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Metabolomic Analysis of Pressure-overloaded and Infarcted Mouse Hearts

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Abstract

Background—Cardiac hypertrophy and heart failure are associated with metabolic dysregulation and a state of chronic energy deficiency. Although several disparate changes in individual metabolic pathways have been described, there has been no global assessment of metabolomic changes in hypertrophic and failing hearts *in vivo*. Here, we investigated the impact of pressure overload and infarction on myocardial metabolism.

Methods and Results—Male C57BL/6J mice were subjected to transverse aortic constriction (TAC) or permanent coronary occlusion (myocardial infarction; MI). A combination of LC/MS/MS and GC/MS techniques was used to measure 288 metabolites in these hearts. Both TAC and MI were associated with profound changes in myocardial metabolism affecting up to 40% of all metabolites measured. Prominent changes in branched amino acids (BCAAs) were observed after 1 week of TAC and 5 days after MI. Changes in BCAAs after MI were associated with myocardial insulin resistance. Longer duration of TAC and MI led to a decrease in purines, acylcarnitines, fatty acids and several lysolipid and sphingolipid species, but a marked increase in pyrimidines as well as ascorbate, heme and other indices of oxidative stress. Cardiac

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remodeling and contractile dysfunction in hypertrophied hearts were associated also with large increases in myocardial, but not plasma, levels of the polyamines putrescine and spermidine as well as the collagen breakdown product prolylhydroxyproline.

Conclusions—These findings reveal extensive metabolic remodeling common to both hypertrophic and failing hearts that are indicative of extensive extracellular matrix remodeling, insulin resistance and perturbations in amino acid, lipid and nucleotide metabolism.

Keywords

heart failure; hypertrophy; metabolism; metabolomics; biomarker

Despite recent advances, heart failure continues to be a leading cause of morbidity and mortality in the Western world. Progressive contractile dysfunction during heart failure is in part due to chronic hemodynamic overload, which is initiated and sustained by hypertension, stenotic valves, or tissue damage due to myocardial infarction. As a result, the heart undergoes profound structural and metabolic changes that remain only partially understood.

Previous work has shown that heart failure is associated with dysregulation of both glucose and fatty acid metabolism^{1, 2}. This metabolic dysregulation has been viewed as a contributory factor to the development of heart failure, and it has been suggested that modification of myocardial metabolism could improve clinical outcomes in heart failure patients^{2, 3}. Although some of the metabolic changes that accompany cardiac hypertrophy and failure have been examined^{2–5}, variations in disease manifestation and treatment history have produced discordant results, and data from animal models are diverse. While some models show increased glucose utilization and suppressed fatty acid oxidation^{6, 7}, others show no major changes, at least during early stages of contractile dysfunction^{8, 9}. Moreover, it remains unclear how hypertrophy affects insulin sensitivity, and how this relates to the changes in intermediary metabolism and the development of heart failure^{10, 11}.

In addition to model-dependent variations, some discrepant findings related to metabolic changes could be due to methodological differences. Because, metabolic changes are dynamic, they are difficult to study and experimental procedures such as *ex vivo* perfusion could introduce significant artifacts. Moreover, in contrast to energetic processes, other metabolic pathways have received less attention, even though there is growing recognition that non-energetic processes could also significantly affect cardiac function and remodeling.

The current study was designed to examine metabolic changes that occur in the heart *in vivo* during hypertrophy and heart failure using mouse models of permanent coronary ligation and pressure overload-induced cardiac hypertrophy. Although global metabolic changes in the diseased heart have been studied before, these studies have been limited to the analysis of transgenic models (e.g., transgenic rats harboring human renin and angiotensin genes¹²) or animals with global metabolic dysfunction (e.g., salt-sensitive Dahl rats¹³). Clinical metabolomic profiling studies, on the other hand, have been restricted to measurements of plasma metabolites¹⁴, which reveal little about the metabolic changes in the heart and are often confounded by metabolites derived from non-cardiac sources or concurrent medical treatment. Hence, we examined metabolic changes in clinically-relevant models of

hypertrophy and heart failure. Our results show extensive changes in amino acid, lipid and nucleotide metabolism and provide new understanding of the profound metabolic remodeling that accompanies hypertrophy and progression to heart failure.

Methods

Detailed Methods are provided in the Supplemental Supplement.

Animal surgeries

Adult, 2.75–4.25-month-old, male C57BL/6J mice were subjected to either TAC or MI, as described^{15, 16}, in accordance with the University of Louisville Animal Care and Use Committee.

Sample preparation for metabolomic analysis

Details of sample preparation and metabolomic data analysis are described in the Supplemental Methods and Supplemental Figure I. After anesthesia, the hearts were excised, washed in ice-cold PBS to remove excess blood, and snap-frozen in liquid nitrogen. Metabolites were extracted with methanol, and then prepared for either LC/MS/MS or GC/MS analysis as described before¹⁷.

Statistical considerations

Data are reported as mean \pm SEM. TAC and sham groups were compared by ANOVA, followed by Bonferroni or Tukey post-tests. Unpaired *t* test was used for direct comparisons between MI and its corresponding sham group or when TAC samples at a specific time were compared with their corresponding sham group (e.g., Figure 1B and the Table). Principal component analysis, hierarchical clustering and heatmap analysis, volcano plot analysis, and Fisher's exact tests were performed using Metaboanalyst 2.0 software (<http://www.metaboanalyst.ca/>), which was used also for basic parametric tests applied to metabolomic data and for calculating false discovery rates (FDR). Regression analyses were performed using GraphPad 5.0 software. $P < 0.05$ was considered significant.

Results

Cardiac Echocardiography

Both TAC and MI led to contractile dysfunction (Figure 1A). Left ventricular (LV) function, as measured by ejection fraction (EF), was significantly reduced at 1 d and 8 weeks of TAC, but not after 1 week of TAC; MI produced more severe reductions in EF (Figure 1B). End diastolic volume (EDV) and end systolic volume (ESV) were increased with MI and TAC after 1 d. These changes and other phenotypic characteristics are shown in the Table. Collectively, these results show that 8 weeks of TAC and 5 d of MI led to ventricular dilation and a significant reduction in EF.

Changes in myocardial metabolites

Using an unbiased, non-targeted metabolomic approach¹⁸, the relative concentrations of myocardial metabolites were measured by mass spectrometry and queried against the

Metabolon reference library. Of the 288 metabolites measured, 41% and 24% of the metabolites were lipids and amino acids, respectively. The remaining metabolite superfamilies represented 3–12% of the total metabolites measured in the study (Supplemental Figure II-A). Pressure overload for 1 d resulted in minimal changes in metabolites, whereas longer durations of TAC caused more significant metabolic changes. In hearts subjected to MI, ~40% of the metabolites differed significantly from metabolites in sham-operated hearts (Supplemental Figure II-B).

TAC-mediated metabolite changes

Principal component analysis (PCA) of global changes showed that sham samples clearly separate from the 1 week and 8 week TAC samples (Figure 2A). This is corroborated by heatmap cluster analysis, which showed that the metabolites significantly different in the TAC groups were sufficient to separate the groups (Figure 2B). Metabolic changes after TAC were time-dependent (Supplemental Table I). The 1 d TAC group, with exception for one sample, was indistinguishable from the sham group, whereas 1 week of TAC showed an increase in amino acids and their metabolites. Polyamine metabolites such as putrescine and spermidine were increased after both 1 week and 8 weeks of TAC as was the tryptophan metabolite, C-glycosyltryptophan. A longer duration of TAC (8 weeks) led to a decrease in the abundance of pyruvate, flavin adenine dinucleotide (FAD⁺), and multiple members of the lipid superfamily. One week after TAC, there was a 2-fold increase in phosphoethanolamine levels, which was decreased by 50% by 8 weeks (Supplemental Table I).

MI-induced metabolic changes

PCA analysis of sham versus MI samples showed a clear separation between groups (Figure 3A): 87 of the 288 metabolites analyzed were significantly different from sham (Supplemental Table II). The false discovery rate (FDR) of most significantly changed metabolites was < 0.1. Volcano plot analysis (Figure 3B) shows that the levels of the osmolyte–betaine, as well as the markers of fibrosis (pro-hydroxy-pro and putrescine) and ascorbate were elevated; pantothenate, creatine, and several lipids, notably acylcarnitines such as oleoylcarnitine and palmitoylcarnitine, were reduced. Heme was detected in all 5 of the MI samples and in some of the TAC samples, but not in any of the sham samples. Similarly, dehydroascorbate was detected in only 1 sham sample, but was found in 4 MI samples.

Metabolic changes unique to TAC or MI

Z-score plots were constructed to identify metabolic changes distinct to each group. One day of TAC resulted in changes in only 4 (out of 288) metabolites (Figure 4A); however, 20 metabolites exclusive to hearts after 1 week of TAC were identified, with remarkable changes in 2-arachidonylglycerophosphethanolamine and several amino acids (Figure 4B). Eight weeks of TAC reduced the levels of several members of the lipid superfamily, although levels of 2-hydroxyglutarate and glycerol-3-phosphate were increased (Figure 4C).

A total of 75 metabolites were changed exclusively in infarcted hearts (Figure 4D). Large increases in glycerophosphorycholine, some pyrimidines and pyrimidine metabolites,

peptides such as γ -glutamylglutamate and TDTEKGEFLSEGGV (fibrinogen alpha polypeptide), and N-formylmethionine were increased in MI samples. However, decreases in metabolites involved in lipid and energy metabolism, such as several lysophospholipids, carnitine, and acylcarnitines, were unique to the failing heart. Levels of metabolites central to energy provision such as pantothenate (and its downstream metabolite phosphopantetheine), FAD⁺, pyruvate, creatine, malate, and succinylcarnitine were also diminished (Figure 4D), suggesting a decrease in biochemical energy transfer processes.

Metabolic changes common to both treatment groups

To examine metabolic changes common to both TAC and MI, metabolites affected in both groups were plotted. As shown in Figure 5A, changes in pro-hydroxy-pro, uracil, asymmetric dimethylarginine (ADMA), and several amino acids occurred in both the 1 week TAC and 5 d MI groups. Pro-hydroxy-pro was not plotted for the 1 week TAC group because this metabolite was undetectable in the 1 week sham group; hence, the z-score could not be calculated. Regardless, pro-hydroxy-pro was detected after 1 week of TAC and was higher after MI than in sham control. Both in MI and 1 week TAC hearts, indices of oxidative stress—oxidized glutathione (GSSG) and cysteine-glutathione disulfide—were increased, whereas 1-pentadecanoylglycerophosphocholine, glycerate, propionylcarnitine, and adenosine-3', 5'-diphosphate were less abundant.

Metabolic changes common to MI and 8 week TAC are shown in Figure 5B. Ergothioneine, an antioxidant and thiourea derivative of the betaine of histidine, was increased in both the MI and 8 week TAC groups. Betaine (trimethylglycine) was also increased, as were glucarate and erythronate, which can be formed when N-acetyl-D-glucosamine (GlcNAc) is oxidized. Interestingly, MI, 1 week and 8 week TAC all led to an increase in the C-mannosylated amino acid, C-glycosyltryptohan and methionine, as well as metabolites indicative of the fibrotic response, i.e., putrescine and spermidine (Figure 5C).

Pathway impact analyses

To examine the data in the context of metabolic pathways, pathway impact was calculated as the sum of the importance measures of the matched metabolites normalized by the sum of the importance measures of all metabolites in each pathway. While little pathway impact was calculated from the 1 d TAC group (Figure 6A), the highest pathway impact values in the 8 week TAC group were related with arginine and proline metabolism, pentose/glucuronate interconversions, cysteine/methionine metabolism, and glutathione metabolism. The highest pathway impact for the MI and 1 week TAC groups (Figure 6B,D) was due to branched chain amino acid (BCAA) metabolism (i.e., Val, Leu, Ile metabolism). Other amino acid metabolic pathways, glycerolipid metabolism, purine and pyrimidine metabolism, and linoleic acid biosynthesis also showed relatively high impact scores.

Confirmation of metabolite changes in the failing heart

Changes in metabolites that showed the highest pathway impact and were prominently associated with MI and TAC were confirmed by additional GC/MS analysis using internal controls. This analysis showed that the absolute abundance of BCAAs was increased significantly in the failing heart (Figure 7A) and was highly correlated with EF (Figure 7B).

Levels of both putrescine and spermidine were elevated at 5 d of MI, and correlated well with myocardial function (Figure 7B–7E). The severity of heart failure was also significantly associated with an increase in the concentration of pro-hydroxy-pro (Figure 7G,H).

Discussion

The results of this study reveal profound and progressive changes in the metabolic profiles of hearts subjected to pressure overload or MI. Hearts subjected to these procedures displayed similar changes in amino acid, glycerolipid, and nucleotide metabolism with little accumulation of fatty acids or glycolytic intermediates, suggesting dysregulation of common metabolic pathways. These findings reveal novel metabolic features of hypertrophic and failing hearts and identify potential biomarkers and therapeutic targets.

Amino acid metabolism

One of the most remarkable metabolic changes we found was an increase in branched chain amino acids (BCAAs). Our results show extensive and pervasive increases in BCAAs in both pressure-overloaded and infarcted hearts that are associated with myocardial insulin resistance (Supplemental Figure III). Previous work has shown that elevated plasma levels of BCAAs are associated with metabolic risk factors¹⁹ and insulin resistance in humans²⁰. Elevated BCAA levels have been linked to the development of insulin in rodents as well²¹. Although myocardial insulin resistance is associated with cardiac hypertrophy in humans²² and dogs²³, experiments in mice have produced conflicting results^{10, 11}. Data presented here show that insulin resistance in the failing heart corresponds with increased cardiac levels of BCAAs with no changes in plasma BCAA levels (Supplemental Figure IV). Changes in other cellular processes such protein synthesis, amino acid transport, or autophagy—all of which are increased during cardiac hypertrophy^{24–27}—could also affect amino acid levels in the heart; however, it is unclear how these processes might contribute to changes in myocardial amino acids. Clearly, further work is required to determine how changes in BCAA metabolism contribute to metabolic dysregulation in heart failure.

Lipid metabolism

Myocardial decompensation and failure were associated with extensive changes in lipid metabolism. We found a significant decrease in the levels of several sphingolipids, which could be due to increased utilization or decreased synthesis. While we cannot firmly distinguish between these possibilities, it is likely that sphingolipids are depleted due to increased utilization. Sphingosine is used for the synthesis of ceramide, which has been shown to be increased in failing and hypertrophied hearts^{28, 29}. Recent studies suggest that *de novo* sphingolipid synthesis is essential for myocardial hypertrophy in mice fed a high-fat diet and that sphingolipids are required for the induction of cardiac autophagy³⁰. Alternatively, sphingolipids could be depleted due to neutral sphingomyelinase (SMase), which is activated during heart failure³¹. The activation of SMase has been linked to changes in the glutathione redox state³², which was a prominent finding in our study. Interestingly, phosphoethanolamine (PE), which is downstream of sphingosine in PE

phospholipid synthesis, was also decreased. Collectively, these data suggest that myocardial decompensation is associated with decreased PE phospholipid synthesis.

The decrease in lysolipids after TAC or MI could be attributed to reduced membrane turnover. However, this is not supported by the observed increase in the products of membrane phospholipid catabolism such as glycerophosphorylcholine and glycerol 3-phosphate. Thus, it seems more likely that there is an increase in phospholipid degradation, which would be consistent with studies showing marked changes in phospholipid metabolism in pressure-overloaded hearts³³ and accelerated phospholipid degradation in the ischemic myocardium³⁴.

Energy metabolism

Previous investigations studying metabolic changes in hypertrophic and failing hearts have shown progressive inhibition of fatty acid metabolism and an increased reliance on carbohydrate metabolism^{3, 35}. We found lower levels of free fatty acids in hearts subjected to MI and 8 weeks of TAC, and decreased abundance of multiple acylcarnitines. While the mechanisms underlying these changes remain unclear, it has been shown that in humans with heart failure, myocardial fatty acid turnover and oxidation rates are increased^{36, 37}, but myocardial uptake and retention of fatty acids are impaired^{38, 39}. Therefore, diminished fatty acid uptake along with increased utilization could account for the diminished abundance of acylcarnitines observed in our study. Nevertheless, further work is required to support this conclusion, because a decrease in acylcarnitine could also be due to more general changes in carnitine metabolism^{40, 41}, decreased acylcarnitine synthesis, or reduced fatty acid oxidation..

Though it is widely held that the hypertrophied and failing heart demonstrate increased glycolysis³⁵, we did not find changes in many glycolytic intermediates. Only pyruvate was observed at lower levels in TAC hearts (see Supplemental Table I), and the infarcted heart showed >2-fold decrease in pyruvate (p=0.053). It has been shown that lactate dehydrogenase activity is increased in experimental models of cardiac hypertrophy^{42, 43}, which could account for pyruvate depletion. In support of this, myocardial lactate/pyruvate ratios were elevated 2.5-fold in infarcted hearts (see Online Data), which is consistent with an increase in anaerobic metabolism. Increased flux of pyruvate through the Krebs cycle by anaplerosis is another possibility. In hypertrophied hearts, an ~80% increase in anaplerotic flux was associated with elevated malate levels^{44, 45}, which suggests that the pyruvate-malate pathway (regulated by malic enzyme) is increased in the hypertrophic heart. However, we found that malate was decreased in the failing heart and unaltered in hearts subjected to TAC. Thus, even though a 2-fold increase in malate has been reported in hypertrophic rat hearts perfused *ex vivo*⁴⁴, no increase in malate content in hearts *in vivo* could indicate limited contribution of anaplerotic flux through malic enzyme. Depletion of pyruvate could also be attributed to transamination to alanine, as pyruvate is an acceptor of amino groups generated from protein breakdown. Therefore, given the profound increase in free amino acids observed in this study, it is possible that pyruvate depletion may be related to increased amino acid formation and the formation of keto-acids for energy metabolism.

In contrast to previous studies, which showed no change in the pentose phosphate pathway in hypertrophied, perfused rat hearts⁴⁶, the current study showed that levels of several pentose phosphate pathway intermediates are lower in hearts subjected to MI and TAC. This suggests a potential decrease in anabolic metabolism during myocardial decompensation, which may be an adaptation to oxidative stress, as previous studies show that pentose phosphate pathway-derived NADPH fuels superoxide production in the failing heart⁴⁷. Increased myocardial stress is also in agreement with elevated UDP-GlcNAc levels in hearts subjected to TAC, which is consistent with increased O-GlcNAc signaling that occurs with pharmacological or TAC-induced cardiomyocyte hypertrophy¹⁶ and heart failure¹⁵.

Oxidative stress

The remarkable 50-fold increase in ascorbate levels in failing hearts provides further evidence of oxidative stress. The immediate precursor of ascorbate, gulono-1,4-lactone, also was elevated to levels approaching statistical significance, suggesting that there might be an increase in ascorbate biosynthesis. Interestingly, infarcted hearts contained high levels of heme, which in the presence of ferrous iron and ascorbate could generate oxidants^{48, 49} and contribute to oxidative stress in the failing heart.

Markers of cardiac remodeling

We found that C-glycosyltryptophan, polyamines, and pro-hydroxy-pro were markedly elevated both after MI and TAC. These metabolites have been found previously to be elevated in the plasma of patients with dilated cardiomyopathy⁵⁰, although the tissue of origin was not identified. Although we found that these metabolites were increased in the heart, which could be indicative of cardiac remodeling, we did not observe a concomitant increase in their plasma levels (Supplemental Figure IV). Thus, elevations of these metabolites in plasma may not be reflective of their changes in the myocardium.

Limitations

Our study has several limitations. First, even though we measured a large number of metabolites, important metabolites such as fructose 1,6- and 2,6-bisphosphate, pyridine nucleotides, ATP/ADP, and malonyl CoA could not be detected because of technical limitations. Moreover, as with any metabolomic study, only the levels, but not the fluxes of the metabolites were measured. This limitation prevented us from estimating changes in the activity of individual metabolic pathways. Lastly, it is possible that isolation of hearts prior to metabolite extraction could have produced artifactual changes; however, we consider this unlikely because ischemia prior to freezing would have induced high variability in lactate/pyruvate ratios, which was not observed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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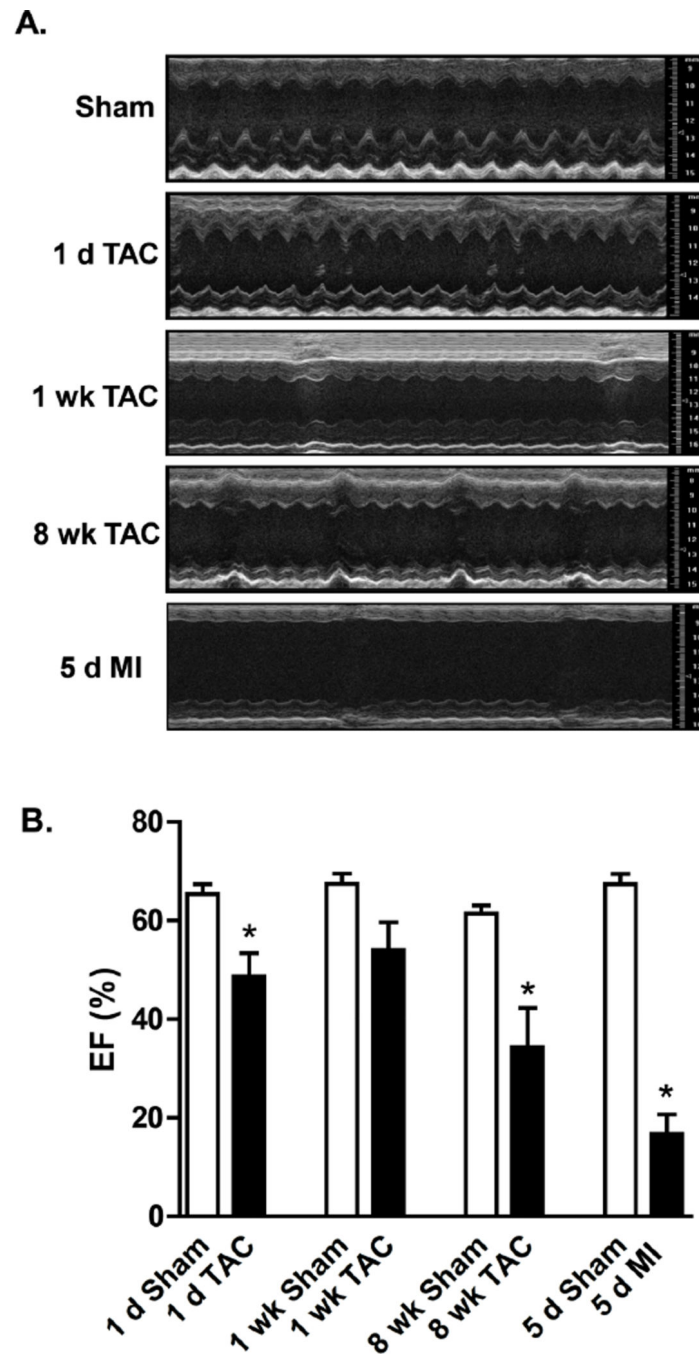


Figure 1. Cardiac echocardiography after sham surgery, transverse aortic constriction (TAC), or permanent coronary ligation (myocardial infarction; MI)

(A) Representative M-mode images. (B) Ejection fraction; n = 5–8 per group, *p<0.05.

Other measurements are listed in the Table.

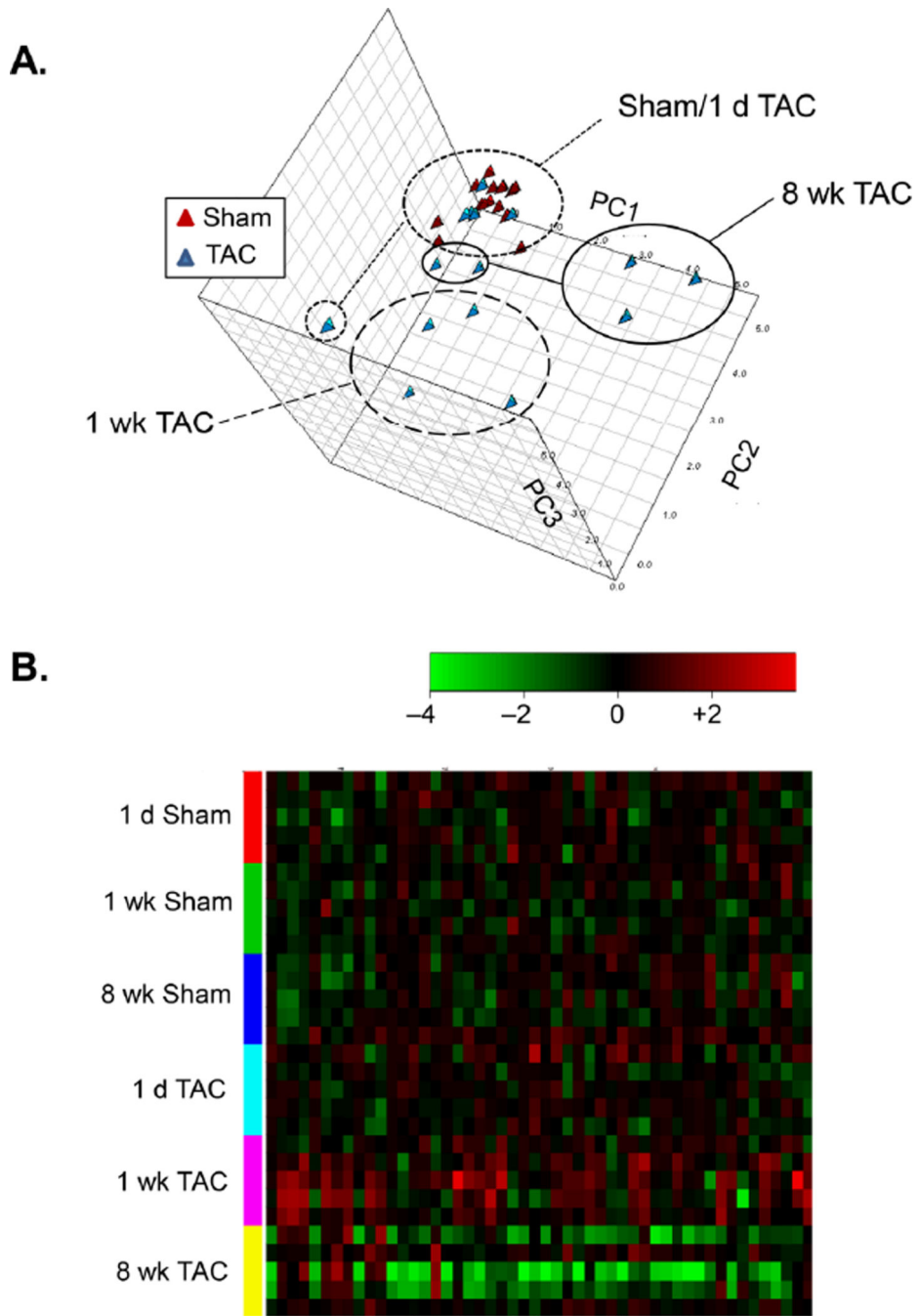


Figure 2. Stage-specific grouping of pressure-overloaded hearts based on metabolic profile (A) PCA analysis of cardiac metabolites after 1 d and 1 and 8 weeks of TAC compared with their respective shams. (B) Hierarchical cluster analysis of the 50 most significant TAC-affected metabolites; n = 5 per group. A complete list of metabolites significantly different in TAC compared with sham hearts is shown in Supplemental Table I.

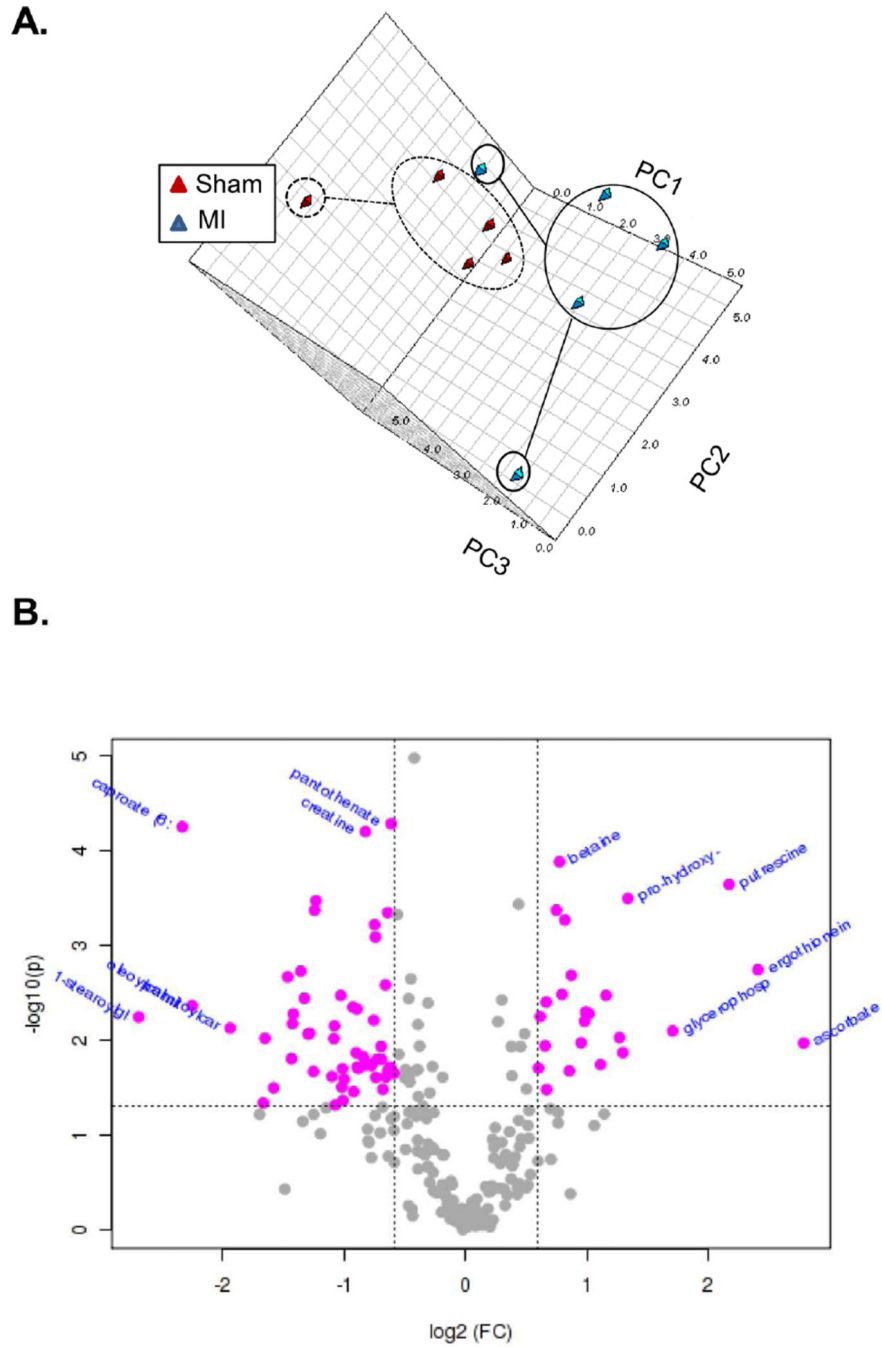
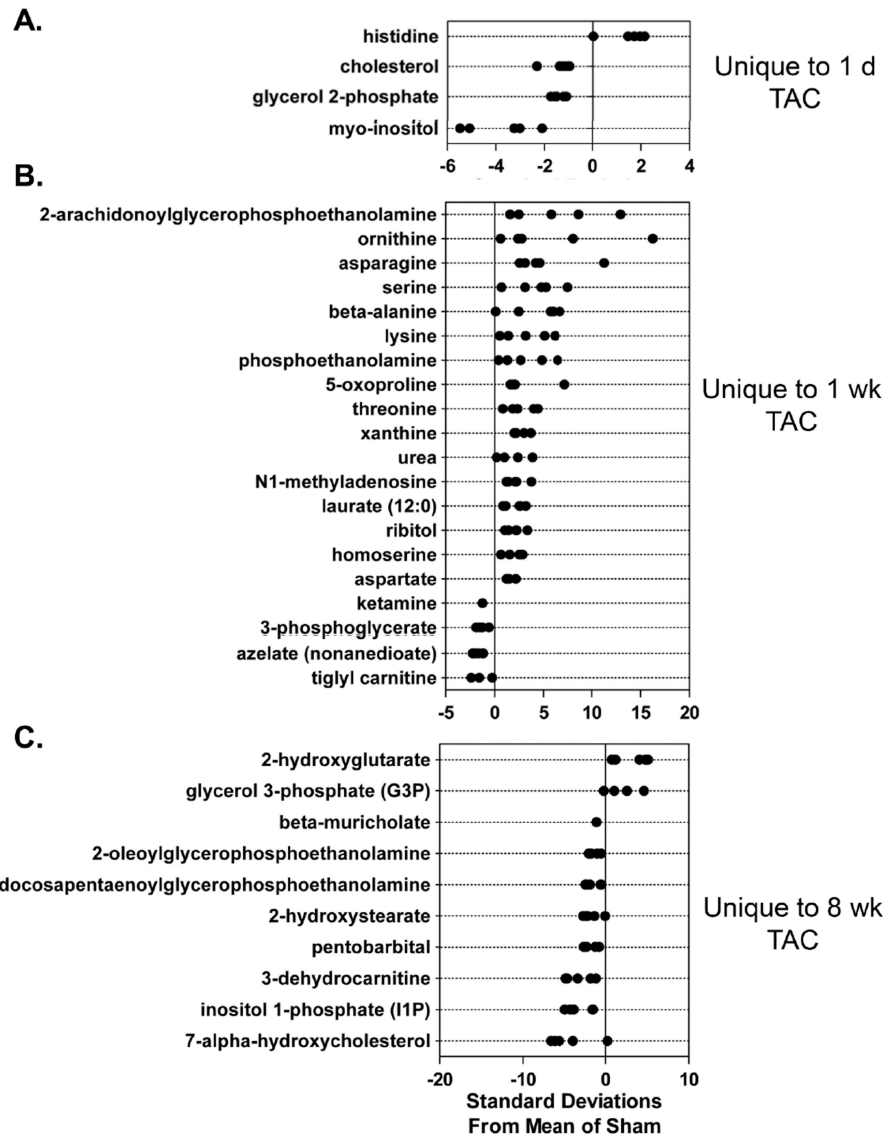


Figure 3. Metabolic changes in myocardial metabolites after infarction
 (A) PCA analysis of MI and sham groups. (B) Volcano plot analysis of metabolic changes in infarcted hearts (compared with sham hearts); n = 5 per group; for volcano analysis, the fold-change threshold was 1.5 (x-axis) and the p value threshold was set at 0.05 (y-axis). Values in pink were found to be significantly different. Metabolites significantly changed after MI are listed in Supplemental Table II.



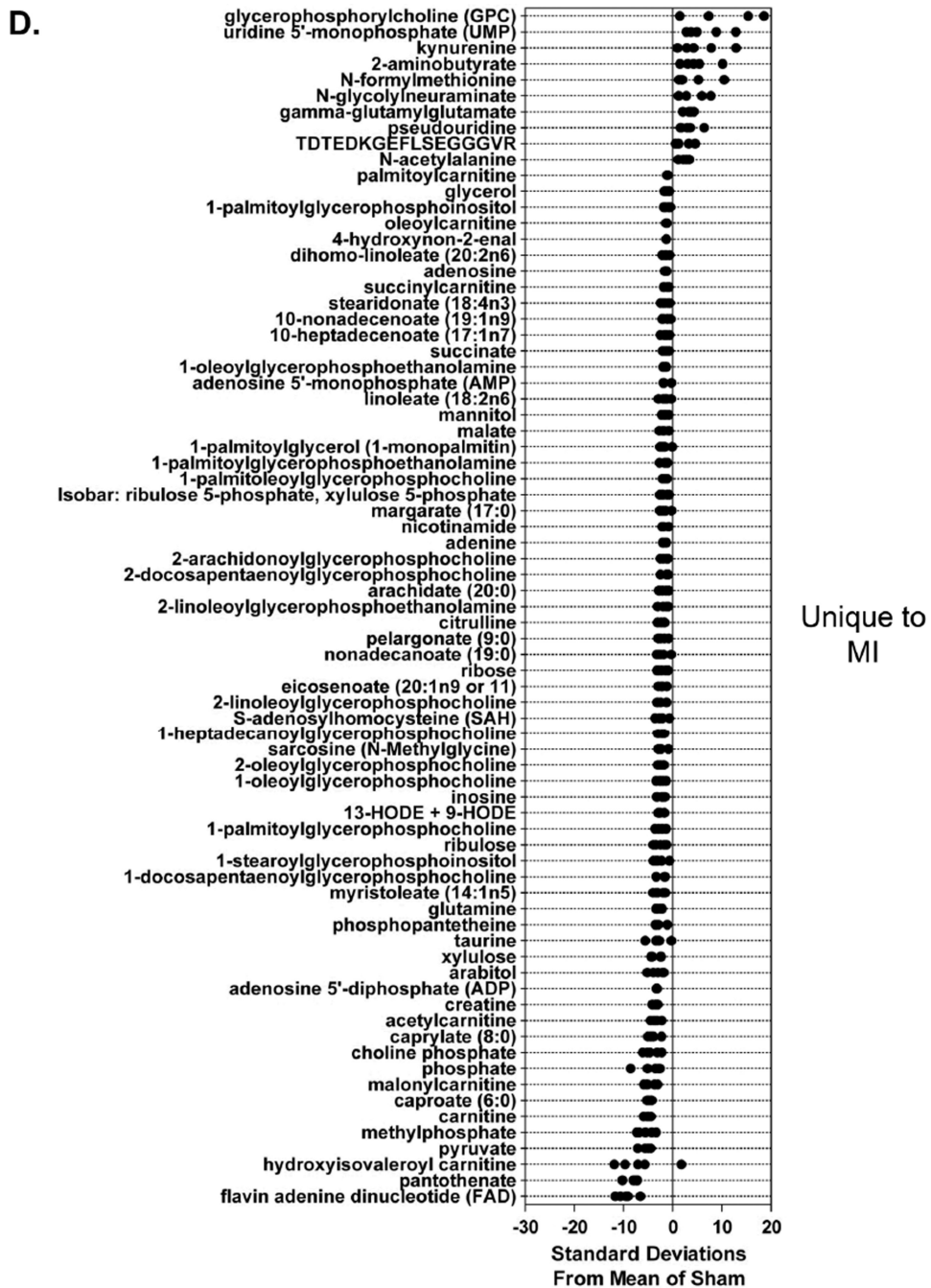
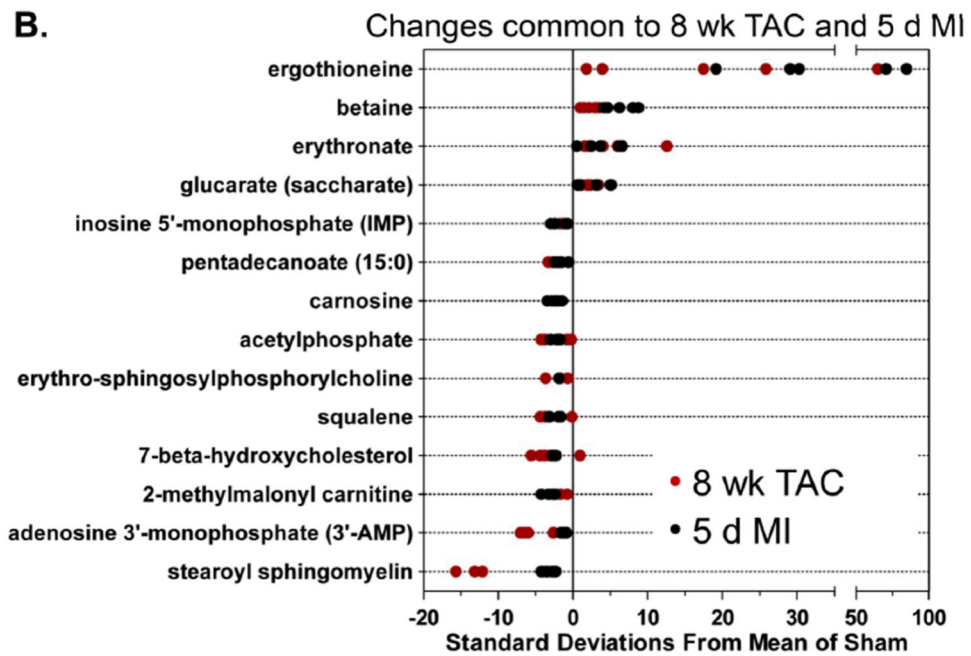
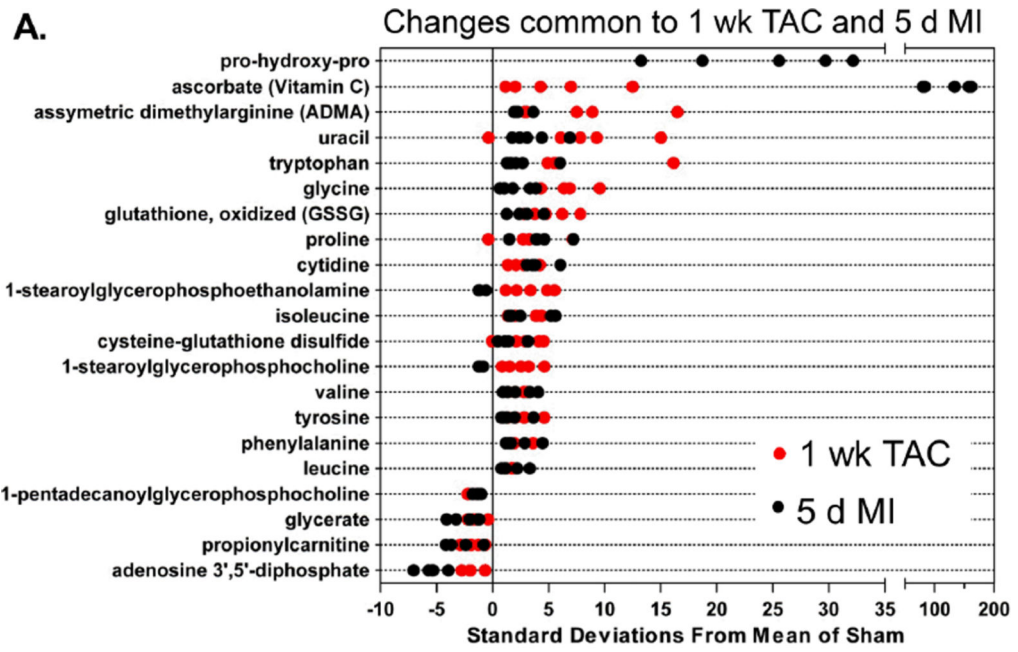


Figure 4. Different stages of ventricular dysfunction show unique metabolic signatures
Z-score plot analysis of metabolic changes unique to TAC or MI: Metabolic changes unique to (A) 1 d; (B) 1 week; and (C) 8 weeks of TAC; and (D) 5 d of MI. Data are shown as standard deviation from the mean of respective sham. Each dot represents a single metabolite in one sample; n = 5 per group.



C.

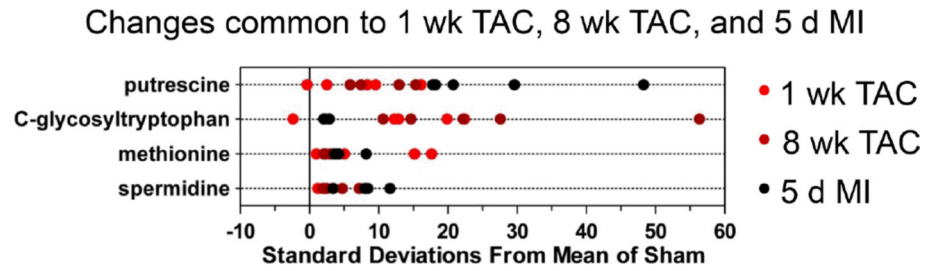


Figure 5. Metabolic changes common to both TAC and MI

Z-score plot analysis of metabolic changes common between: (A) 1 week TAC and 5 d MI; (B) 8 week TAC and 5 d MI; and (C) 1 week TAC, 8 week TAC and 5 d MI. There were no shared changes with 1 d TAC group. In each plot, the data are shown as standard deviation from the mean of their respective sham. Each dot represents a single metabolite in one sample. n = 5 per group.

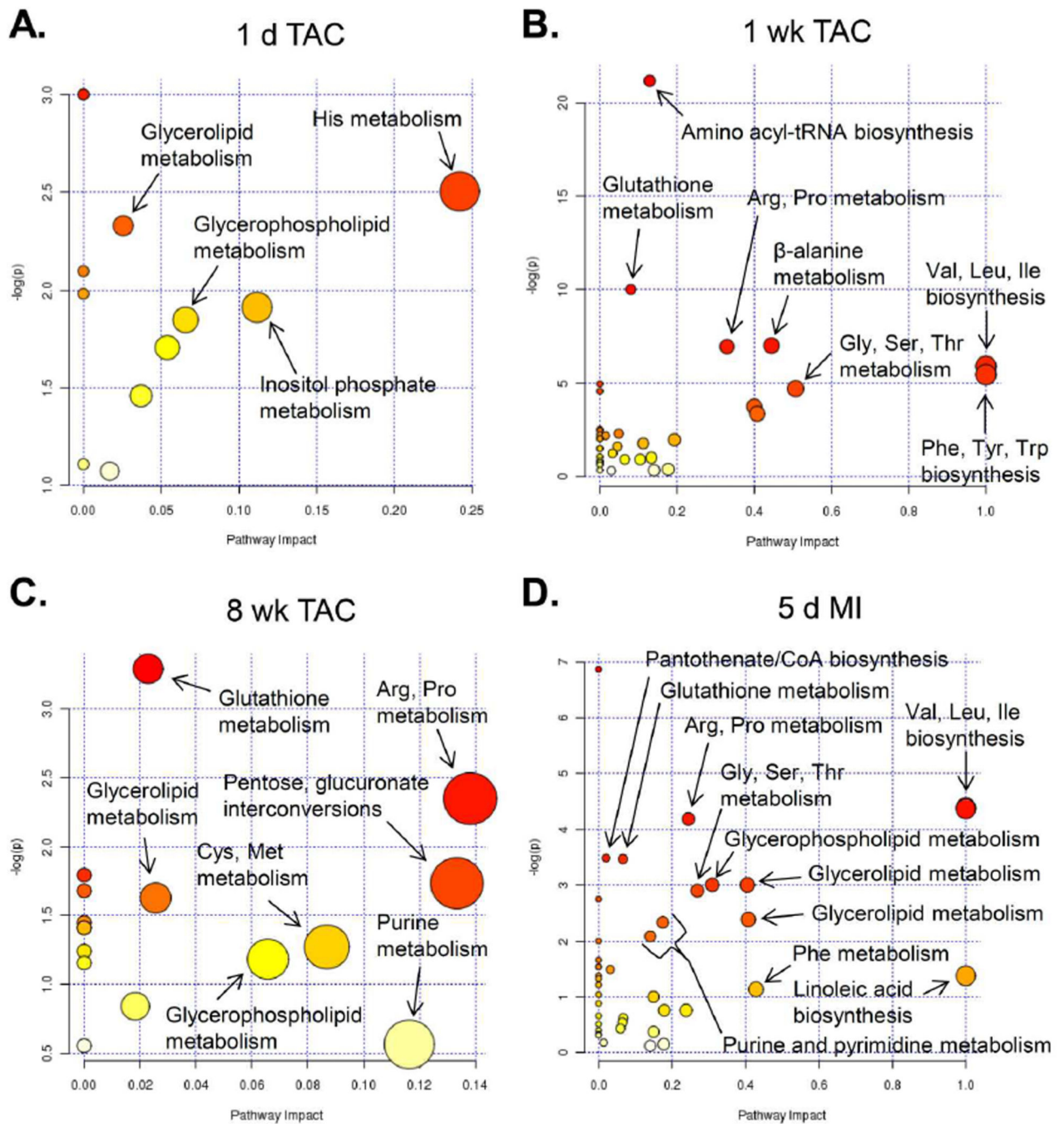


Figure 6. Pathway impact analysis of metabolic changes
 Metabolites showing significant changes were analyzed using Metaboanalyst MetPA and the *Mus musculus* pathway library: (A) 1 d; (B) 1 week; and (C) 8 weeks of TAC; and (D) 5 d of MI. Fisher's exact test was used for overrepresentation analysis, and relative betweenness centrality was used for pathway topology analysis; n = 5 per group.

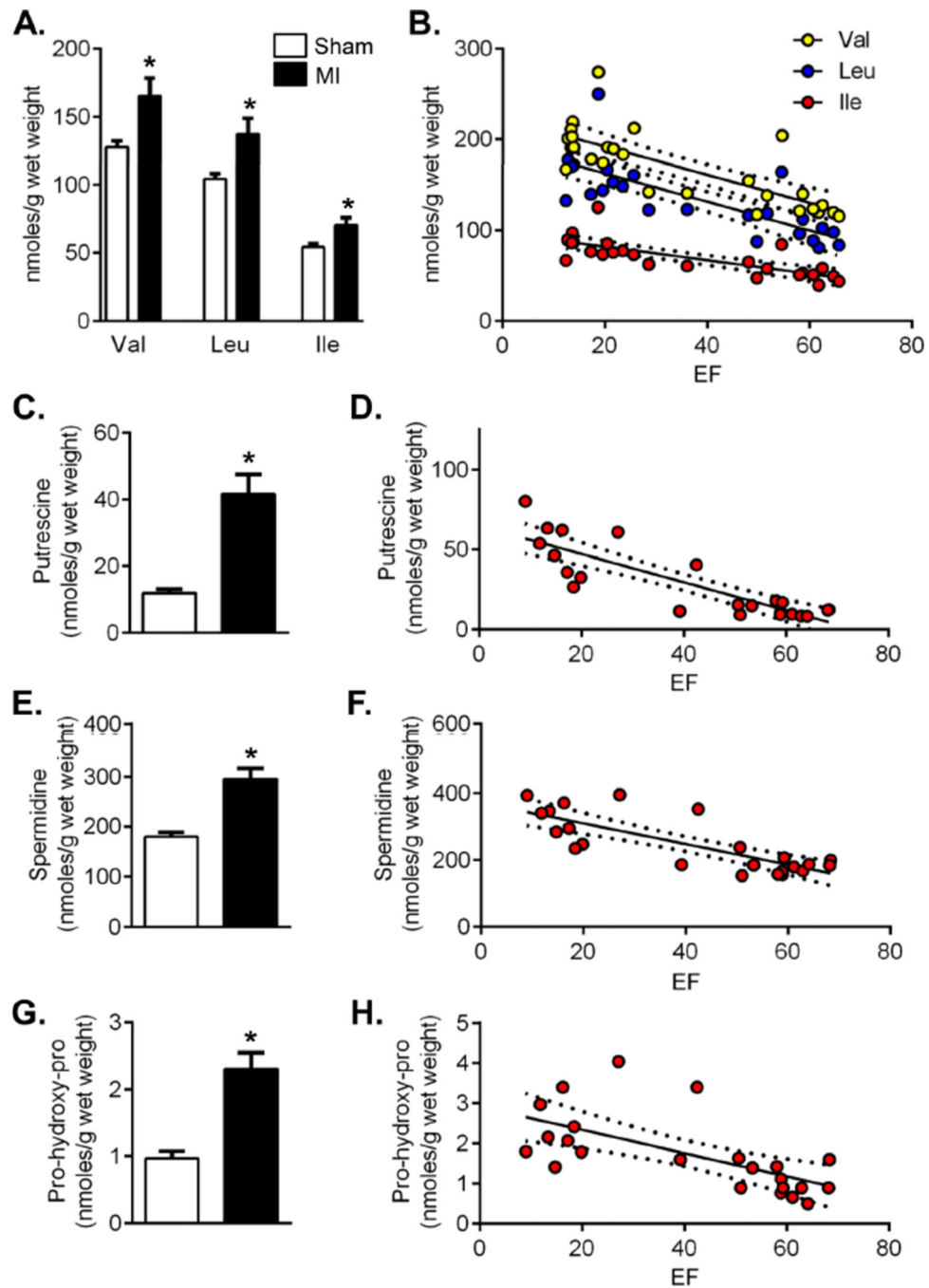


Figure 7. Heart failure increases BCAAs, polyamines, and prolylhydroxyproline
 GC/MS analysis of cardiac metabolites after 5 d sham and 5 d MI: **(A)** BCAA levels; **(B)** Regression analysis of BCAA levels vs. ejection fraction (EF): R² = Val, 0.59; Leu, 0.61; Ile, 0.59; all p < 0.0001; **(C)** Putrescine levels; **(D)** Regression analysis of putrescine levels vs. EF: R² = 0.73, p < 0.0001; **(E)** Spermidine levels; **(F)** Regression analysis of spermidine levels vs. EF: R² = 0.63, p < 0.0001; **(G)** Pro-hydroxy-pro levels; **(H)** Regression analysis of

pro-hydroxy-pro levels vs. EF: $R^2 = 0.41$, $p=0.0009$; $n = 10-13$ per group; $*p<0.05$ vs. sham (unpaired t-test); in regression analyses, dotted lines indicate 95% confidence intervals.

Table

Phenotypic characteristics of C57BL/6J mice subjected to sham treatment, transverse aortic constriction (TAC), or permanent coronary ligation (MI).

Group	n	Age (wk)	BW (g)	HR (bpm)	LVIDD (mm)	LVIDS (mm)	IVSTD (mm)	LVPWD (mm)	LV mass (g)	Vcfc (circ/sec)	EDV (µl)	ESV (µl)
1 d Sham	5	12	28.6±1.3	535±11	3.77±0.11	2.41±0.07	0.87±0.04	0.75±0.05	110±6	25.2±1.0	39.8±2.4	13.9±1.5
1 d TAC	8	12	29.1±0.7	576±12*	4.11±0.14	3.13±0.21*	0.96±0.06	0.73±0.05	134±11	17.6±1.9*	54.9±4.6*	29.5±5.2*
1 wk Sham	6	12	28.8±1.0	516±27	3.87±0.10	2.32±0.19	0.72±0.06	0.55±0.04	83±7	27.5±3.5	49.1±2.4	16.0±1.5
1 wk TAC	7	12	29.6±0.6	541±11	3.84±0.10	2.87±0.13*	0.93±0.04*	0.75±0.08*	119±8*	15.9±1.1*	46.3±6.7	23.4±6.3
8 wk Sham	5	20	27.2±1.1	493±7	4.02±0.17	2.69±0.15	0.80±0.09	0.65±0.07	106±15	19.7±1.4	57.7±5.0	22.1±1.8
8 wk TAC	5	20	26.7±0.9	575±28*	5.05±0.30*	4.21±0.40*	0.95±0.04	0.74±0.05	190±25*	12.6±2.4*	74.6±9.0	50.9±10.9*
5 d Sham	5	18	27.5±0.8	542±27	3.70±0.09	2.50±0.15	0.90±0.06	0.76±0.07	109±4	23.6±1.8	41.9±2.4	13.7±1.4
5 d MI	7	18	28.8±0.9	598±20	5.84±0.33*	5.53±0.36*	0.67±0.08	0.52±0.09	160±26	4.8±1.2*	119.0±15.4*	102.0±16.6*

* p<0.05 vs. matched sham group (unpaired t-test); BW, body weight; HR, heart rate; LVIDD, left ventricular internal diameter in diastole; LVIDS, left ventricular internal diameter in systole; IVSTD, interventricular septal thickness in diastole; LVPWD, left ventricular posterior wall in diastole; LV mass, left ventricular mass; Vcfc, velocity of circumferential shortening; EDV, end diastolic volume; ESV, end systolic volume