The heart after AKI looks like it had an MI (and, how to use and interpret metabolomics data)

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Ben Fox, BS

Outline

- AKI has an independent effect on mortality
- AKI has independent effects
  - traditional
  - non-traditional
  - even in sick people
- AKI and the heart, mice and people
- Metabolomics in the heart after AKI in mice

AKI is an independent predictor of mortality in every setting

ARF characteristics	Adjusted OR of death	Study
Aortic surgery	9.1	Kashyap, 1997
Cardiac surgery	7.9	Chertow, 1998
ICU	1.6	De Mendonca, 2000
Hospital-acquired	2.5	Obialo, 2000
Amphotericin B	6.6	Bates, 2000
Liver cirrhosis	4.3	Aggarwal, 2001
Bone marrow transplant	6.8	Parikh, 2005

So what is the mortality of AKI, and how does it compare to other ICU diseases?

No AKI, No ESRD

Acute lung injury

Myocardial infarction

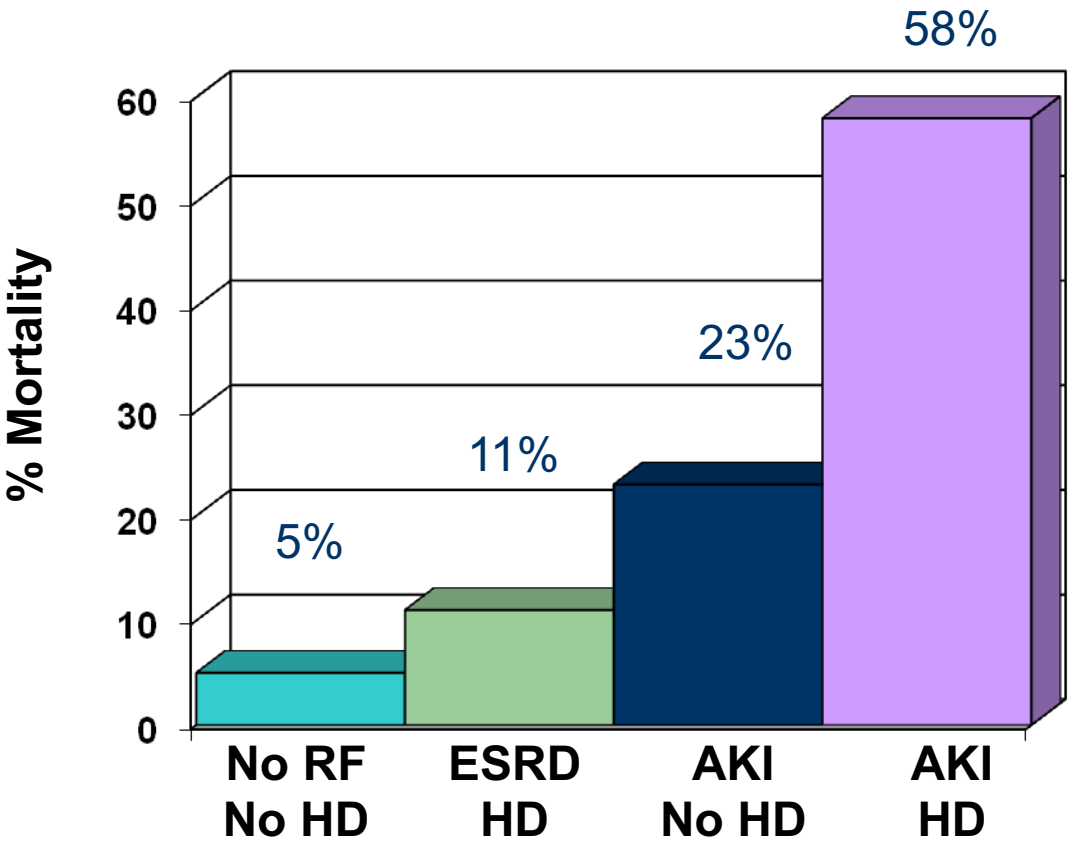
AKI not requiring dialysis

ESRD

AKI requiring dialysis

No AKI, No ESRD	5%
Myocardial infarction	5 to 13%
ESRD	5 to 26%
Acute lung injury	< 20%
AKI not requiring dialysis	20 to 40%
AKI requiring dialysis	50 to 60%

Mortality of AKI versus ESRD in the ICU



APACHE III and APS scores of ARF and ESRD were the same

Clermont G, et al KI. 2002 62:986-996

In fact…AKI mortality is consistently higher than ESRD mortality

- Retrospective, Riyadh ICU database (41,972)
  - 54% vs. 21 (n= 797, 935)[1]
- Case control (n = 54)
  - 50% vs. 24%[2]
- For patients only receiving CRRT for RRT
  - 63% vs. 46%[3]
  - 61% vs. 36%[4]

1) Rocha, E. NDT. 2009. 2) Ostermann, M. Critical Care. 2008.

3) Hussain, S Hemodial Int 20009. 4) Walcher, A Renal Failure, 2011.

What then, are the independent complications of AKI that contribute to mortality?

Complications of AKI – What UpToDate thinks (i.e., traditional):

- Vol
- M
- elet
- function)
  - ing, bleeding,
- Perid itis, neuropathy, altered mental status

1) These complications are also complications of ESRD

2) These complications can be corrected by RRT

3) AKI is not acute ESRD

Independent systemic complications of AKI (non-traditional complications)

- Respiratory complications
- Lung injury (Rabb, Faubel)
- Heart failure (CRS 3)
- Cardiac injury (Kelly, Doi)
- Liver injury (Lee)
- Metabolic effects
- Sepsis/Infection (PICARD, Jovanovich, Gist)
- Confusion
- Brain injury (Rabb)
- Intestinal injury (Lee)

Independent systemic complications of AKI identified in sick people using omics

- Community Acquired Pneumonia and Sepsis Outcome Diagnostic (CAPSOD) study
- 150 patients with critical illness, (sepsis or SIRS) at the time of presentation to the ED
- Designed to identify markers to identify community acquired sepsis from non-infectious SIRS and to differentiate 28-day survivors from non-survivors at the time of initial presentation
- Blood collected within 24 hours of hospital admission

Tsalik, KI, 2015

Importance of kidney function in the analysis

- 370 metabolites were measured (241 annotated)
- Original analysis found that **renal dysfunction** was the **greatest** contributor to variance in the plasma metabolome
- Kidney function explained **44%** of variance

Table 1 | Patient demographics

Acute kidney injury stage	Stage 0	Stage 1	Stage 2/3	HD
n	65	41	20	24
APACHEII	13.8 ± 7.5	18.5 ± 7.9	22.8 ± 8.3	17.7 ± 5.0
Staphylococcus aureus (%)	20.0	9.8	10.0	33.3
Staphylococcus pneumonia (%)	21.5	19.5	35.0	8.3
Escherichia coli (%)	12.3	14.6	5.0	4.2
Other etiologic agent[a] (%)	12.3	22.0	20.0	33.3
Unidentified etiologic agent (%)	10.8	12.2	25.0	4.2
No infection (%)	23.1	22.0	5.0	16.7
Death (%)	20.0	29.3	40.0	12.5
Age	59.6 ± 17.7	64.9 ± 16.6	67.4 ± 18.6	51.6 ± 12.1
Gender (male) (%)	50.8	61.0	45.0	62.5
Race (B/W/O)	40/23/2	27/11/3	11/6/3	22/2/0
Liver disease (%)	6.2	7.3	20.0	4.2
Heart failure (%)	6.2	9.8	15.0	4.2
Chronic lung disease (%)	30.8	29.3	30.0	20.8
Malignancy (%)	13.8	19.5	5.0	8.3

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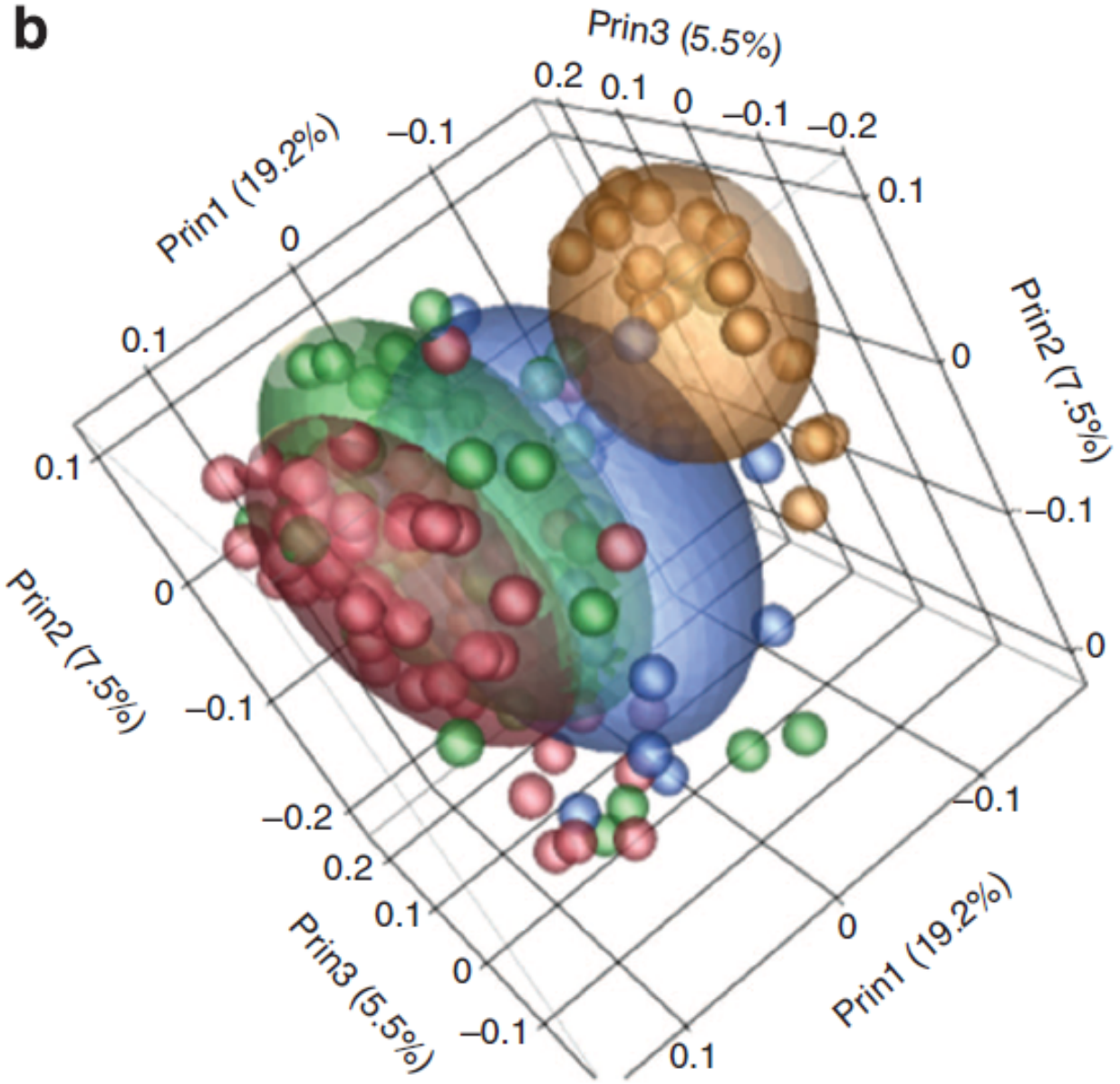


Unsupervised principal components analysis by Pearson's correlation demonstrated primary segregation by renal function category

(red: no AKI, green AKI1, blue AKI2/3, brown: ESRD)

How the heart works – A review, by and for nephrologists

- The heart pumps blood to the kidney
- The heart needs ATP to contract to pump blood
- The heart makes ATP via oxidative phosphorylation (requires oxygen/mitochondria)
- No oxygen = no ox phos = bad
  - ATP sores can use used up in seconds
  - Decreased cardiac contraction
  - Increased ROS -> increased apoptosis
  - Compensation: alternative sources of energy production

Cardiorenal syndrome (Ronco, 2008)

- Cardiorenal syndrome type 1
  - Acute cardiac dysfunction leading to AKI
- Cardiorenal syndrome type 2
  - Chronic cardiac dysfunction leading to CKD
- Cardiorenal syndrome type 3
  - AKI leading to acute cardiac dysfunction
- Cardiorenal syndrome type 4
  - CKD leading to chronic cardiac dysfunction
- Cardiorenal syndrome type 5 (Secondary)

AKI and the heart, epi data

- AKI with cardiac failure (de Mendonca, 2000)
  - 16 countries, 40 ICUs
  - 64% mortality
  - Highest of any other organ failure
- Mortality of MI, HF, post-cardiac surgery is higher with AKI (many studies)

Ischemic AKI and the heart, acute effects, rats– Kelly, JASN, 2003

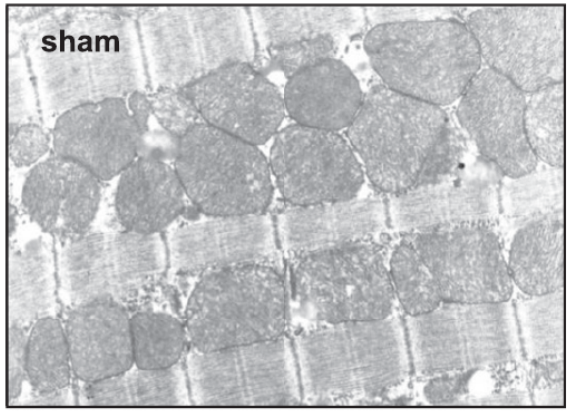
- First major report to look at the heart after AKI
- Increased TNF, IL-1β, ICAM-1 (6, 24, 48 hrs)
- Increased MPO activity (6 hrs)
- Increased apoptosis (48 hrs)
- Echo: decreased fractional shortening (48 hrs)
  - (A marker of impaired contractility)
- Anti-TNF treatment reduced apoptosis

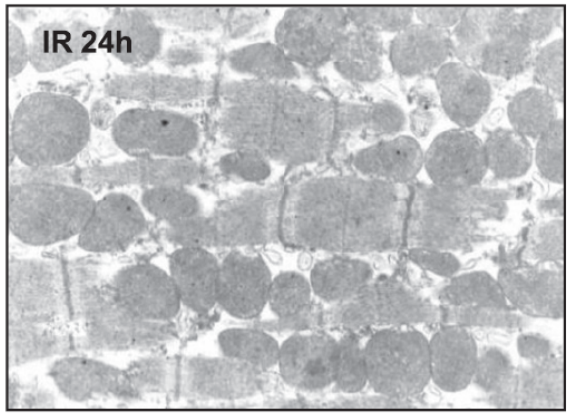
Other studies

- Nath, Am J Pathology, 2005
  - Ischemic AKI, mice, 24 hours
  - Vascular congestion in the heart is increased in AKI
- Tokuyama, Nephron Physiology, 2007
  - Permanent unilateral renal artery ligation, mice, 24 hrs
  - Increased macrophages and osteopontin
  - Reversed with Losartan

Ischemic AKI and the heart, acute effects, mice– Sumida, Doi, JASN, 2015

- Apoptosis (24 and 72 hrs)
- Mitochondrial fragmentation (24 hrs)
- DRP 1 (fission) increased (24 hrs)
- Echo: decreased fractional shortening (not 24; but 72 hrs)
- Mitochondrial division inhibitor-1 (mdivi-1) reduced apoptosis and improved cardiac function by echo
- DRP1 also increased during MI, and inhibition protective (Sharp, FASEB, 2014)





Hypothesis

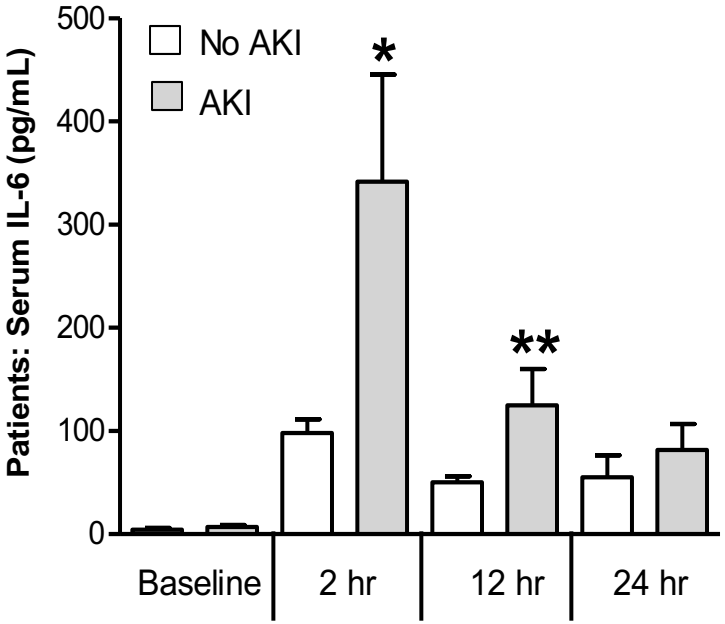
- Since decreased cardiac contractility occurs in the heart during AKI, and
- Since contractility is normally maintained by ATP production via ox phos in mitochondria
- Then, hypothesize that metabolomics in the heart after AKI will be characterized by the hallmarks of hypoxia and impaired ox phos (alternate energy production)
  - e.g., perturbations in purine/pyrimidine metabolism, amino acid metabolism, oxidative stress

Method

- C57Bl/6 mice, male, between 8 and 10 weeks
- 7 groups:
  - Normal
  - Sham at 4 hours, 24 hours, 7 days
    - Anesthesia, laparotomy
  - Ischemic AKI at 4 hours, 24 hours, 7 days
    - Anesthesia, laparotomy
    - 22 minutes of bilateral clamping and then clamp release
- 124 metabolites measured in heart in all groups

Sham = surgical stress
Ischemic AKI = surgical stress + AKI

IL-6 in patients with AKI



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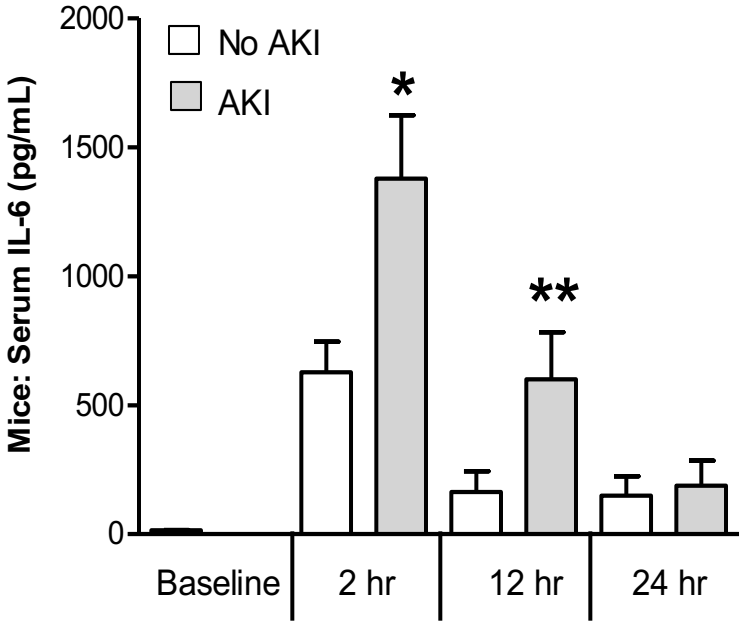
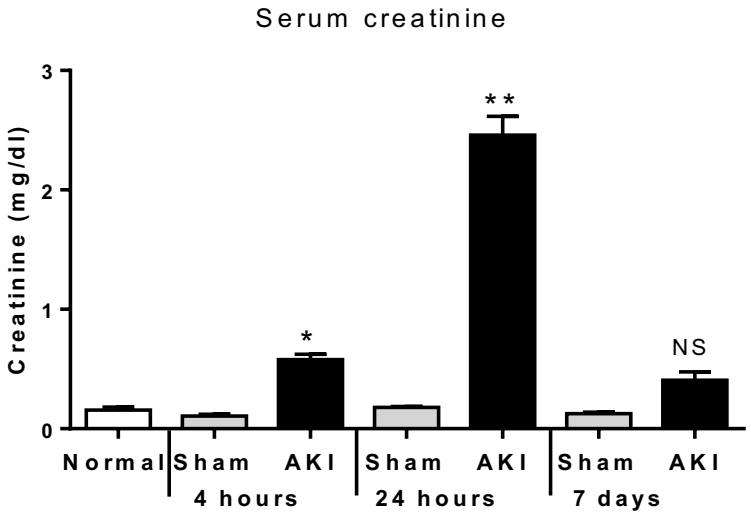
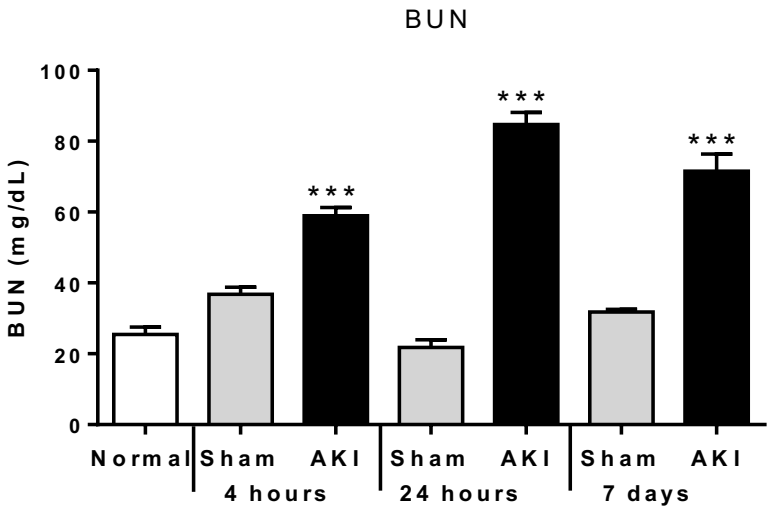


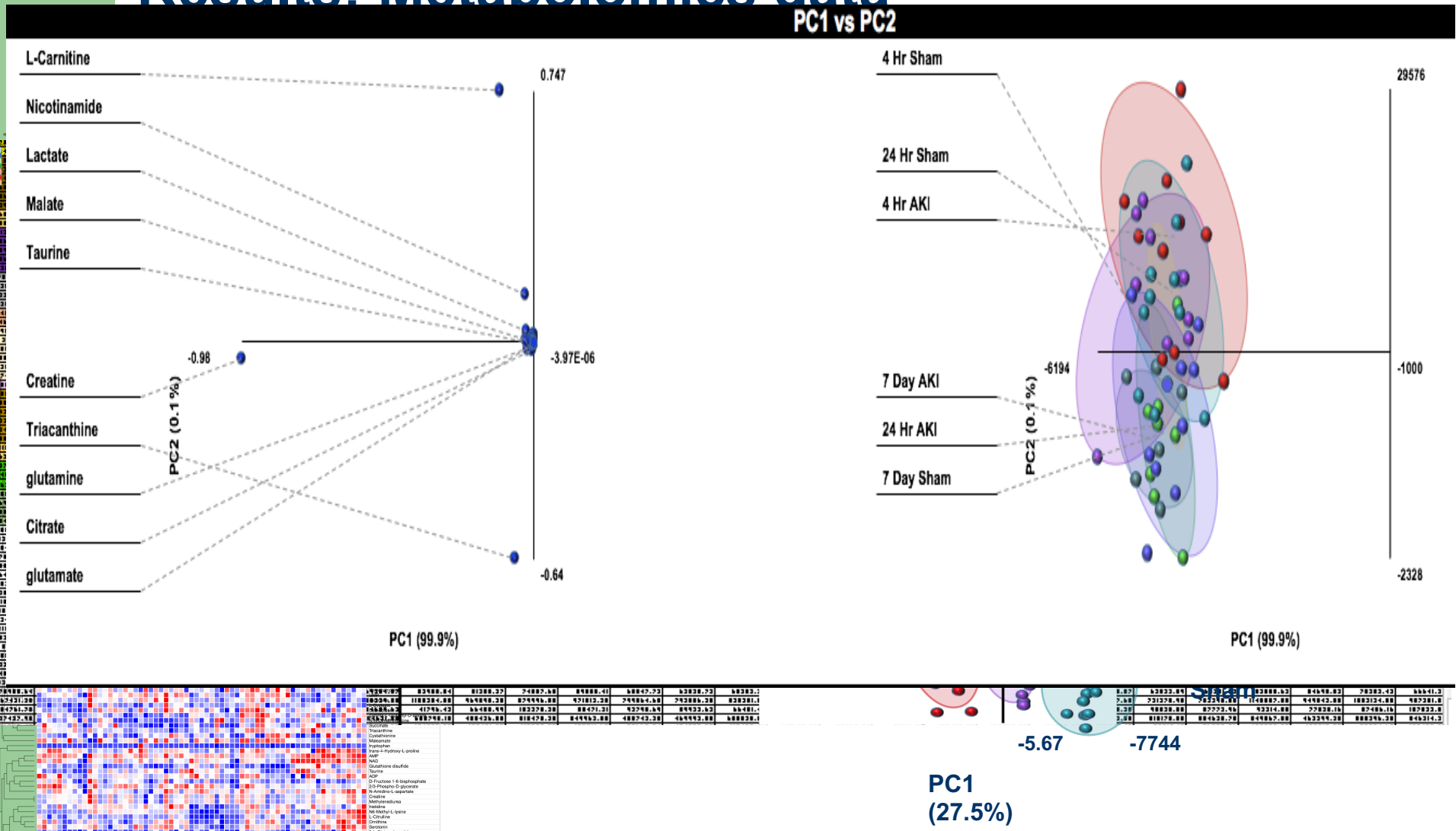
Liu, KD, Altmann, C, Smits, G, Krawzeski CD, Edelstein, CL, Devarajan, P, Faubel, S. Critical Care, 2009.

Results: Time course of kidney dysfunction





Results: Metabolomics data



Now what??

arginine

Google Search I'm Feeling Lucky

Shop on Google

Sponsored

NOW Foods
L Arginine | 500
...
$7.39
Swanson Health

NOW Foods
Arginine ...
$22.40
Jet.com
Special offer

NOW Foods
L Arginine ...
$23.12
Swanson Health...
(115)

NOW Foods
Tri Amino | 120
...
$14.79
Swanson Health

Doctor's Best
L Arginine ...
$14.02
Jet.com
Special offer

Puritan's Pride
L Arginine 1000
...
$14.39
Puritan's Pride

More on Google

Arginine - Wikipedia

https://en.wikipedia.org/wiki/Arginine

Arginine encoded by the codons CGU, CGC, CGA, CGG, AGA, and AGG i
ginine is classified as a ...
· ADMA · Arginine glutamate

the Heart - Healthline

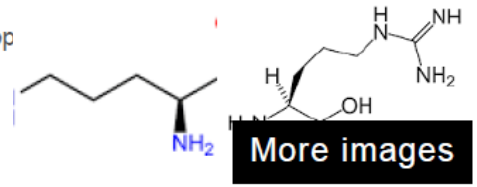
th/arginine-heart-health

or L-**arginine**, is one of the 20 amino acids we get from pı
i-essential amino acid. Find out more.

n and L-arginine: Get the Facts - Healthline

th/erectile-dysfunction/l-arginine

erectile dysfunction, you may be interested in herbal supp
s about this supplement.



N4O2 - PubChem

.nih.gov/compound/L-arginine

mino acid in juvenile humans, **Arginine** is a complex amin
eins and enzymes due to its ...

$_{14}N_4O_2$ **PubChem CID**: 6322
)4 g/mol **Chemical Names**: L-arginine; Arginine; 74-79-3

rginine and exercise. - NCBI

gov/pubmed/18090659

ted by 58 - Related articles
ent studies have shown that resting growth hormone respo
ginine and the dose range is 5-9 g ...

Heart Health & Performance - Dr. Axe

ne/

is an important amino acid found in protein foods. L-argin
ormance and so much more, with ...

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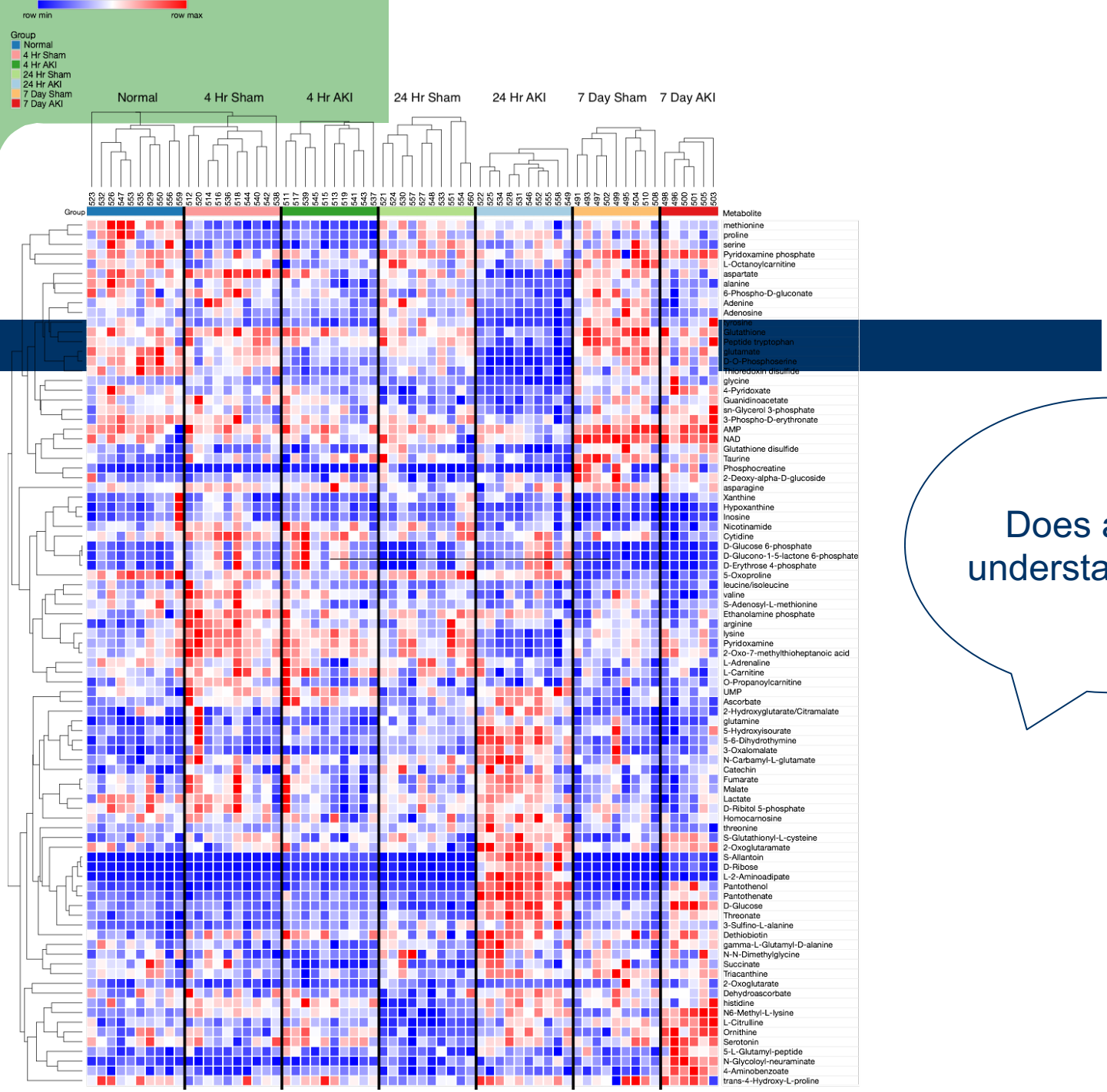
the codons CGU, CGC, CGA,
G is an α-amino acid that is used in
roteins. Wikipedia

O_2

g/mol

ino-5-guanidinopentanoic acid

7



Does anyone understand this??

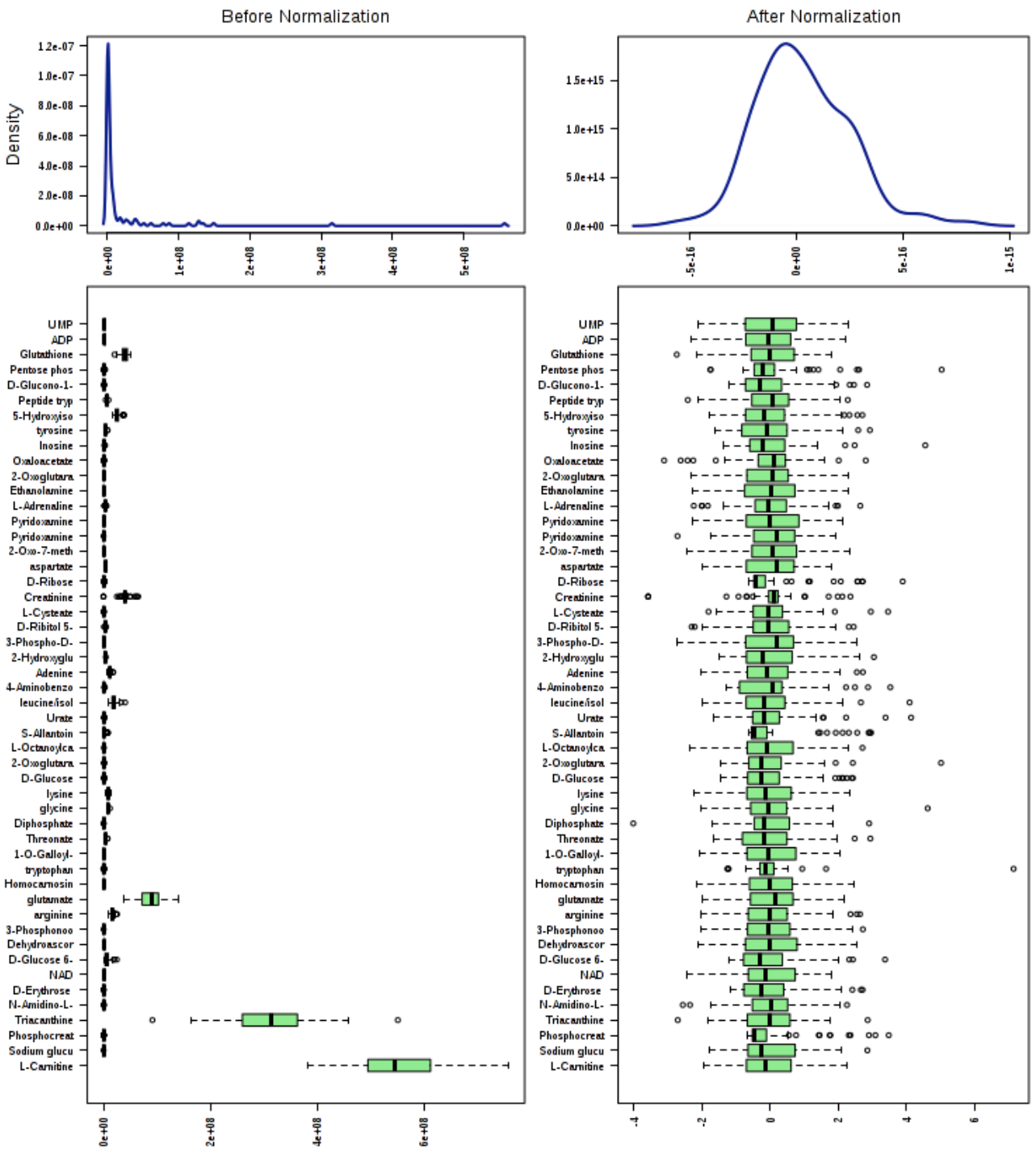
7500 light years away

Data Analysis

1. Normalize and scale data: Autoscaling
2. Univariate statistical analysis
   - ANOVA
3. Unsupervised analysis
   - Principal Component Analysis (PCA)
   - Hierarchical Clustering Analysis (HCA)
4. Supervised analysis
   - Projection to Latent Structures Discriminant Analysis (PLS-DA)
5. Significant Metabolites – Post-hoc analysis
6. Significant Pathways- pathway enrichment and pathway topology analysis/network analysis

Autoscaling

- For every peak value, autoscaling subtracts the mean and divides by the standard deviation of the metabolite
- It makes the variance of each metabolite equal to 1 and centers it around 0
- Allows metabolites to be compared based on **correlations** rather than covariances
  - Metabolites with small changes become equally important as metabolites with large changes
- This allows for homoscedastic statistical tests


Before Normalization
After Normalization
Density
1.2e-07
1.0e-07
8.0e-08
6.0e-08
4.0e-08
2.0e-08
0.0e+00
0e+00
1e+08
2e+08
3e+08
4e+08
5e+08
1.5e+15
1.0e+15
5.0e+14
0.0e+00
-5e-16
0e+00
5e-16
1e-15
UMP
ADP
Glutathione
Pentose phos
D-Glucono-1-
Peptide tryp
5-Hydroxyiso
tyrosine
Inosine
Oxaloacetate
2-Oxoglutara
Ethanolamine
L-Adrenaline
Pyridoxamine
Pyridoxamine
2-Oxo-7-meth
aspartate
D-Ribose
Creatinine
L-Cysteate
D-Ribitol 5-
3-Phospho-D-
2-Hydroxyglu
Adenine
4-Aminobenzo
leucine/isol
Urate
S-Allantoin
L-Octanoylca
2-Oxoglutara
D-Glucose
lysine
glycine
Diphosphate
Threonate
1-O-Galloyl-
tryptophan
Homocarnosin
glutamate
arginine
3-Phosphonoo
Dehydroascor
D-Glucose 6-
NAD
D-Erythrose
N-Amidino-L-
Triacanthine
Phosphocreat
Sodium glucu
L-Carnitine
0e+00
2e+08
4e+08
6e+08
-4
-2
0
2
4
6

Univariate Statistical Analysis

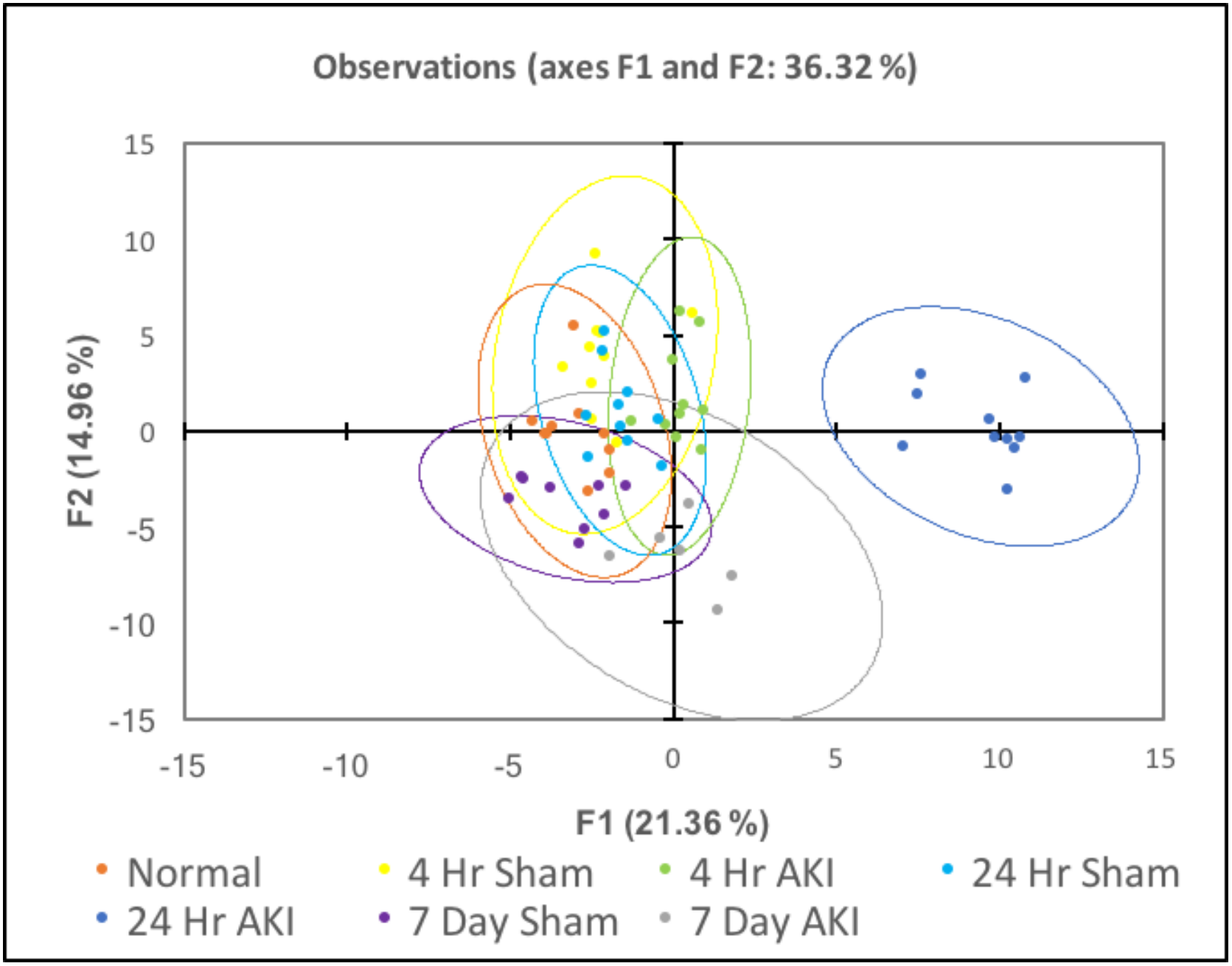
- Use autoscaled data
- ANOVA (analysis of variance) to find individual metabolites that vary significantly among the groups
- 89 significant metabolites (124 total)

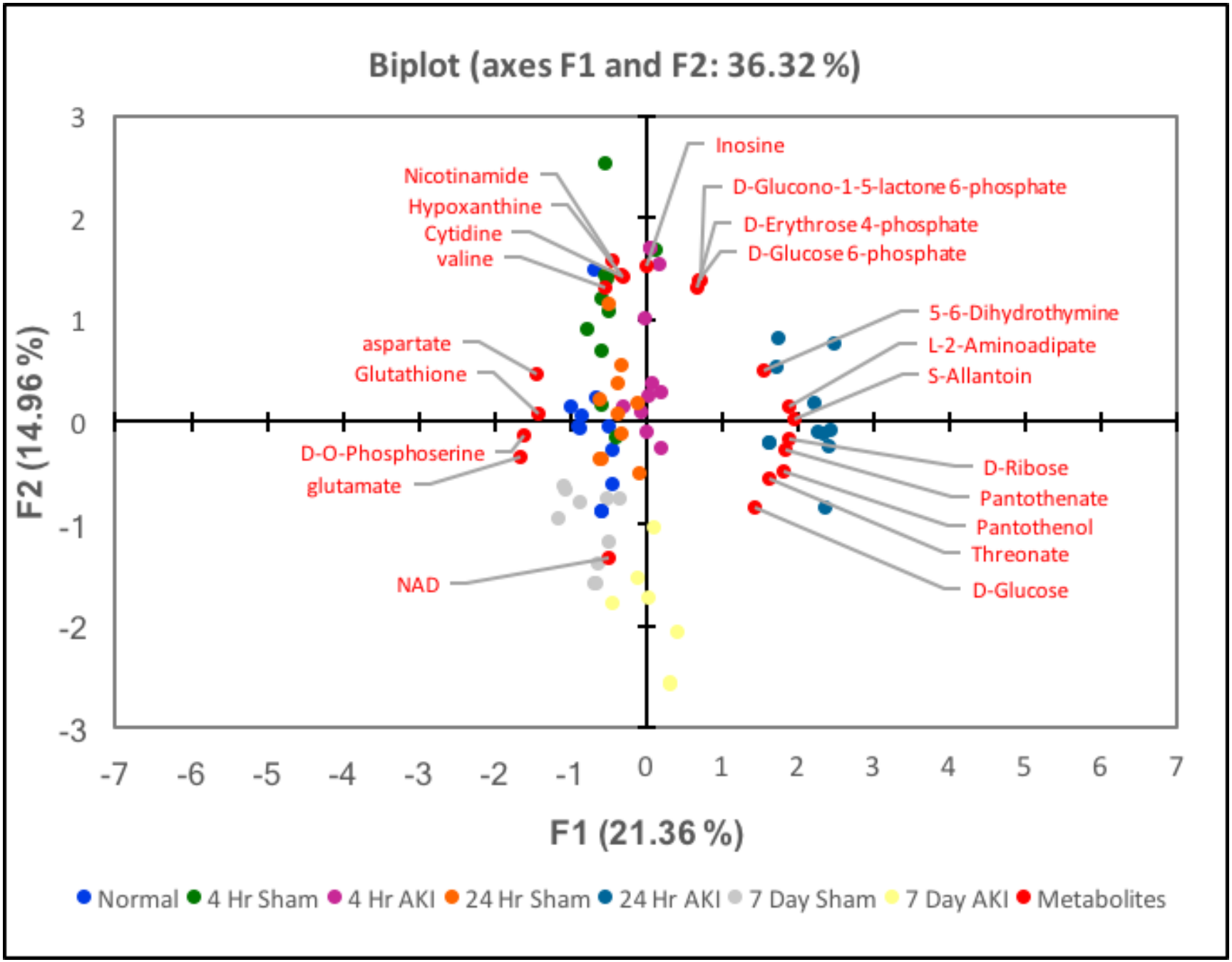
Unsupervised Multivariate Analysis

- Unsupervised- **does not** consider group membership (e.g. normal, sham, etc)
- Clusters samples and metabolites that show similar trends
- Provides an overview of the groups in the data, metabolites' correlations, and the metabolites contributing to the variation in the groups

Principal Component Analysis (PCA)

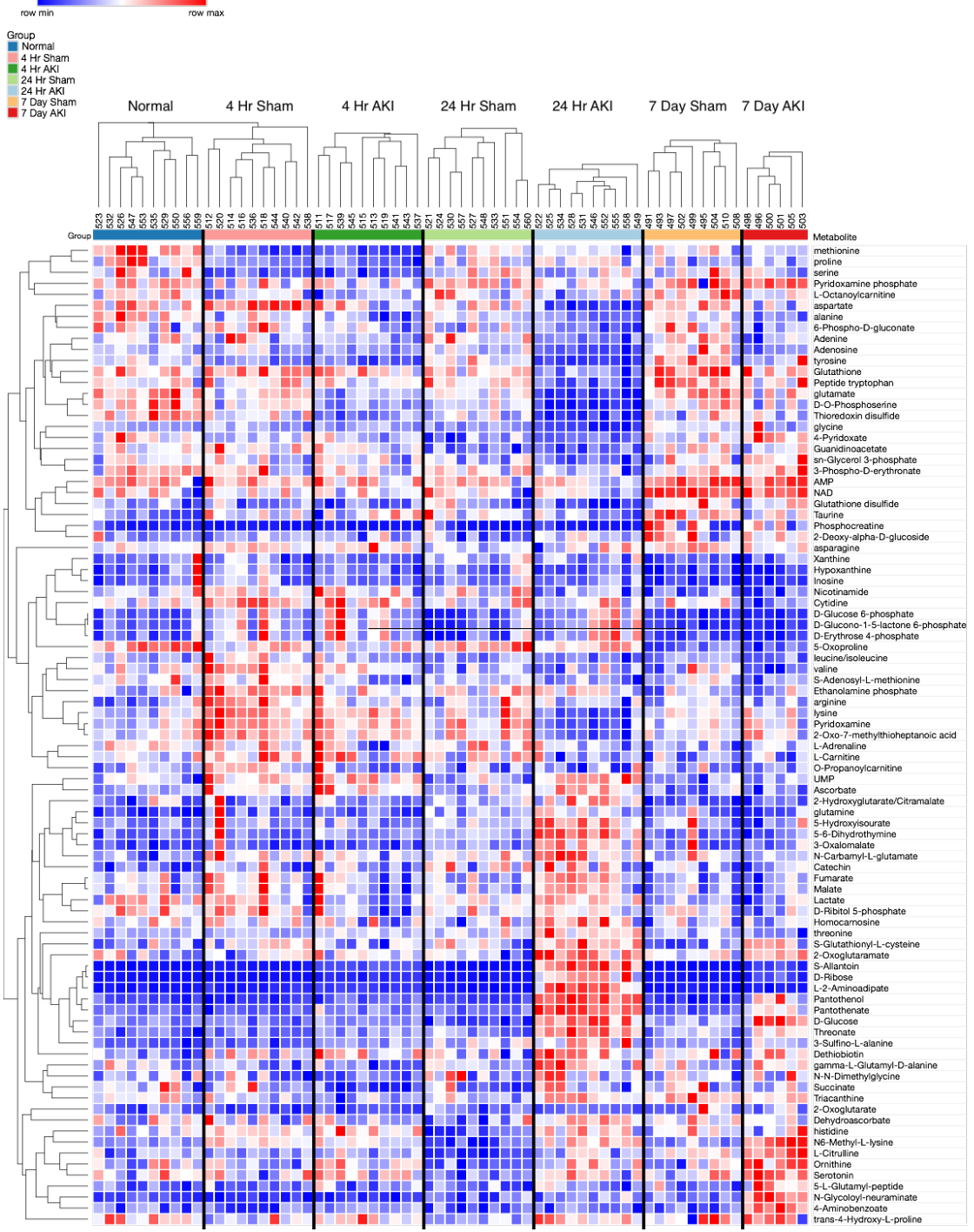
- Reduces the dimensionality of the variables measured to principal components through a linear transformation
  - We wanted to analyze 89 metabolites. Instead of analyzing each metabolite individually, we analyzed 3 principal components
  - Principal components comprise variability in the data
- Samples are projected onto scores plots. Principal components are the new dimensionless axes
- Scores plots help show sample group variation, correlations between samples, and outliers
- Biplots help show metabolite correlations (Pearson) and metabolites contributing to sample groups separation
  - Sum of squared cosines > 0.5


Observations (axes F1 and F2: 36.32 %)
15
10
5
0
-5
-10
-15
F2 (14.96 %)
-15
-10
-5
0
5
10
15
F1 (21.36 %)
Normal
4 Hr Sham
4 Hr AKI
24 Hr Sham
24 Hr AKI
7 Day Sham
7 Day AKI


Biplot (axes F1 and F2: 36.32 %)
Inosine
Nicotinamide
D-Glucono-1-5-lactone 6-phosphate
Hypoxanthine
D-Erythrose 4-phosphate
Cytidine
D-Glucose 6-phosphate
valine
5-6-Dihydrothymine
aspartate
L-2-Aminoadipate
Glutathione
S-Allantoin
D-O-Phosphoserine
D-Ribose
glutamate
Pantothenate
Pantothenol
Threonate
NAD
D-Glucose
F2 (14.96 %)
F1 (21.36 %)
3
2
1
0
-1
-2
-3
-7
-6
-5
-4
-3
-2
-1
0
1
2
3
4
5
6
7
Normal
4 Hr Sham
4 Hr AKI
24 Hr Sham
24 Hr AKI
7 Day Sham
7 Day AKI
Metabolites

Hierarchical Clustering Analysis (HCA)

- Uses a hierarchical dendogram to display trends in the data
- Often used with a heat map
- Pearson correlation metric- finds linear correlations between samples and metabolites
- Average linkage method- how the distance between the clusters is computed

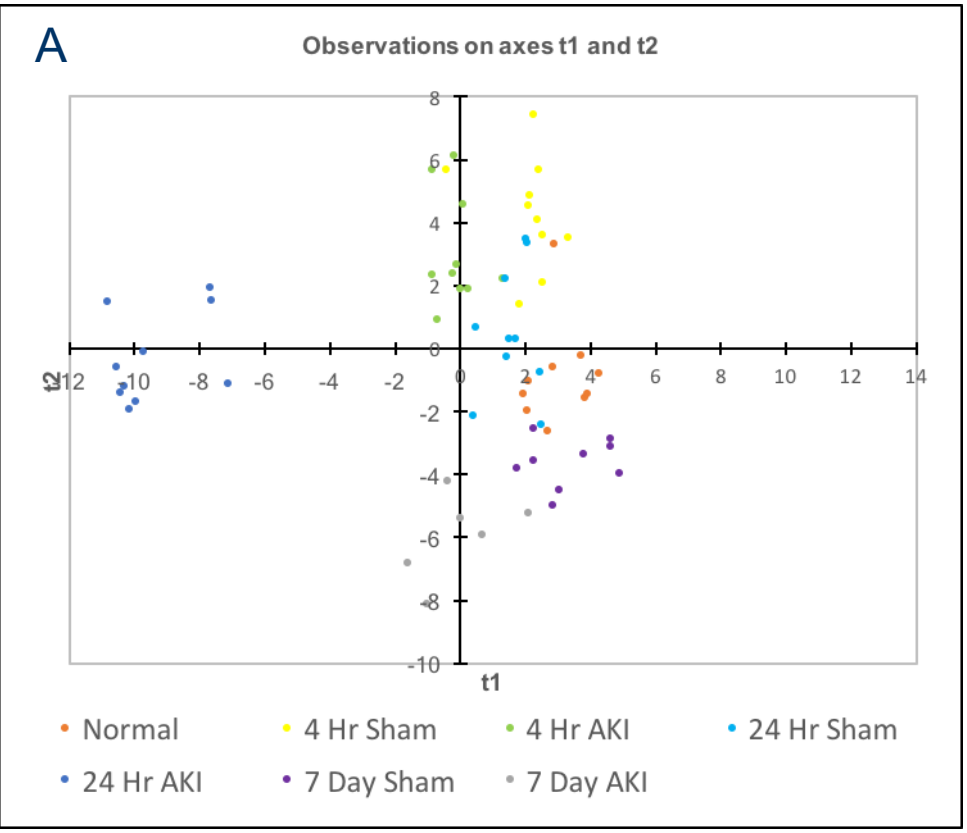

row min
row max
Group
Normal
4 Hr Sham
4 Hr AKI
24 Hr Sham
24 Hr AKI
7 Day Sham
7 Day AKI
Normal
4 Hr Sham
4 Hr AKI
24 Hr Sham
24 Hr AKI
7 Day Sham
7 Day AKI
523 532 526 547 553 535 529 550 556 559
512 520 514 545 536 518 544 540 542 538
511 517 539 545 515 513 519 541 543 537
521 524 530 557 527 548 533 551 554 560
522 525 534 528 541 546 552 555 558 549
491 493 497 502 499 495 504 510 508 498
486 500 501 505 503
Group
Metabolite
methionine
proline
serine
Pyridoxamine phosphate
L-Octanoylcarnitine
aspartate
alanine
6-Phospho-D-gluconate
Adenine
Adenosine
tyrosine
Glutathione
Peptide tryptophan
glutamate
D-O-Phosphoserine
Thioredoxin disulfide
glycine
4-Pyridoxate
Guanidinoacetate
sn-Glycerol 3-phosphate
3-Phospho-D-erythronate
AMP
NAD
Glutathione disulfide
Taurine
Phosphocreatine
2-Deoxy-alpha-D-glucoside
asparagine
Xanthine
Hypoxanthine
Inosine
Nicotinamide
Cytidine
D-Glucose 6-phosphate
D-Glucono-1-5-lactone 6-phosphate
D-Erythrose 4-phosphate
5-Oxoproline
leucine/isoleucine
valine
S-Adenosyl-L-methionine
Ethanolamine phosphate
arginine
lysine
Pyridoxamine
2-Oxo-7-methylthioheptanoic acid
L-Adrenaline
L-Carnitine
O-Propanoylcarnitine
UMP
Ascorbate
2-Hydroxyglutarate/Citramalate
glutamine
5-Hydroxyisourate
5-6-Dihydrothymine
3-Oxalomalate
N-Carbamyl-L-glutamate
Catechin
Fumarate
Malate
Lactate
D-Ribitol 5-phosphate
Homocarnosine
threonine
S-Glutathionyl-L-cysteine
2-Oxoglutaramate
S-Allantoin
D-Ribose
L-2-Aminoadipate
Pantothenol
Pantothenate
D-Glucose
Threonate
3-Sulfino-L-alanine
Dethiobiotin
gamma-L-Glutamyl-D-alanine
N-N-Dimethylglycine
Succinate
Triacanthine
2-Oxoglutarate
Dehydroascorbate
histidine
N6-Methyl-L-lysine
L-Citrulline
Ornithine
Serotonin
5-L-Glutamyl-peptide
N-Glycoloyl-neuraminate
4-Aminobenzoate
trans-4-Hydroxy-L-proline

Supervised Multivariate Analysis

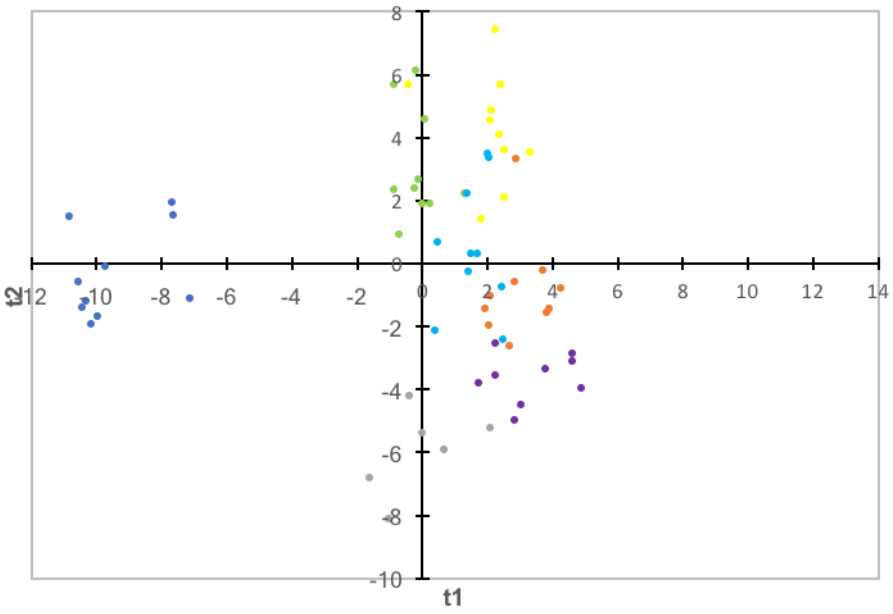
- Supervised- **does** consider group membership (e.g. normal, sham, etc)
- Looks for a way to divide up the sample groups based on metabolites' variations
- Machine learning- with the knowledge of the sample groups, algorithm can guess group membership for future samples based off of the metabolome

Projections to Latent Structures Discriminant Analysis (PLS-DA)

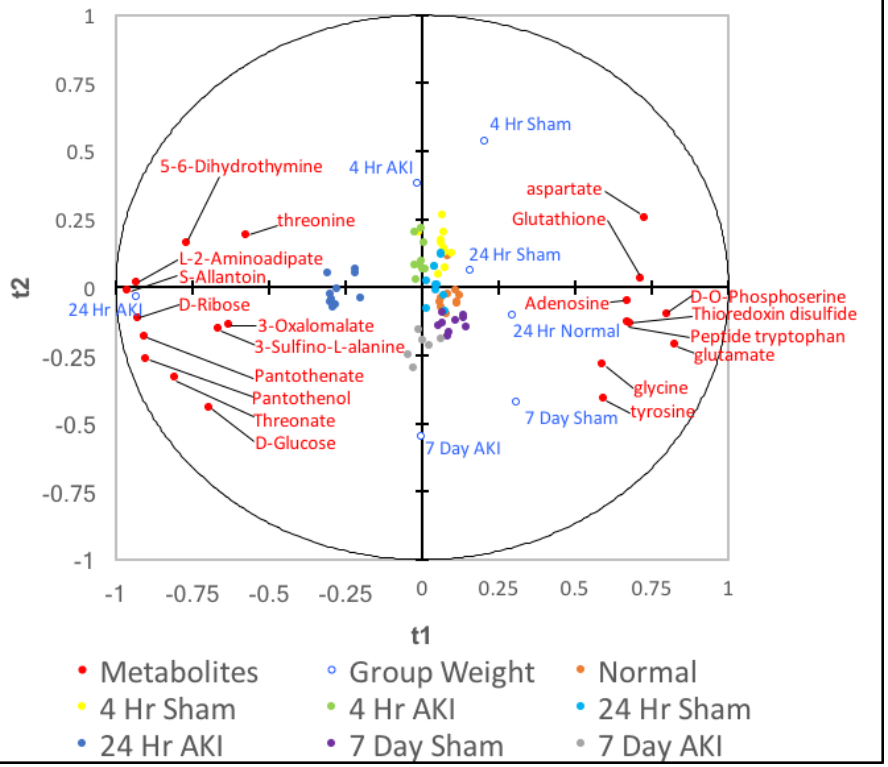
- Projects the X variables (metabolites) that best explain the variation in the Y variables (sample groups)
- Finds correlations between metabolites and metabolites significantly contributing to the projection
  - VIP- variable importance in the projection (top 20)
- Produces both scores and correlation biplots (same interpretation as PCA)


A
Observations on axes t1 and t2
8
6
4
2
0
-2
-4
-6
-8
-10
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
t2
t1
Normal
4 Hr Sham
4 Hr AKI
24 Hr Sham
24 Hr AKI
7 Day Sham
7 Day AKI

A


Observations on axes t1 and t2
t2
t1
8
6
4
2
0
-2
-4
-6
-8
-10
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
Normal
4 Hr Sham
4 Hr AKI
24 Hr Sham
24 Hr AKI
7 Day Sham
7 Day AKI

B


Correlations on axes t1 and t2
t2
t1
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-1
-0.75
-0.5
-0.25
0
0.25
0.5
0.75
1
4 Hr Sham
4 Hr AKI
5-6-Dihydrothymine
aspartate
threonine
Glutathione
24 Hr Sham
L-2-Aminoadipate
S-Allantoin
24 Hr AKI
D-Ribose
Adenosine
D-O-Phosphoserine
Thioredoxin disulfide
24 Hr Normal
Peptide tryptophan
3-Oxalomalate
3-Sulfino-L-alanine
glutamate
Pantothenate
glycine
Pantothenol
tyrosine
7 Day Sham
Threonate
D-Glucose
7 Day AKI
Metabolites
Group Weight
Normal
4 Hr Sham
4 Hr AKI
24 Hr Sham
24 Hr AKI
7 Day Sham
7 Day AKI

Post Hoc Tests

- Pre-selected groups (e.g. normal vs sham at every time point, normal vs AKI at every time point, etc) were compared with the Bonferonni-Sidak correction
  - We found the Bonferonni-Sidak method to be the most stringent method considering our comparisons
  - Avoid type 1 errors (false positives)
  - Control family wise error rate (FWER)
  - Many papers do not include a correction and even use a significance threshold of $p < 0.1$, which could lead to multiple false positives
- This helped distinguish the effect of AKI and surgery

	Metabolites Affected By Surgery (Sham).		
	4 Hr	24 Hr	7 Day
Decreased	**Alanine (Amino Acid)**	**Alanine (Amino Acid)**	**Methionine (Amino Acid)**
	Methionine (Amino Acid)	**Methionine (Amino Acid)**	**Proline (Amino Acid)**
	Proline (Amino Acid)	Histidine (Amino Acid)	**5-Oxoproline (Glutathione Metabolism)**
	Tyrosine (Amino Acid)	Glutamate (Amino Acid)	Lactate (Glycolysis)
	Serine (Amino Acid)	4-Pyridoxate (Vitamin B6 Metabolism)	
	5-Oxoproline (Glutathione Metabolism)	D-O-Phosphoserine (Glycine, Serine, And Threonine Metabolism)	
	Thioredoxin Disulfide (Redox Homeostasis)	**Thioredoxin Disulfide (Redox Homeostasis)**	
		L-Citrulline (Urea Cycle)	
		Ornithine (Urea Cycle)	
		Serotonin (Signaling)	
		Trans-4-Hydroxy-L-Proline (Arginine And Proline Metabolism)	
		Guanidinoacetate (Arginine And Proline Metabolism)	
Increased	Arginine (Amino Acid)	3-Sulfino-L-Alanine (Cysteine And Methionine Metabolism)	Glycine (Amino Acid)
	Glutamine (Amino Acid)	**Glutamine (Amino Acid)**	Adenosine (Purine Metabolism)
	Leucine/Isoleucine (Amino Acid)	**Taurine (Taurine and Hypotaurine Metabolism)**	**Taurine (Taurine and Hypotaurine Metabolism)**
	Lysine (Amino Acid)	Catechin (Other)	Glutathione Disulfide (Glutathione Metabolism)
	N-Carbamyl-L-Glutamate (Histidine Metabolism)	**N-Carbamyl-L-Glutamate (Histidine Metabolism)**	Phosphocreatine (Arginine And Proline Metabolism)
	Ascorbate (Glutathione Metabolism)		NAD (Nicotinate And Nicotinamide Metabolism)
	Ethanolamine Phosphate (Glycerophospholipid Metabolism)		Peptide Tryptophan (Other)
	2-Oxo-7-Methylthioheptanoic Acid (Carboxylate Metabolism)		2-Deoxy-Alpha-D-Glucoside (Other)
	N6-Methyl-L-Lysine (Lysine Metabolism)		
	Pyridoxamine (Vitamin B6 Metabolism)		
	UMP (Pyrimidine Metabolism)		

	Metabolites Affected by AKI		
	4 Hr	24 Hr	7 Day
Decreased	**Alanine (Amino Acid)***	**Alanine (Amino Acid)***	Valine (Amino Acid)
	Glutamate (Amino Acid)	**Glutamate (Amino Acid)***	Cytidine (Pyrimidine Metabolism)
	Leucine/Isoleucine (Amino Acid)†	Aspartate (Amino Acid)	
	D-O-Phosphoserine (Glycine, Serine, And Threonine Metabolism)	Glycine (Amino Acid)	
	Succinate (TCA Cycle)	Lysine (Amino Acid)	
	Thioredoxin Disulfide (Redox Homeostasis)*	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)†	
		D-O-Phosphoserine (Glycine, Serine, And Threonine Metabolism)*	
		Thioredoxin Disulfide (Redox Homeostasis)*	
		3-Phospho-D-Erythronate (Other)	
		Peptide Tryptophan (Other)	
Increased	**S-Allantoin (Purine Metabolism)**	Threonine (Amino Acid)	L-Citrulline (Urea Cycle)
		S-Allantoin (Purine Metabolism)	Ornithine (Urea Cycle)
		5-Hydroxyisourate (Purine Metabolism)	**S-Glutathionyl-L-Cysteine (Glutathione Meta**
		5-6-Dihydrothymine (Pyrimidine Metabolism)	5-L-Glutamyl-Peptide (Glutathione Metabolisr
		UMP (Pyrimidine Metabolism)	**Threonate (Ascorbate Metabolism)**
		D-Glucose (Glycolysis)	Serotonin (Signaling)
		D-Glucose 6-Phosphate (Glycolysis)	**Pantothenate (Pantothenate And CoA Biosyr**
		2-Oxoglutaramate (TCA Cycle)	**Pantothenol (Pantothenate And CoA Biosynt**
		D-Glucono-1-5-Lactone 6-Phosphate (Pentose Phosphate Pathway)	**D-Glucose (Glycolysis)**
		D-Ribose (Pentose Phosphate Pathway)	N-Glycoloyl-Neuraminate (Aminosugar Metab
		Ascorbate (Glutathione Metabolism)	**N6-Methyl-L-Lysine (Lysine Metabolism)**
		Gamma-L-Glutamyl-D-Alanine (Glutathione Metabolism)	Adenosine (Purine Metabolism)†
		S-Glutathionyl-L-Cysteine (Glutathione Metabolism)	4-Aminobenzoate (Other)
		Threonate (Ascorbate Metabolism)	
		3-Oxalomalate (Glycoxylate And Dicarboxylate Metabolism)	
		Pantothenate (Pantothenate And CoA Biosynthesis)	
		Pantothenol (Pantothenate And CoA Biosynthesis)	
		Dethiobiotin (Biotin Metabolism)	
		L-2-Aminoadipate (Lysine Metabolism)	
		N6-Methyl-L-Lysine (Lysine Metabolism)	
	† Sig To Sham Vs Normal And Sham Vs AKI But Not AKI Vs Normal		
	*** Effect Of AKI And Sham, But Empahsized More In AKI**		

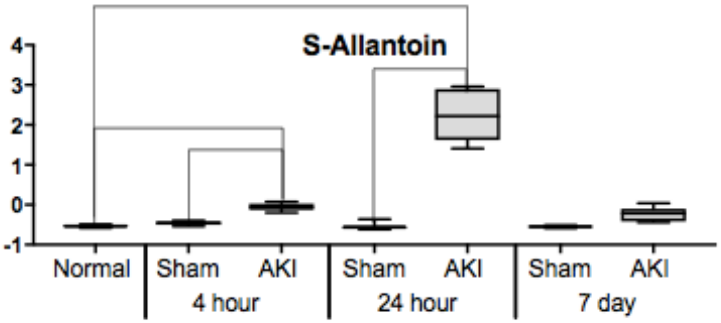
Metabolomics Studies in other Heart Diseases

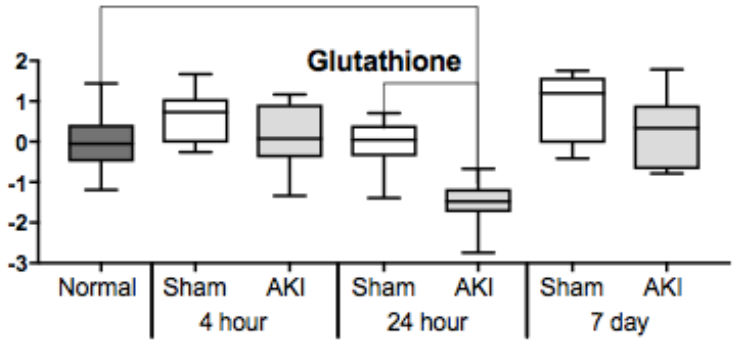
1. Metabolomics Study of Heart Homogenates From Myocardial Infarction (MI) in Rats (Sun 2016) 24 hours
   - Altered purine and pyrimidine pathways
     - Decreases in purines inosine and guanosine, and increases in pyrimidines uracil and uridine
     - Nucleotide metabolism
   - Oxidative stress
     - Increase in Uric acid, decrease in glutathione
2. Metabolomics Analysis of Pressure-overloaded and Infarcted Mouse Hearts (Sansbury 2014)
   - At 24 hours, there were ONLY 4 metabolites that were changed
   - 1 week after pressure overload
     - Perturbations in amino acids
       - Increases in branch chained amino acids, lysine, serine, asparagine, threonine
     - Oxidative stress
       - 50 fold increase in ascorbate

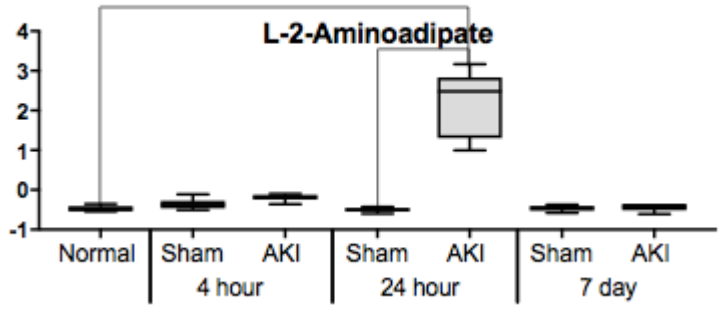
Metabolomics Studies in other Heart Diseases

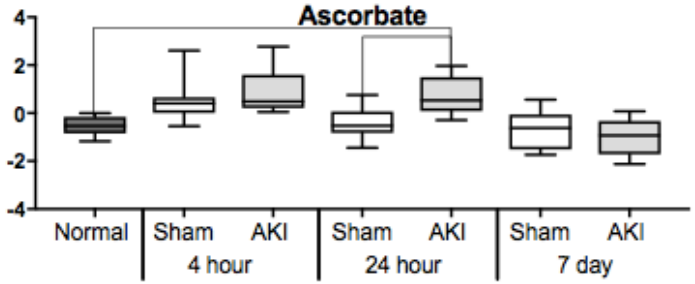
3. Global Metabolomic Profiling of Acute Myocarditis Caused by T cruzi Infection (Girones 2014)
   - 14 and 21 days after infection
   - Altered energy metabolic pathways
     - Increases in glucose, glucose 6 phosphate, fructose 6 phosphate
     - Increased glycolysis
     - Decreases in adenine, adenosine
     - Nucleotide metabolism
   - Oxidative Stress
     - increases in allantoin, xanthine, xanthonise
4. Cardiac Metabolism in Heart Failure – Implications Beyond ATP Production (Doenst 2014)
   - Altered energy metabolic pathways
     - Decrease in fatty acid oxidation
     - Increase in glycolysis
   - Oxidative stress
     - Pentose phosphate pathway

Oxidative Stress

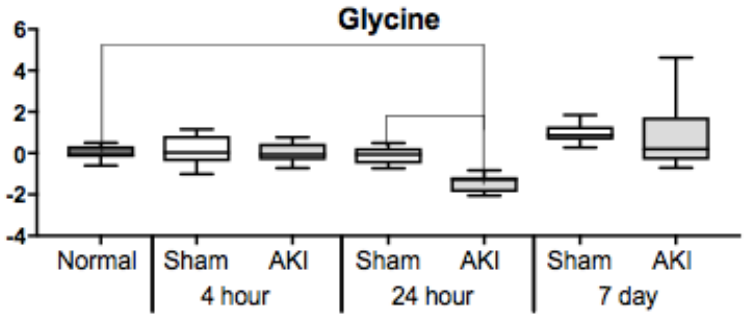


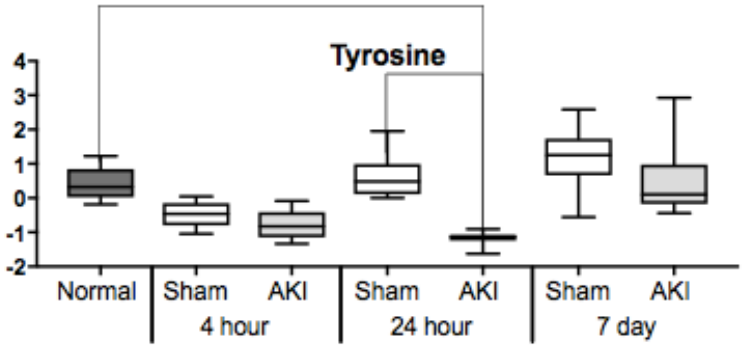


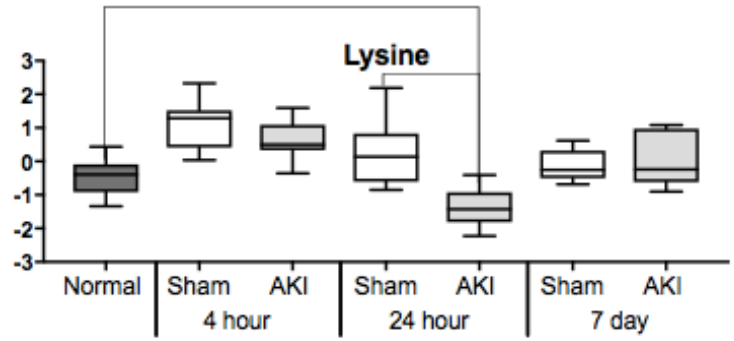


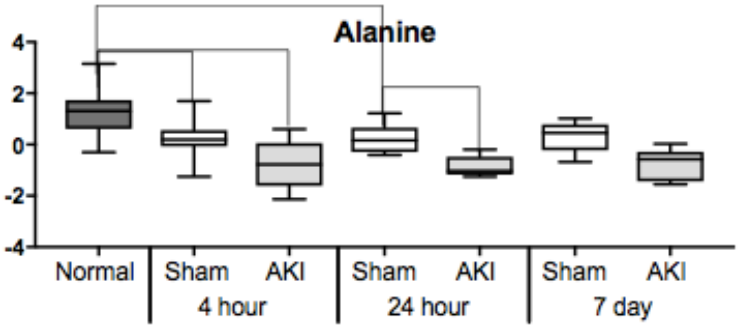


Amino Acids

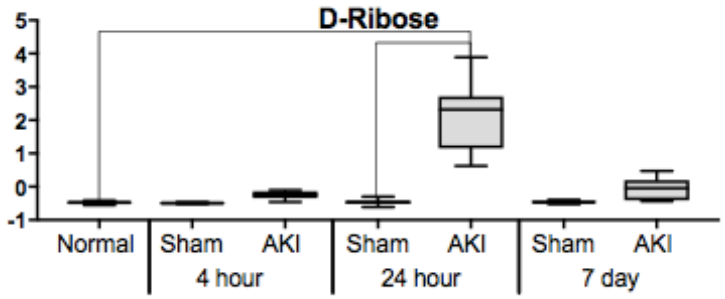

Glycine
6
4
2
0
-2
-4
Normal
Sham
AKI
Sham
AKI
Sham
AKI
4 hour
24 hour
7 day

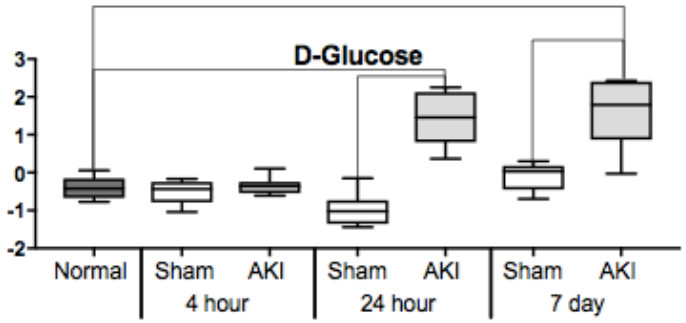

Tyrosine
4
3
2
1
0
-1
-2
Normal
Sham
AKI
Sham
AKI
Sham
AKI
4 hour
24 hour
7 day

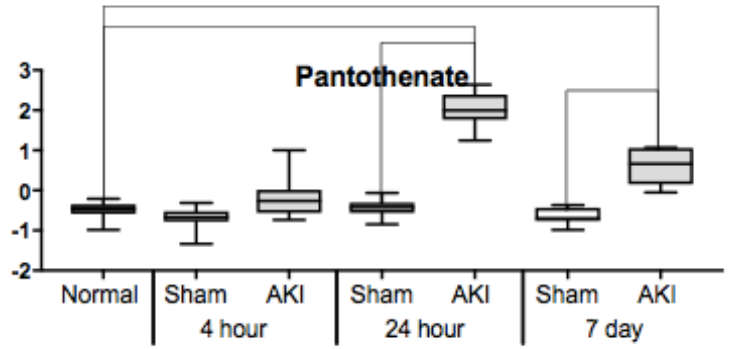

Lysine
3
2
1
0
-1
-2
-3
Normal
Sham
AKI
Sham
AKI
Sham
AKI
4 hour
24 hour
7 day

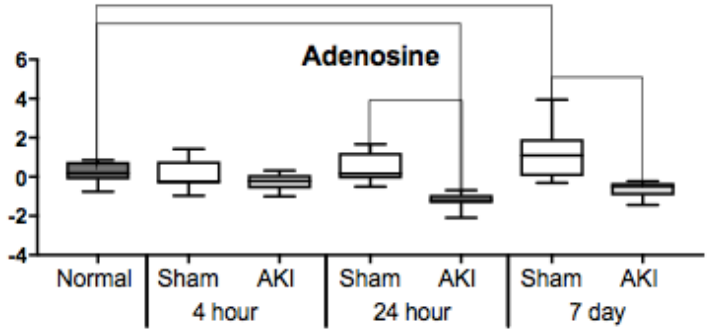

Alanine
4
2
0
-2
-4
Normal
Sham
AKI
Sham
AKI
Sham
AKI
4 hour
24 hour
7 day

Energy Metabolism









Pathway Enrichment/Pathway Topology Analysis

- Pathway Enrichment Analysis
  - Finds significant pathways based on concentration changes between two sample groups or “hits” of metabolites in pathways
  - Quantitative enrichment analysis (QEA)- utilizes concentration data to determine pathways. It looks for pathways where a few metabolites are significantly changed or a lot of metabolites are slightly changed
- Pathway Topology Analysis
  - Visual depiction of the pathways with concentration data overlaid for metabolites in pathway
    - Visualize the significant pathways from enrichment analysis
  - Ability to see the full picture of the pathway and deduce what is happening
  - It is not always clear if a pathway is active or inactive based on any of these analyses

Pathways Affected By Surgery (Sham).		
4 Hr	**24 Hr**	**7 Day**
Aminoacyl-tRNA biosynthesis	D-Glutamine and D-glutamate metabolism	Aminoacyl-tRNA biosynthesis
Lysine biosynthesis		Glutathione metabolism
Lysine degradation		
Cysteine and methionine metabolism		
Biotin metabolism		
Phenylalanine, tyrosine and tryptophan biosynthesis		
Phenylalanine metabolism		
Ubiquinone and other terpenoid-quinone biosynthesis		
Alanine, aspartate and glutamate metabolism		
Pyrimidine metabolism		
Vitamin B6 metabolism		

Metabolites Affected by AKI		
4 Hr	**24 Hr**	**7 Day**
	Alanine, aspartate and glutamate metabolism	Amino sugar and nucleotide sugar metabolism
	Purine metabolism	Starch and sucrose metabolism
	Ubiquinone and other terpenoid-quinone biosynthesis	Galactose metabolism
	Pantothenate and CoA biosynthesis	Pantothenate and CoA biosynthesis
	Tyrosine metabolism	
	Phenylalanine, tyrosine and tryptophan biosynthesis	
	Phenylalanine metabolism	
	Porphyrin and chlorophyll metabolism	
	Primary bile acid biosynthesis	
	Lysine biosynthesis	
	Lysine degradation	
	Glycine, serine and threonine metabolism	
	Starch and sucrose metabolism	
	Galactose metabolism	
	Vitamin B6 metabolism	
	Methane metabolism	
	Cyanoamino acid metabolism	
	Glutathione metabolism	
	Selenoamino acid metabolism	
	Histidine metabolism	
	beta-Alanine metabolism	
	Pentose phosphate pathway	
	Ascorbate and aldarate metabolism	
	Biotin metabolism	
	Pyrimidine metabolism*	
	Aminoacyl-tRNA biosynthesis*	
	Arginine and Proline Metabolism*	
	Nitrogen metabolism*	
	*** Effect Of AKI And Sham, But Empahsized More In AKI**	

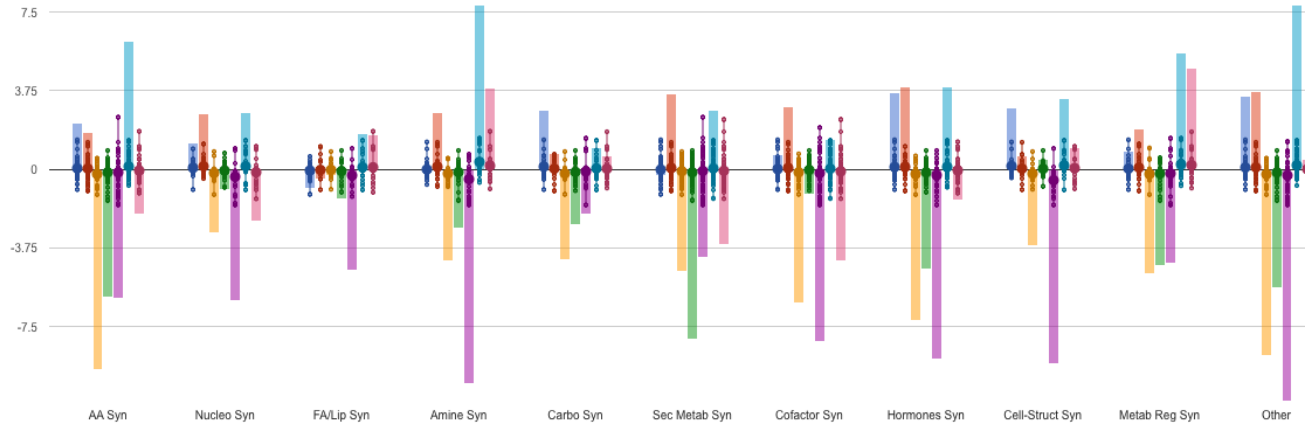
Dashboard Help

Pathway Tools Omics Dashboard for *MetaCyc*

Normal 4 Hr Sham 4 Hr AKI 24 Hr Sham 24 Hr AKI 7 Day Sham 7 Day AKI

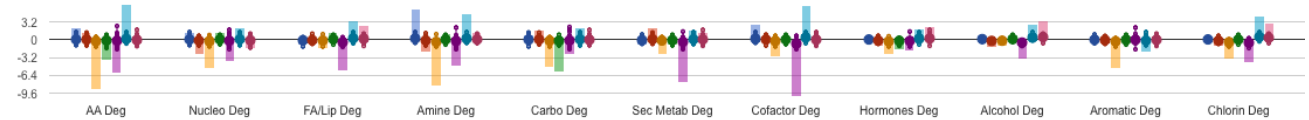
- Display Preferences
- Series Selection
- Series Grouping
- Enrichment Analysis
- Customize Contents

Biosynthesis



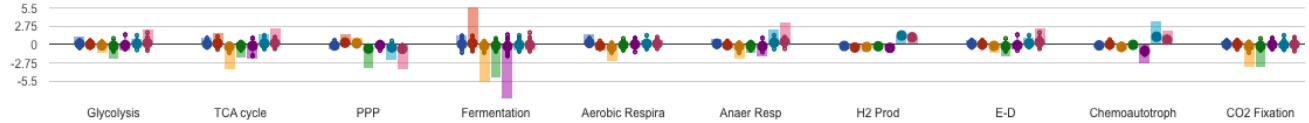
Options Show Sums

Degradation



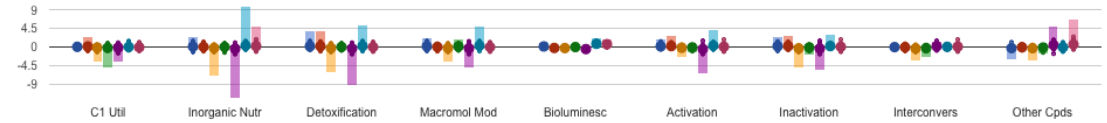
Options Show Sums

Energy

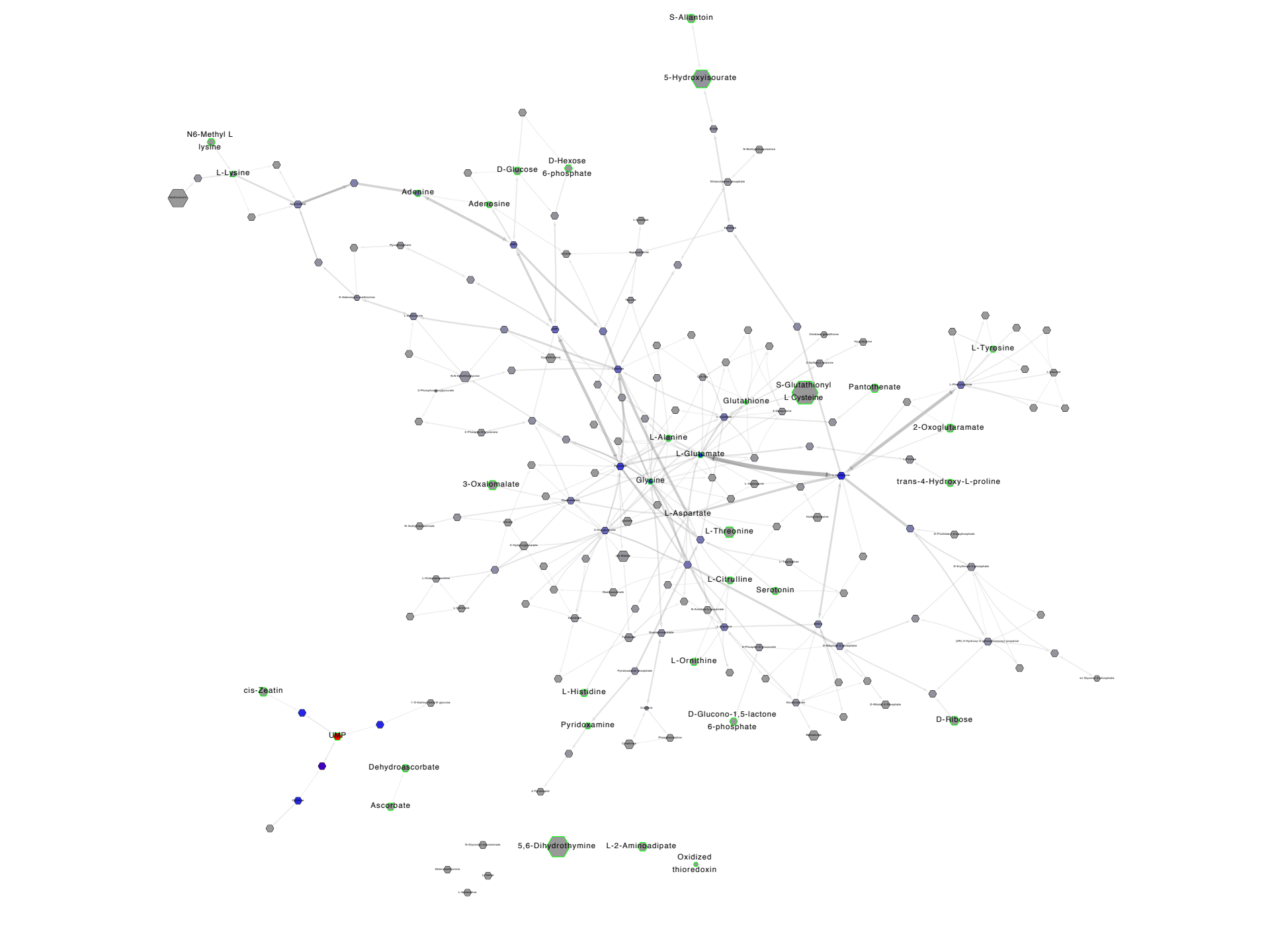


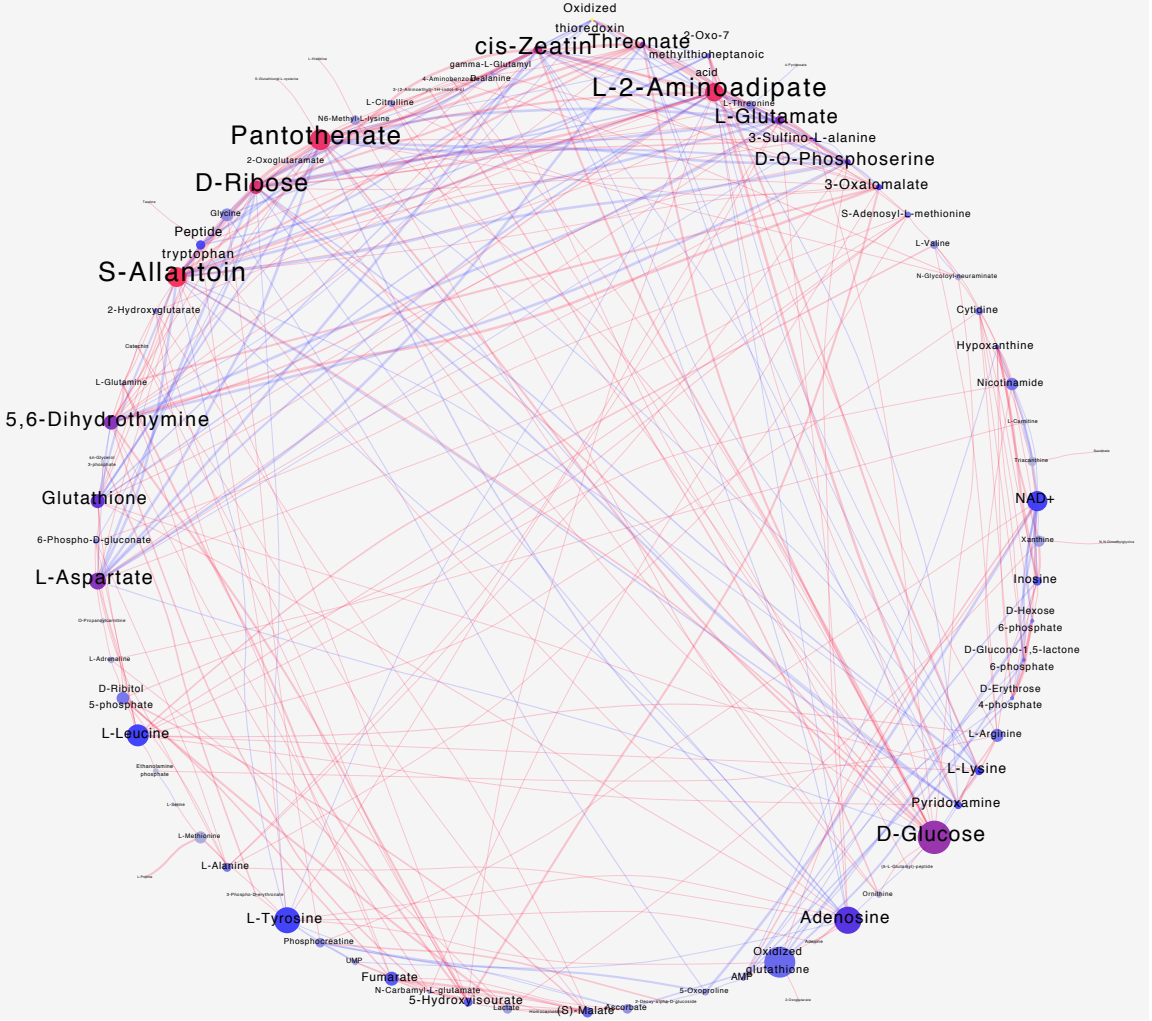
Options Show Sums

Other Pathways



Options Show Sums


S-Allantoin
5-Hydroxyisourate
N6-Methyl L
lysine
L-Lysine
D-Glucose
D-Hexose
6-phosphate
Adenine
Adenosine
L-Tyrosine
S-Glutathionyl
L Cysteine
Pantothenate
Glutathione
2-Oxoglutaramate
L-Alanine
L-Glutamate
Glycine
3-Oxalomalate
trans-4-Hydroxy-L-proline
L-Aspartate
L-Threonine
L-Citrulline
Serotonin
L-Ornithine
L-Histidine
cis-Zeatin
UMP
Pyridoxamine
D-Glucono-1,5-lactone
6-phosphate
D-Ribose
Dehydroascorbate
Ascorbate
5,6-Dihydrothymine
L-2-Aminoadipate
Oxidized
thioredoxin



Metabolite	Degree	Betweenness Centrality
S-Allantoin	25	0.07502456
Pantothenol	24	0.07967866
L-2-Aminoadipate	24	0.0685001
D-Ribose	23	0.04677428
Pantothenate	22	0.02438779
D-Glucose	19	0.13674489
Threonate	18	0.00893323
L-Glutamate	18	0.0250032
L-Aspartate	18	0.06159535
5,6-Dihydrothymine	18	0.05202338
Glutathione	16	0.04841644
D-O-Phosphoserine	16	0.01143997
Adenosine	15	0.1083844
L-Tyrosine	12	0.103345
NAD+	12	0.07672097
L-Lysine	12	0.021898
L-Leucine	12	0.08474106
3-Oxalomalate	12	0.01263174
Pyridoxamine	11	0.02035089
Peptide tryptophan	11	0.02659497
3-Sulfino-L-alanine	11	8.67E-04

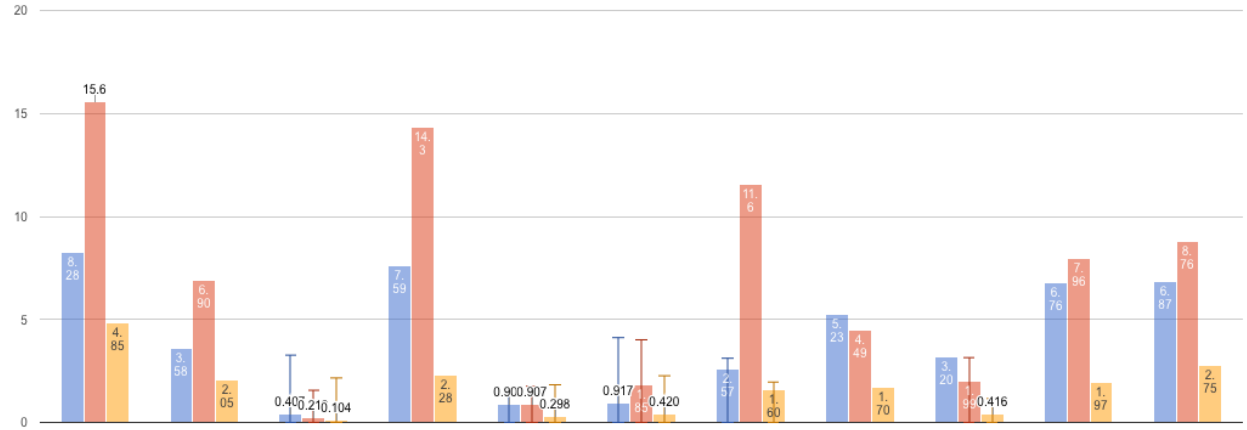
Dashboard Help

Pathway Tools Omics Dashboard for *MetaCyc*

4 Hr AKI 24 Hr AKI 7 Day AKI

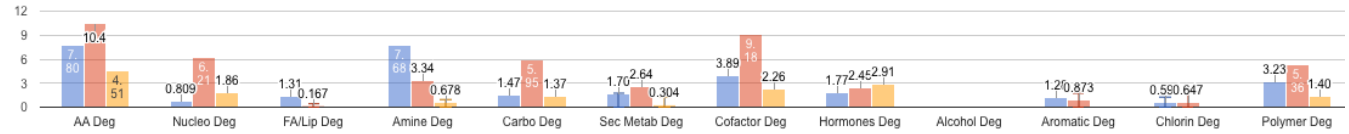
- Display Preferences
- Series Selection
- Series Grouping
- Enrichment Analysis
- Customize Contents

Biosynthesis



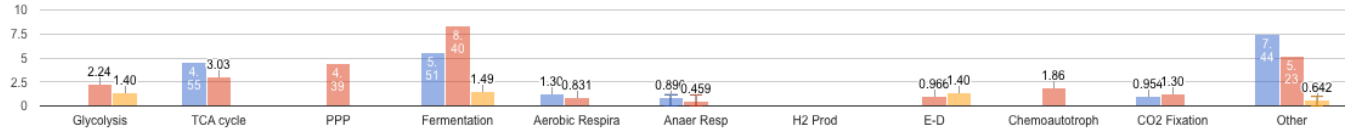
Options

Degradation



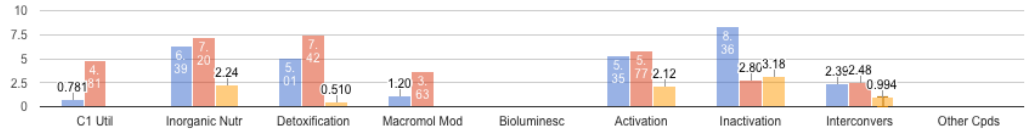
Options

Energy

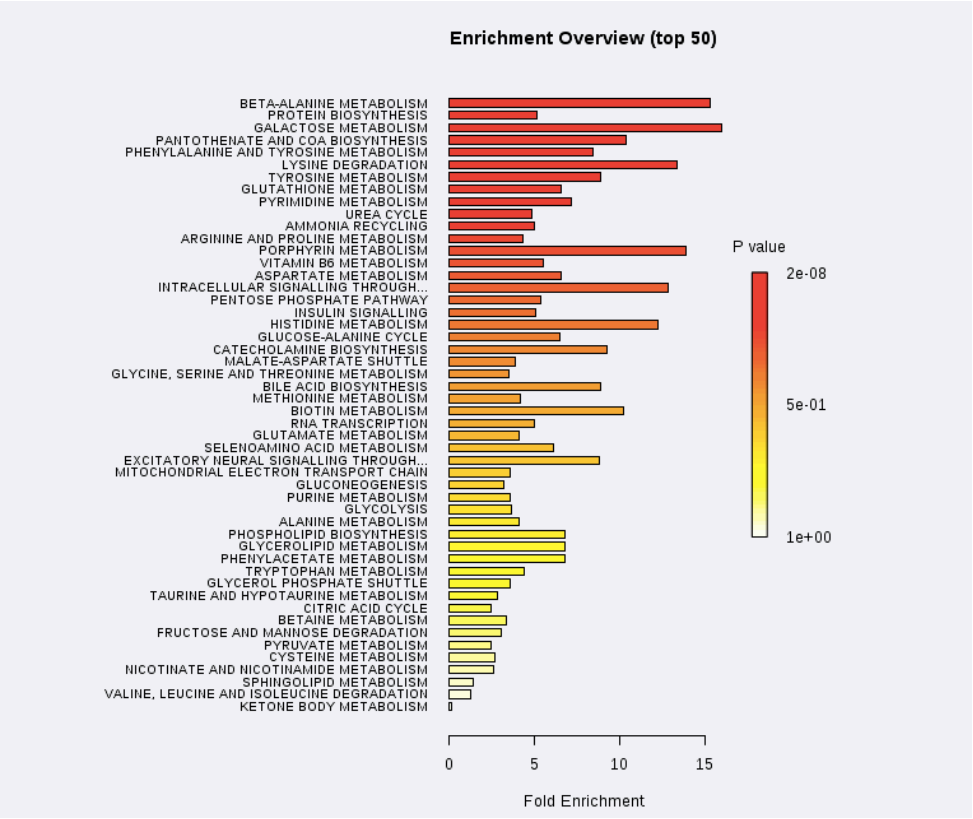


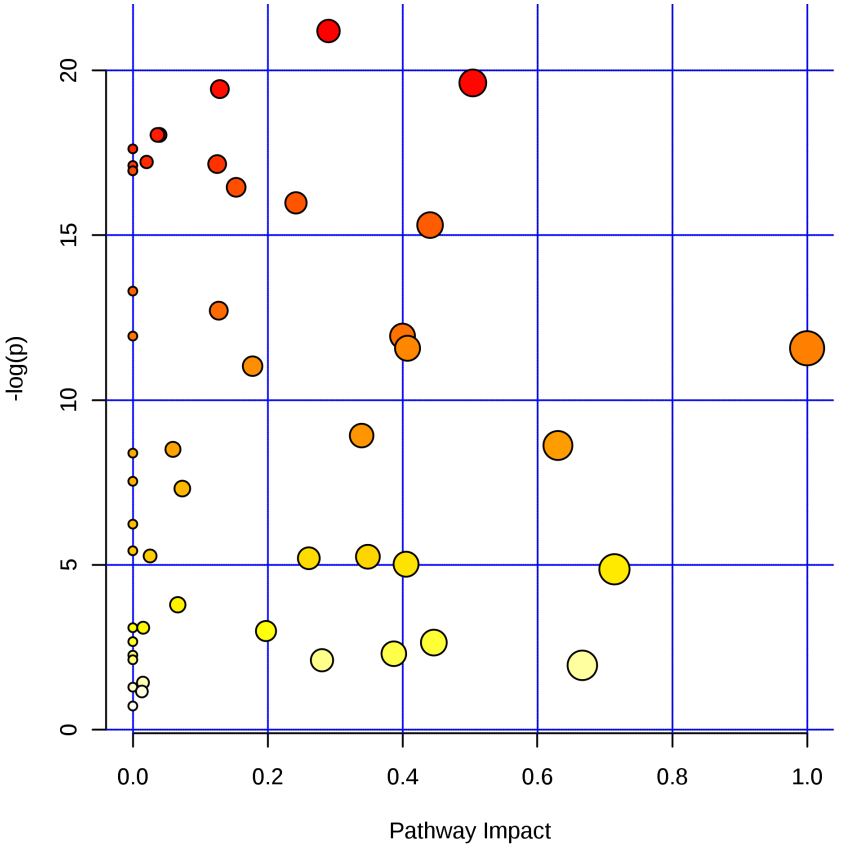
Options

Other Pathways



Options

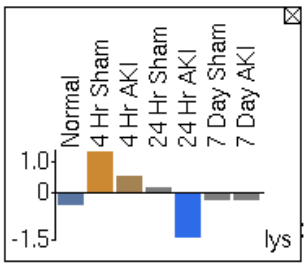

Enrichment Overview (top 50)
BETA-ALANINE METABOLISM
PROTEIN BIOSYNTHESIS
GALACTOSE METABOLISM
PANTOTHENATE AND COA BIOSYNTHESIS
PHENYLALANINE AND TYROSINE METABOLISM
LYSINE DEGRADATION
TYROSINE METABOLISM
GLUTATHIONE METABOLISM
PYRIMIDINE METABOLISM
UREA CYCLE
AMMONIA RECYCLING
ARGININE AND PROLINE METABOLISM
PORPHYRIN METABOLISM
VITAMIN B6 METABOLISM
ASPARTATE METABOLISM
INTRACELLULAR SIGNALLING THROUGH...
PENTOSE PHOSPHATE PATHWAY
INSULIN SIGNALLING
HISTIDINE METABOLISM
GLUCOSE-ALANINE CYCLE
CATECHOLAMINE BIOSYNTHESIS
MALATE-ASPARTATE SHUTTLE
GLYCINE, SERINE AND THREONINE METABOLISM
BILE ACID BIOSYNTHESIS
METHIONINE METABOLISM
BIOTIN METABOLISM
RNA TRANSCRIPTION
GLUTAMATE METABOLISM
SELENOAMINO ACID METABOLISM
EXCITATORY NEURAL SIGNALLING THROUGH...
MITOCHONDRIAL ELECTRON TRANSPORT CHAIN
GLUCONEOGENESIS
PURINE METABOLISM
GLYCOLYSIS
ALANINE METABOLISM
PHOSPHOLIPID BIOSYNTHESIS
GLYCEROLIPID METABOLISM
PHENYLACETATE METABOLISM
TRYPTOPHAN METABOLISM
GLYCEROL PHOSPHATE SHUTTLE
TAURINE AND HYPOTAURINE METABOLISM
CITRIC ACID CYCLE
BETAINE METABOLISM
FRUCTOSE AND MANNOSE DEGRADATION
PYRUVATE METABOLISM
CYSTEINE METABOLISM
NICOTINATE AND NICOTINAMIDE METABOLISM
SPHINGOLIPID METABOLISM
VALINE, LEUCINE AND ISOLEUCINE DEGRADATION
KETONE BODY METABOLISM
0
5
10
15
Fold Enrichment
P value
2e-08
5e-01
1e+00

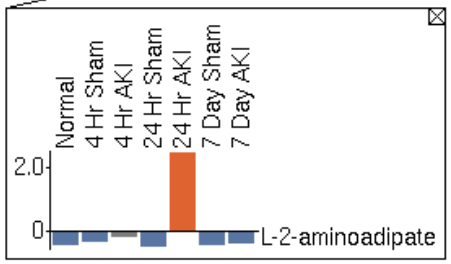

-log(p)
0
5
10
15
20
0.0
0.2
0.4
0.6
0.8
1.0
Pathway Impact

MetaCyc Pathway: L-lysine degradation XI (mammalian)

More Detail | Less Detail

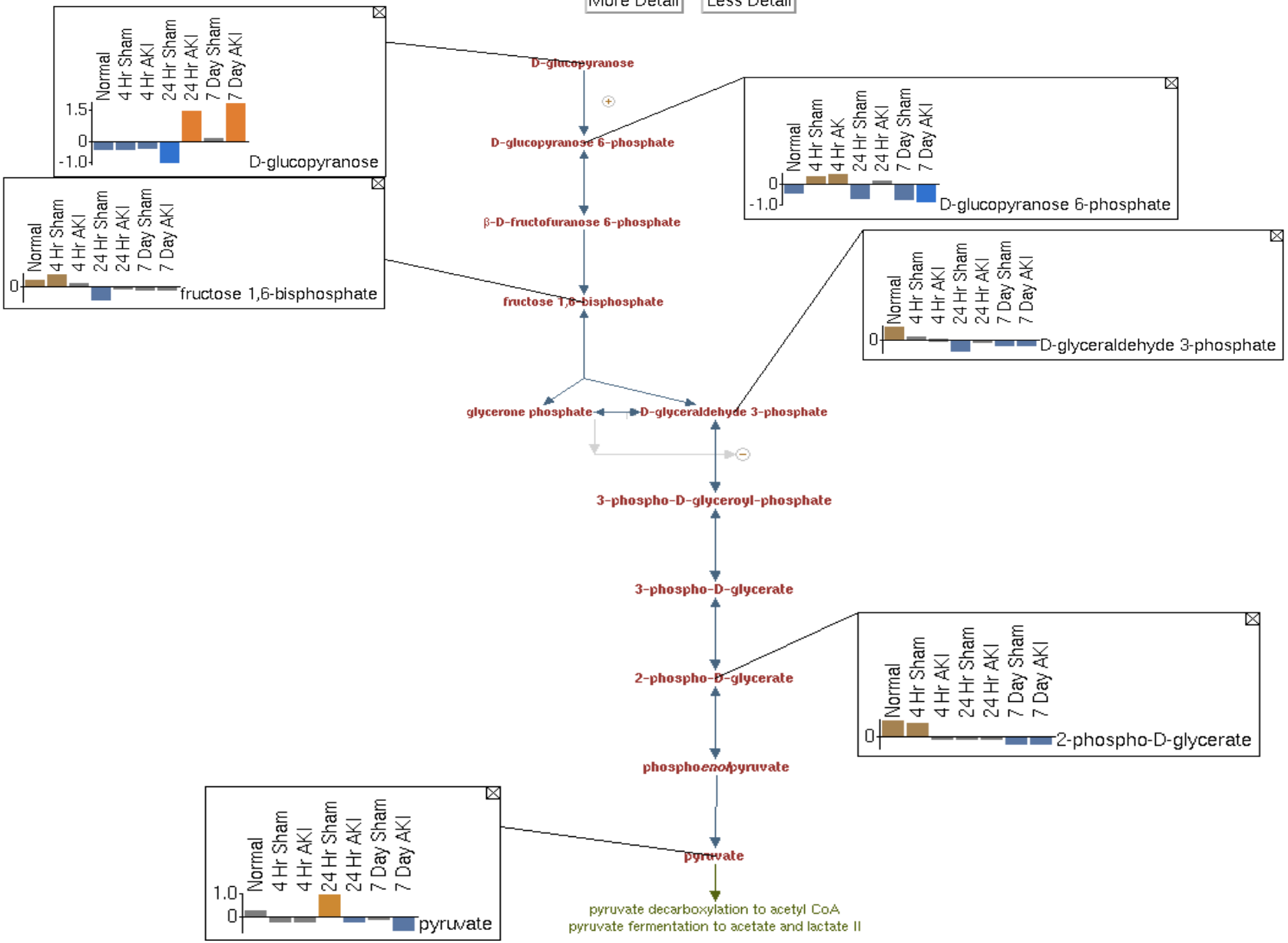
L-lysine → L-saccharopine → (*S*)-2-amino-6-oxohexanoate → L-2-aminoadipate → 2-oxoadipate → glutaryl-CoA → glutaryl-CoA degradation



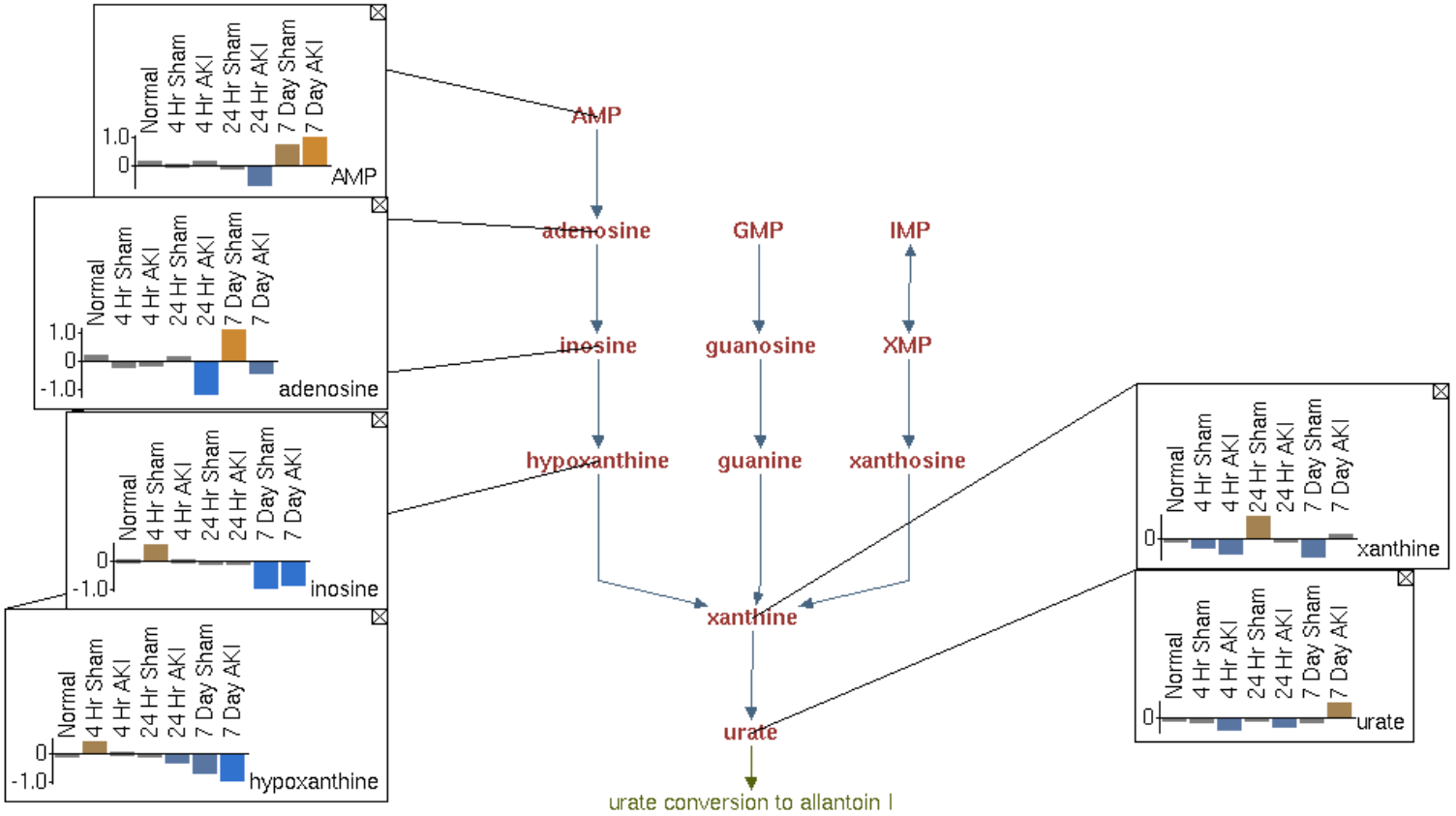


MetaCyc Pathway: glycolysis III (from glucose)

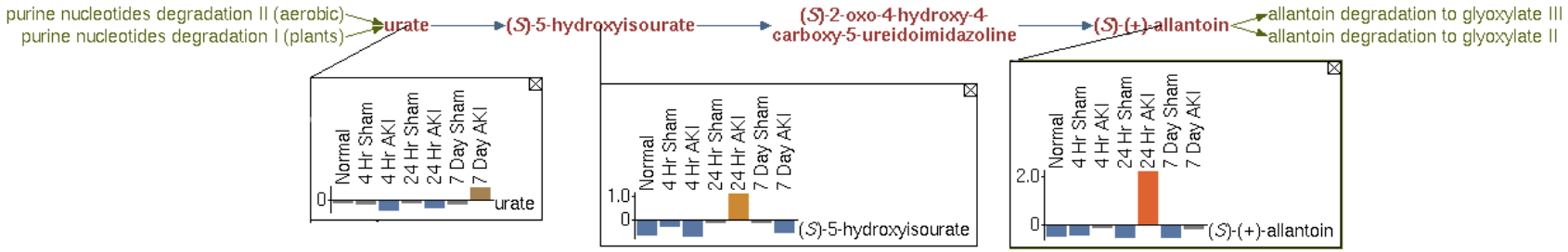
More Detail | Less Detail



MetaCyc Pathway: purine nucleotides degradation II (aerobic)



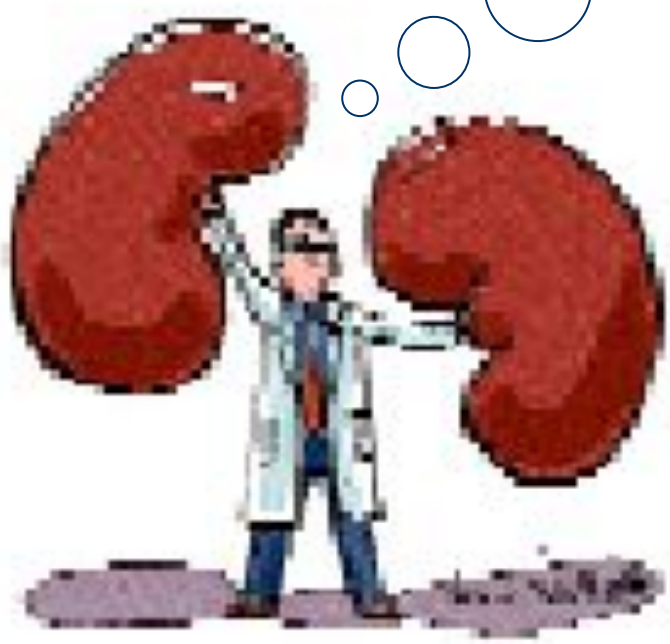
MetaCyc Pathway: urate conversion to allantoin I



Take home messages

- Reducing mortality in AKI will require more than managing volume, potassium and acidosis (traditional complications)
- Must recognize that AKI is a specific and systemic disease (non-traditional complications)
- AKI has 3 dramatic effects on the heart
  - Increased oxidative stress
  - Altered energy metabolism
  - Decreased contractility

Thoughts?



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