heart at 4 hours			
Kidney	**Plasma**	**Heart**	**Metabolite type or pathway**
1-Hydroxy-2-aminoethylphosphonate			Other
Ectoine			Other
25 total	**3 total**	**6 total**	

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Table 5.

Comparison of metabolites that were increased in the kidney, plasma, and/or heart 4 hours after AKI.

*Top:*Metabolites that were increased in more than one site 4 hours after AKI are presented in the top section. One metabolite, S-Allantoin, was increased in both the plasma and heart; 7 metabolites were increased in both the kidney and plasma. *Bottom:* All of the remaining metabolites that were increased in the kidney, plasma or heart 4 hours after AKI are included in the bottom section, and grouped by metabolite type/pathway. In total, 4 hours after AKI, 27 metabolites were increased in the kidney, 21 were increased in the plasma, and 1 was increased in the heart.

Increased metabolites in the kidney, plasma, and heart at 4 hours			
Kidney	**Plasma**	**Heart**	**Metabolite type or pathway**
	S-Allantoin	S-Allantoin	Purine metabolism
2-Deoxy-alpha-D-glucoside	2-Deoxy-alpha-D-glucoside		Other
6-Phospho-D-gluconate	6-Phospho-D-gluconate		Pentose phosphate pathway
Creatinine	Creatinine		Arginine and proline metabolism
L-Homocitrulline	L-Homocitrulline		Lysine metabolism, urea cycle
D-Ribose	D-Ribose		Pentose phosphate pathway
N-Glycoloyl-neuraminate	N-Glycoloyl-neuraminate		Aminosugar metabolism
Tricanthine	Tricanthine		Other
Kidney	**Plasma**	**Heart**	**Metabolite type or pathway**
Leucine	Asparagine		Amino acid
	Histidine		Amino acid
	Tryptophan		Amino acid
4-Acetamidobutanoate	5-Guanidino-2-oxopentanoate		Arginine and proline metabolism
Carnosine	Phosphocreatine		Arginine and proline metabolism
Homocarnosine			Arginine and proline metabolism
N-Amidino-L-aspartate			Arginine and proline metabolism
L-Citrulline			Arginine metabolism, urea cycle
N-Carbamyl-L-glutamate			Histidine metabolism
N6-Methyl-L-lysine			Lysine metabolism
	Threonate		Ascorbate metabolism
	5-L-Glutamyl-Peptide		Glutathione metabolism
	Hypoxanthine		Purine metabolism
	Xanthine		Purine metabolism
UMP			Pyrimidine metabolism
D-glucono-1,5-lactone			Pentose Phosphate Pathway
	Nicotinamide (NAM)		Vitamin B3 analog
Pyridoxal			Vitamin B6 metabolism
D-Arabitol	D-Rhamnose		Other sugar
Maltose			Other sugar

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Increased metabolites in the kidney, plasma, and heart at 4 hours			
Kidney	**Plasma**	**Heart**	**Metabolite type or pathway**
L-Arabinose			Monosaccharide
N-Acetylneuraminate			Aminosugar metabolism
3D-3–5/4-Trihydroxycyclohexane-1–2-dione	Beta-D-Glucuronoside		Other
6-Aminohexanoate	N-Acyl-D-Aspartate		Other
Ferric gluconate	Triacanthine		Other
Homomethionine			Other
Serotonin			Other
27 total	**21 total**	**1 total**	

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Table 6.

Comparison of metabolites that were decreased in the kidney, plasma, and/or heart 24 hours after AKI.

*Top:*Metabolites that were decreased in more than one site 24 hours after AKI are presented in the top section. One metabolite, tyrosine, was decreased in all three sites. 6 metabolites were decreased in both the plasma and the heart. *Bottom:* All of the remaining metabolites that were decreased in the kidney, plasma or heart 24 hours after AKI are included in the bottom section, and grouped by metabolite type/pathway. In total, 24 hours after AKI, 37 metabolites were decreased in the kidney, 34 were decreased in the plasma, and 20 were decreased in the heart.

Increased metabolites in the kidney, plasma, and heart at 4 hours			
Kidney	**Plasma**	**Heart**	**Metabolite type or pathway**
Tyrosine	Tyrosine	Tyrosine	Amino acid
	Alanine	Alanine	Amino acid
	Lysine	Lysine	Amino acid
	D-O-Phosphoserine	D-O-Phosphoserine	Amino acid metabolite
	Glutathione	Glutathione	Antioxidant
	Pyridoxamine	Pyridoxamine	Vitamin B6 metabolism
	2-Oxo-7-Methylthioheptanoic acid	2-Oxo-7-Methylthioheptanoic acid	Carboxylate metabolism
Glutamate		Glutamate	Amino acid
Glycine		Glycine	Amino acid
Adenine		Adenine	Purine nucleotide
Adenosine		Adenosine	Purine nucleotide
Cysteine	Cysteine		Amino acid
Methionine	Methionine		Amino acid
Valine	Valine		Amino acid
L-Carnitine	L-Carnitine		Carnitine and fatty acid metabolism
cis-p-Coumarate	Cis-P-Coumarate		Other
Kidney	**Plasma**	**Heart**	**Metabolite type or pathway**
Cystine	Arginine	Aspartate	Amino acid
	Proline	Histidine	Amino acid
	Phenylalanine	Peptide Tryptophan	Amino acid
	Threonine		Amino acid
		Trans-4-Hydroxy-L-Proline	Amino acid metabolite
	Indolepyruvate		Tryptophan metabolism
	N6-Methyl-L-Lysine		Lysine metabolism
Guanidinoacetate			Arginine and proline metabolism
		L-Citrulline	Arginine metabolism, urea cycle
L-Homocysteine			Serine metabolism
S-Adenosyl-L-homocysteine			Serine metabolism

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Increased metabolites in the kidney, plasma, and heart at 4 hours			
Kidney	**Plasma**	**Heart**	**Metabolite type or pathway**
5-Oxoproline	Gamma-L-Glutamyl-D-Alanine		Glutathione metabolism
5-L-Glutamyl-taurine	Glutathione Disulfide		Glutathione metabolism
gamma-Glutamyl-gamma-aminobutyrate	S-Glutathionyl-L-Cysteine		Glutathione metabolism
gamma-L-Glutamyl-L-cysteine			Glutathione metabolism
		Thioredoxin disulfide	Antioxidant
AMP	Purine		Nucleotide/nucleotide metabolism
GMP	Cytidine		Nucleotide/nucleotide metabolism
Hypoxanthine	Guanine		Nucleotide/nucleotide metabolism
Inosine			Nucleotide/nucleotide metabolism
alpha-D-Ribose 1-phosphate			Pentose Phosphate Pathway
Sedoheptulose 1-phosphate			Pentose Phosphate Pathway
NAD			Vitamin B3 metabolism, NAD/NADH reactions
Nicotinamide (NAM)			Vitamin B3, NAD/NADH reactions
	2-Methyleneglutarate		Vitamin B3 metabolism
Lactate	D-Glucose		Glycolysis
Pyruvate	Lactate		Glycolysis
	Succinate		TCA cycle
Ethanolamine phosphate			Glycerophospholipid biosynthesis
Glycerol 3-phosphate			Glycerophospholipid biosynthesis
	O-Propanoylcarnitine		Carnitine and fatty acid metabolism
	2-Hydroxyglutarate/Citramalate		Butanoate Metabolism
		Ornithine	Urea cycle
1-Hydroxy-2-aminoethylphosphonate	3D-3–5/4-Trihydroxycyclohexane-1–2-Dione		Other
4-Aminobenzoate	L-Adrenaline	Serotonin	Other
Poly-gamma-D-glutamate	N-Acyl-D-Mannosaminolactone	3-Phospho-D-Erythronate	Other
Ectoine	Sodium Glucuronate		Other
Anthranilate			Other
Hypotaurine			Other
Phosphate			Other
37 total	**34 total**	**20 total**	

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Table 7.

Comparison of metabolites that were increased in the kidney, plasma, and/or heart 24 hours after AKI.

*Top:*Metabolites that were increased in more than one site 24 hours after AKI are presented in the top section. Four metabolites were increased in all three sites. *Bottom:* All of the remaining metabolites that were increased in the kidney, plasma or heart 24 hours after AKI are included in the bottom section, and grouped by metabolite type/pathway. In total, 24 hours after AKI, 39 metabolites were increased in the kidney, 42 were increased in the plasma, and 20 were increased in the heart.

Increased metabolites in the kidney, plasma, and heart at 24 hours			
Kidney	**Plasma**	**Heart**	**Metabolite type or pathway**
Ascorbate	Ascorbate	Ascorbate	Vitamin C (anti-oxidant)
D-Ribose	D-Ribose	D-Ribose	Pentose phosphate pathway
2-Oxoglutarate	2-Oxoglutaramate	2-Oxoglutaramate	TCA cycle
3-Oxalomalate	3-Oxalomalate	3-Oxalomalate	TCA cycle
	Threonate	Threonate	Vitamin C metabolite
	Dethiobiotin	Dethiobiotin	Biotin (Vitamin B7) metabolism
	S-Allantoin	S-Allantoin	Purine metabolism
	D-Glucose 6-Phosphate	D-Glucose 6-Phosphate	Glycolysis
N6-Methyl-L-lysine		N6-Methyl-L-lysine	Lysine Metabolism
Gamma-L-Glutamyl-D-alanine		Gamma-L-Glutamyl-D-alanine	Glutathione metabolism
Histidine	Histidine		Amino acid
4-Acetamidobutanoate	4-Acetamidobutanoate		Arginine and proline metabolism
Creatinine	Creatine		Arginine and proline metabolism
Creatinine	Creatinine		Arginine and proline metabolism
N-Acetyl-L-citrulline	N-Acetyl-L-Citrulline		Arginine and proline metabolism
L-Citrulline	L-Citrulline		Arginine metabolism, urea cycle
Dimethylglycine	Dimethylglycine		Glycine, serine, and threonine metabolism
L-Homocitrulline	L-Homocitrulline		Lysine metabolism, urea cycle
Pyridoxal	Pyridoxal		Vitamin B6 metabolism
N-Glycoloyl-Neuraminate	N-Glycoloyl-Neuraminate		Aminosugar
Ornithine	Ornithine		Urea cycle
Homomethionine	Homomethionine		Other
Methylenediurea	Methylenediurea		Other
Kidney	**Plasma**	**Heart**	**Metabolite type or pathway**
Arginine	Cystine	Threonine	Amino acid

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Increased metabolites in the kidney, plasma, and heart at 24 hours			
Kidney	**Plasma**	**Heart**	**Metabolite type or pathway**
Peptide tryptophan	Glutamine		Amino acid
N-Amidino-L-aspartate			Arginine and proline metabolism
Phosphocreatine			Arginine and proline metabolism
	5-Guanidino-2-Oxopentanoate		Arginine and proline metabolism
	3-Sulfino-L-Alanine		Cysteine and methionine metabolism
N-Carbamyl-L-glutamate			Histidine metabolism
Dehydroascorbate	5-L-Glutamyl-peptide	S-Glutathionyl-L-Cysteine	Glutathione metabolism
	5-Oxoproline		Glutathione metabolism
gamma-L-Glutamylputrescine			Glutathione metabolism
5-Hydroxyisourate	Hypoxanthine	5-Hydroxyisourate	Purine metabolism
	Xanthine		Purine metabolism
5–6-Dihydrothymine	Thymine	5–6-Dihydrothymine	Pyrimidine metabolism
		UMP	Pyrimidine metabolism
		D-Glucono-1–5-Lactone 6-Phosphate	Pentose phosphate pathway
	Maleamate		Vitamin B3 metabolism
		Pantothenate	Vitamin B5
		Pantothenol	Vitamin B5 analog
Pyridoxamine	4-Pyridoxate		Vitamin B6 metabolism
		D-Glucose	Glycolysis
Oxalosuccinate	Fumarate		TCA Cycle
L-Arabinose			Monosaccharide
N-Acetylneuraminate			Aminosugar metabolism
D-Arabitol	D-Rhamnose		Other sugars
	Mannitol		Other sugars
			Other sugars
Putrescine	2-Deoxy-Alpha-D-Glucoside		Other
Serotonin	Beta-D-Glucuronoside		Other
Triacanthine	N-Acyl-D-Aspartate		Other
Hydroxyacetone phosphate	Taurine		Other
N5-Methyl-L-glutamine	Orthophosphate		Other
Acetylcholine	N-N-Dimethylglycine		Other
	Phosphate		Other
39 total	**42 total**	**20 total**	