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ORIGINAL RESEARCH

Metabolomics Analysis Reveals Deranged Energy Metabolism and Amino Acid Metabolic Reprogramming in Dogs With Myxomatous Mitral Valve Disease

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BACKGROUND: Myxomatous mitral valve disease (MMVD), a naturally occurring heart disease, affects 10% to 15% of the canine population. Canine MMVD shares many similarities with human MMVD. Untargeted metabolomics was performed to identify changes in metabolic pathways and biomarkers with potential clinical utilities.

METHODS AND RESULTS: Serum samples from 27 healthy, 22 stage B1, 18 stage B2 preclinical MMVD dogs, and 17 MMVD dogs with a history of congestive heart failure (CHF) were analyzed. Linear regression analysis identified 173 known metabolites whose concentrations were different among the 4 groups (adjusted $P<0.05$), of which 40% belonged to amino acid super pathways, while 30% were lipids. More than 50% of significant metabolites were correlated with left atrial diameter but not left ventricular dimension. Acylcarnitines, tricarboxylic acid cycle intermediates, and creatine accumulated in proportion to MMVD severity. α-Ketobutyrate and ketone bodies were increased as MMVD advanced. Nicotinamide, a key substrate of the main nicotinamide adenine dinucleotide (NAD^+) salvage pathway, was decreased, while quinolinate of the de novo NAD^+ biosynthesis was increased in CHF dogs versus healthy dogs. 3-Methylhistidine, marker for myofibrillar protein degradation, was higher in CHF dogs than non-CHF dogs. Trimethylamine N-oxide (TMAO) and TMAO–producing precursors, including carnitine, phosphatidylcholine, betaine, and trimethyllysine, were increased in CHF dogs versus non-CHF dogs. Elevated levels of uremic toxins, including guanidino compounds, TMAO, and urea, were observed in CHF dogs. Pathway analysis highlighted the importance of bioenergetics and amino acid metabolism in canine MMVD.

CONCLUSIONS: Our study revealed altered energy metabolism, amino acid metabolic programming, and reduced renal function in the development of MMVD and CHF. Complex interplays along the heart-kidney-gut axis were implicated.

Key Words: amino acids ■ canine ■ congestive heart failure ■ dog ■ energy metabolism ■ heart failure ■ metabolomics ■ mitral valve ■ mitral valve regurgitation ■ uremic toxin

See Editorial by Scheibe and Grueter

Myxomatous mitral valve disease (MMVD), the most common naturally occurring heart disease in dogs, is characterized by progressive valvular degeneration that can cause mitral regurgitation and lead to congestive heart failure (CHF).[1] MMVD affects 10% to 15% of the canine population, with a greater frequency in small- and medium-breed geriatric dogs.[2] A staging scheme for classifying canine MMVD has been adopted by the consensus committee established by the American College of

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CLINICAL PERSPECTIVE

What Is New?

- We reported the first untargeted serum metabolomic analysis comparing all stages of naturally occurring myxomatous mitral valve disease in dogs.

What Are the Clinical Implications?

- Eighty-two differential metabolites were identified between healthy dogs and dogs with preclinical stage B1 myxomatous mitral valve disease, while 12 were identified between B1 and B2 stages, and the results may offer novel insights into early transition, development of volume overload, and diagnostic potentials.
- Canine myxomatous mitral valve disease is considered a model for human myxomatous mitral valve disease, and information gained in this study may have clinical relevance for human patients.

Nonstandard Abbreviations and Acronyms

3-MH	3-methylhistidine
BCS	body condition score
BHBA	3-hydroxybutyrate/β-hydroxybutyrate
FC	fold-change
FDR	false discovery rate
KEGG	Kyoto Encyclopedia of Genes and Genomes
MetPA	Metabolomics pathway analysis
MMVD	myxomatous mitral valve disease
NAD+	nicotinamide adenine dinucleotide
nLAD	normalized left atrial diameter
PC	principal component
PCA	principal component analysis
qMSEA	quantitative metabolite set enrichment analysis
TCA	tricarboxylic acid
TMAO	trimethylamine N-oxide
TMAP	N,N,N-trimethyl-L-alanyl-L-proline betaine
TMAVA	N,N,N-trimethyl-5-aminovalerate

Veterinary Internal Medicine.[3] Dogs at risk for developing MMVD but otherwise healthy are considered stage A; dogs with a heart murmur caused by mitral regurgitation but no clinical signs of CHF are classified as stage B; dogs with MMVD and overt clinical signs of CHF are classified as stage C; and MMVD dogs with CHF refractory to treatment are classified as stage D. Stage B dogs are further classified into B1 or B2 based on the absence or presence of cardiac remodeling, respectively. In general, MMVD dogs have a lengthy preclinical stage B. Once progressed to late stage B2 and stage C, the disease advances more rapidly, with a mean survival time of <12 months in dogs with CHF.[4]

Metabolomics has been increasingly used to interrogate molecular and metabolic changes in cardiovascular diseases and is regarded as one of the signposts on the path to clinical utility.[5–7] Metabolomic analysis combined with other systems approaches provides an opportunity to investigate the mechanism of disease development and progression.[8] The metabolic machinery of cardiac energy metabolism includes 3 interconnected components: substrate utilization, oxidative phosphorylation, and energy transfer to and utilization by myofibrils.[9] A wealth of evidence supports a link between energy substrate metabolism and cardiac functions.[9,10] In healthy heart, the main energy substrate used by myocardial cells is fatty acids (FAs). The failing heart experiences reduced capacity of mitochondrial FA oxidation as the main energy source and increases its reliance on alternative energy substrates such as ketone bodies and glucose.[11–15] In recent years, other gut microbe–related pathways have been associated with pathogenesis of cardiovascular diseases including heart failure (HF) in humans.[16,17] For instance, circulating levels of trimethylamine N-oxide (TMAO), a gut microbial metabolite, were linked to increased risks of major adverse cardiovascular events in humans and MMVD in dogs.[18–20]

To date, there are only 2 metabolomic profiling studies on canine MMVD. One study compared serum samples between 18 healthy dogs and 11 age- and sex-matched dogs with preclinical MMVD, while the other reported changes in serum metabolome in response to diet intervention in dogs with preclinical MMVD.[21,22] Direct comparisons in metabolome between various stages of MMVD are lacking. Metabolomic study of human MMVD is also scarce, with only 1 published investigation, which reported differential metabolites in affected versus healthy patients.[23] Canine MMVD is considered a model for human MMVD in that they share many similarities at the molecular and pathophysiological levels,[23–28] and information gained from canine studies may therefore have relevance in human patients. Our hypothesis was that serum metabolomics changes reflect adaptations of energy substrates and interruptions of energy metabolic machinery during MMVD progression, and that gut microbial mediators and uremic toxins are associated with stage B2 or C/D MMVD. Our goal was to further our understanding of MMVD pathogenesis at the molecular and systemic levels, to identify future therapeutic targets

and new biomarkers with diagnostic and prognostic potential through cross-sectional comparisons.

MATERIALS AND METHODS

The authors declare that all of the supporting data are available as Data S1 and S2.

Animals and Study Approval

The study protocol was reviewed and approved by the University of Pennsylvania Institutional Animal Care and Use Committee, and informed owner consent was obtained. Clinically healthy dogs without a heart murmur and without concurrent systemic disease were prospectively enrolled as controls (group A). This group of dogs primarily consisted of systemically healthy dogs owned by students and staff of the hospital. A cohort of dogs with a left apical systolic murmur, echocardiographic diagnosis of thickened and prolapsing mitral valve leaflet(s), and mitral regurgitation, as well as clinical history and physical examination consistent with stage B1, B2, C, or D MMVD were considered for group B1, group B2, and group C/D, respectively.[3] Any dog with severe concurrent systemic disease including diabetes mellitus, cancer, or renal failure, or those with any congenital heart disease, were excluded.

Serum Sample Collection

Venous blood samples of 2 to 3 mL were collected in plain red-topped tubes. The blood was allowed to clot and centrifuged at 1600*g* for 5 minutes to yield serum samples, which were stored at −80°C until use.

Echocardiography

Echocardiographic studies (iE33, Philips Healthcare) were performed without sedation. Left ventricular internal dimensions in end-diastole and left ventricular internal dimensions in end-systole, normalized left atrial diameter (nLAD), and normalized aortic root diameter were measured from right parasternal short-axis 2-dimensional images and normalized to body weight.[29] The ratio of the left atrial diameter to the aortic root diameter was calculated.

Metabolomics Assay

Untargeted metabolomics assays were performed at a commercial laboratory (Metabolon, Inc.). Sample preparation and extraction, liquid chromatography, and mass spectrometry followed Metabolon standard protocols as previously described (Supplemental Methods).[22,30] Compound detection and identification were performed using Metabolon proprietary software and database. A total of 1033 metabolites were identified, including 912 known and 121 unknown (Data S1).

Data Processing

The raw data were generated based on the area-under-the-curve formula using ion counts that provide relative quantification (Data S2). Metabolites with missing values in >80% of samples were removed. The remaining missing data were imputed with a value equal to half of the minimal value in the raw data under the assumption that missing data were those below the detection limit. Metabolites that comprised the bottom 25th percentile in the interquartile range represented near-constant values and were removed. The data were further transformed using the logarithm to the base 2, and autoscaled to achieve a zero mean and unit variance for all metabolites.

Statistical Analysis

Principal component (PC) analysis (PCA) for high-dimensional multivariate data was performed using the R function "prcomp" and PCs were calculated. The first 2 PCs, PC1 and PC2, which capture more data variations than other PCs, were examined for their ability to separate the 4 groups. A multiple linear regression using PC1 or PC2 as the dependent variable and group as the independent variable, adjusted for age, body condition score (BCS), and body weight was performed. Tukey's post hoc test was performed to compare the means between groups and *P* values were adjusted for multiple testing error.

To identify differential metabolites, a multiple linear regression, adjusted for age, BCS, and body weight was performed. *P* values were adjusted to control the false discovery rate (FDR) using the Benjamini-Hochberg method. Significant metabolites were subjected to pairwise comparisons using the R function "pairwise.t.test" with pooled SD and Benjamini-Hochberg adjustment for multiple testing. FDR ≤0.05 was considered significant.

To compare the means of continuous variables, ANOVA and Tukey's post hoc tests were performed. To test the null hypothesis that the 2 categorical variables were independent, chi-square test was performed if all expected numbers were >5. Otherwise, a Fisher's exact test was used instead. Fold-change (FC) was defined as the ratio of the mean of group 2 (g2) over that of group 1 (g1): $FC = 2^{(\log_2 g2 - \log_2 g1)}$.

Pearson's correlation coefficients between echocardiographic variables and significant metabolites were calculated. The 8 dogs without echocardiographic measurements from group A were excluded from the analysis. *P* values were adjusted for multiple testing errors using the Benjamini-Hochberg method. Adjusted $P \leq 0.05$ was considered significant. Pairwise

Pearson's correlation analysis among significant metabolites was also performed.

Analysis of Confounding Effects

To account for any potential confounding effect from age or body weight, 500 iterations of bootstrap resampling without replacement were performed where 10 samples from each group were randomly selected. Those bootstrapped subsamples with no difference in age or body weight were selected for further analysis. PCA and multiple linear regression were performed on each subsample set using PC1 or PC2 as the dependent variable and group as the explanatory variable, adjusted for age, BCS, and sex. *P* value distributions were analyzed.

Potential confounding effect from cardiac medications was also evaluated using data from groups B1 and B2 dogs. Dogs taking ≥1 of 4 commonly used cardiac medications, pimobendan, furosemide, angiotensin-converting enzyme inhibitors, and spironolactone, for at least 2 weeks before the time of sampling were considered for group Y, while the rest of the dogs were considered for group N. PCA and Student *t* test were performed to test the null hypothesis that the means of PC1 or PC2 between group Y and group N were not different.

Metabolomics Pathway Analysis and Quantitative Metabolite Set Enrichment Analysis

Metabolomics pathway analysis (MetPA) was performed using the Human Metabolome Database IDs of the significant metabolites.[31] The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway library for *homo sapiens* was searched and pathway overrepresentation analysis was performed using hypergeometric test. The 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. Quantitative metabolite set enrichment analysis (qMSEA) was performed to identify enriched metabolite sets between group C/D and group A using the PubChem IDs and the concentration data of all metabolites.[32] Metabolite sets with at least 2 compounds in the Small Molecule Pathway Database (www.smpdb.ca) was searched. Pathways with $P \leq 0.05$ were considered significant.

Data processing and statistical analysis were performed in the statistical computing software R (version 3.5.0). Partial least square discriminant analysis, MetPA and qMSEA were performed in MetaboAnalyst 4.0.[33]

RESULTS

Eighty-four client-owned dogs, including 27 group A, 22 group B1, 18 group B2, and 17 group C/D dogs, were enrolled in the study (Table). Significant differences in age and body weight were found (both $P_{ANOVA}<0.001$). Dogs in group A were significantly younger than dogs in any other group, and had a greater mean body weight than those in groups B2 and C/D (adjusted $P<0.05$ in all comparisons, Figure S1). No difference was found in sex or BCS.

There were significant differences in left ventricular internal dimensions in end-diastole, nLAD, and ratio of the left atrial diameter to the aortic root diameter ($P_{ANOVA}<0.0001$, Table), where differences were found in all pairwise group comparisons (adjusted $P<0.05$) except between groups A and B1 (Figure S1). No difference was observed in left ventricular internal dimensions in end-systole or normalized aortic root diameter.

Global Metabolome Changes

PCA showed clear separation of the 4 groups along PC1 ($P=1.93\times10^{-11}$; Figure 1A, top panel), but not along PC2 ($P=0.18$). Differences were found in all pairwise group comparisons (adjusted $P<0.01$; Figure 1A, lower panel) except between groups B1 and B2. In partial least square discriminant analysis, separations were evidenced along component 1 and component 2 ($P=3.2\times10^{-16}$ and 1.1×10^{-5} respectively, Figure S2).

Multiple linear regression analysis identified 201 significant metabolites, of those 173 of 201 (86.1%) were known (Table S1). Sixty-nine metabolites (69/173) belonged to amino acid super pathways, while 52 of 173 (30.1%) were lipids (Figure 1B).

Analysis of Confounding Effects

In 1 computational simulation, 500 bootstrap experiments generated 177 subsamples with no difference in age or body weight ($P>0.05$ in both cases, Table S2). Among those, 17 (17/177) had a significant difference in BCS but none (0/177) had a sex effect. The minimum, median, and maximum of the *P* values for PC1 were 1.7×10^{-7}, 2.5×10^{-4}, and 0.02, respectively (Figure S3A).

Forty dogs from group B1 and B2 were analyzed for potential effects of cardiac medications. No difference in PC1 or PC2 was found between group Y and group N ($P=0.38$, 0.69 respectively; Figure S3B).

Energy Metabolism

Metabolites in all 3 components of the energy metabolic machinery, including energy substrate transfer,

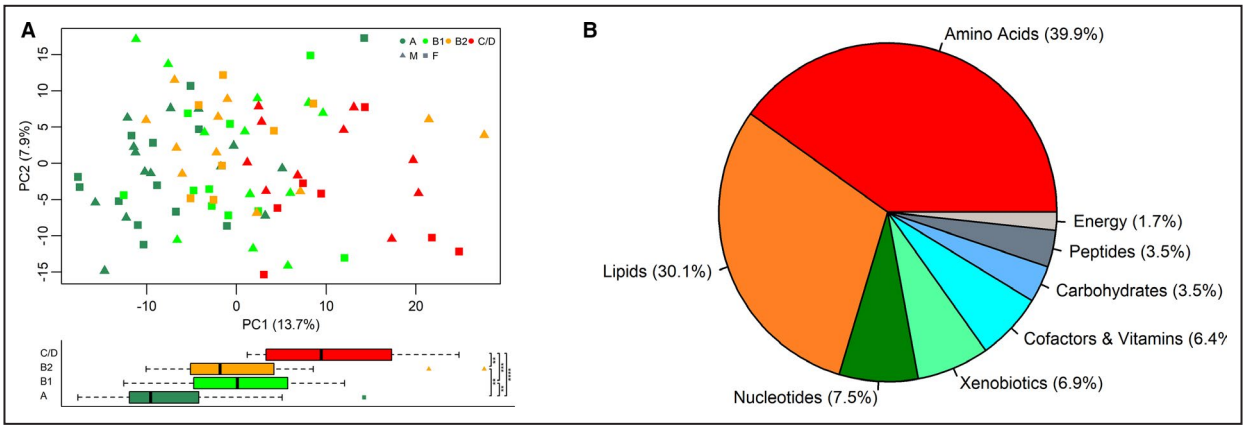

Figure 1. Principal component analysis (PCA) and differential metabolites.
The PCA plot (**A**, top panel) and boxplots along the first principal component (PC1) (**A**, bottom panel). The percentages of data variation explained by PC1 and PC2 are indicated on the x and y axes, respectively. Tukey tests were performed to compare between groups. *P* values were adjusted for multiple testing. ***P*<0.01; ****P*<0.001; *****P*<0.0001. **B**, The pie chart shows percentages of classes of the 173 known metabolites. F indicates female; and M, male.

oxidative phosphorylation, and high-energy phosphate bond transfer and utilization, were examined. Significant changes were found in 22 acylcarnitines, all of which showed >1.8-fold increases in group C/D compared with group A, while 11of 22 (50%) and 8 of 22 (36.4%) of the acylcarnitines showed >1.5-fold increases in group C/D versus group B1 or B2, respectively (FDR<0.05 in all cases; Figure 2, Figure S4). Carnitine concentration was increased by >2-fold in group C/D versus any other group (Figure 3A).

Citrate and aconitate, 2 intermediates of the tricarboxylic acid (TCA) cycle, were increased in groups B1, B2, and C/D versus group A, and in group C/D versus group B1 (FC>1.5 in all cases, Figure 3B and 3C). The level of α-ketobutyrate, a precursor of succinyl-coenzyme A, was higher in groups B2 and C/D than that of group A (FC>1.9 in all cases, Figure 3D). Inorganic phosphate, which is taken up by ADP to form ATP in the TCA cycle, was increased in group C/D compared with group A or B1 (FC>1.8 in both cases, Figure 3E). The level of creatine, a principal component of the creatine kinase energy shuttle system, was also elevated with MMVD progression (FC>1.5 in all cases, Figure 3F).

Significant changes in ketone bodies were observed: 3-hydroxybutyrate/β-hydroxybutyrate (BHBA) and acetoacetate were increased in both group B2 and group C/D compared with group A (FC≥2 in all cases, Figure 3G and 3H). Acetoacetate level was also higher in group B1 than that of group A (FC=1.7). An increase in BHBA was observed in group B1 versus group A, but it did not reach statistical significance after adjusting for multiple testing (FC=1.5, FDR=0.06).

Nicotinamide, the precursor for nicotinamide adenine dinucleotides (NAD^+) salvage pathway, was decreased in group B1 and group C/D compared with group A (FC=1.6 in both cases, Figure 3I). The concentration of quinolinate, a key intermediate in the NAD^+ de novo biosynthesis pathway from L-tryptophan, was higher in group C/D than that of any other group (FC >2.0 in all cases, Figure 3J).

Amino Acid Metabolism

The concentrations of methionine, proline, glycine, and glutamine were decreased as MMVD progressed (FC >1.5 in all cases, Figure 4A through 4D), while the levels of 1-methylhistidine and 3-methylhistidine (3-MH) were increased in group C/D compared with other groups (FC >2.0 in all cases, Figure 4E and 4F). Several compounds in the lysine degradation pathway were also changed. Pipecolate and 2-aminoadipate, key intermediates of lysine degradation, were increased in groups B1 and B2 when compared with group A (FC>1.8 in all cases, Figure 4G and 4H). The level of 2-aminoadipate was also higher in group C/D versus group A (FC=2.1, FDR=0.001).

The concentrations of several uremic toxins including TMAO were changed. The circulating levels of 3 guanidino compounds, including argininate, 2-oxoarginate, and 4-guanidinobutanoate, were higher in MMVD dogs compared with healthy dogs (Figure 4I through 4K). The level of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, was also higher in group C/D than that of other groups (Figure 4L). In addition, 2 nitrogenous waste products, urea and urate, were increased in MMVD dogs versus healthy dogs (Figure 4M and 4N).

TMAO and Its Precursors

The concentration of TMAO was higher in group C/D versus all other groups, and in group B2 versus group A

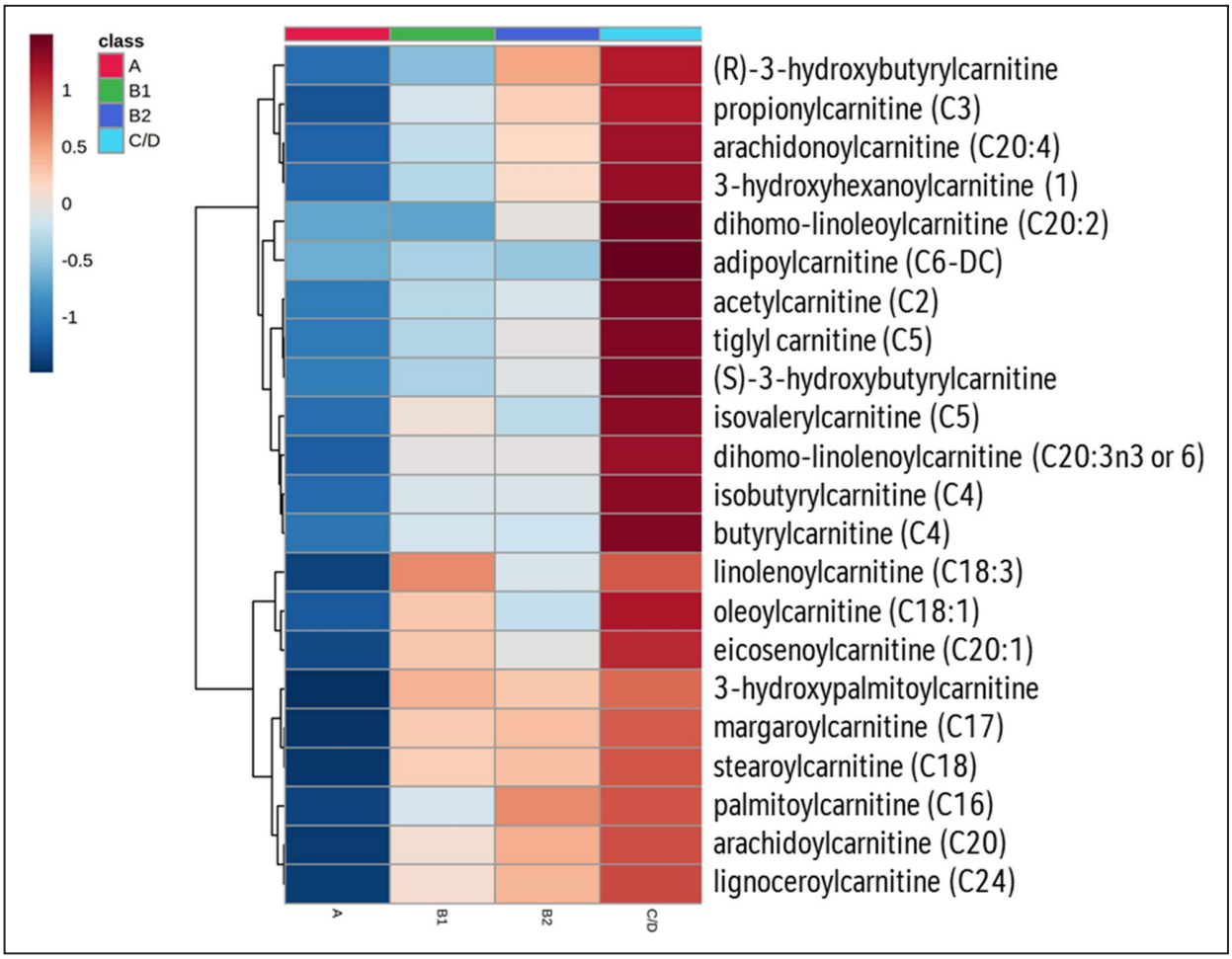

Figure 2. Heat map of 22 significant acylcarnitines.
Hierarchical clustering on the features was performed using the Euclidean distances calculated from the group means. The color scale corresponds to concentrations in log scale from low (deep blue) to high (maroon).

(FC>1.5 in all cases, Figure 5A). Several TMAO-producing nutrient precursors, including carnitine, trimethyllysine, phosphatidylcholines, and betaines, were also increased in MMVD dogs versus healthy dogs (Figures 3A and 5B through 5D). N,N,N-trimethyl-5-aminovalerate (TMAVA, also known as 5-aminovaleric acid betaine), another metabolite of intestinal microbes, was higher in groups B2 and C/D versus group A (Figure 5E). In addition, the concentration of N,N,N-trimethyl-L-alanyl-L-proline betaine (TMAP), a novel marker for kidney function, was higher in group C/D than those of the other groups (FC>1.9 in all cases, Figure 5F).

Correlation Analysis

Among 173 known metabolites, 96 of 173 (55.5%) and 103 of 173 (59.5%) were significantly correlated with nLAD and ratio of the left atrial diameter to the aortic root diameter, respectively (FDR<0.05, Table S3). Of them, 88 of 173 (50.9%) were in common. In contrast, only 3 of 173 (1.7%) metabolites were correlated with left ventricular internal dimensions in end-diastole. Correlations between nLAD and key metabolites from energy metabolism, and amino acid metabolisms are shown in Figure 6.

Aconitate and citrate of the TCA cycle had a near-perfect correlation with each other (r=0.93, Figure S5A). Aconitate was also positively correlated with numerous metabolites including quinolinate, urea, BHBA, TMAO, 3-MH, and carnosine (Figure S5B through S5G). A strong positive correlation was also found between urea and 3-MH (Figure S5H), and between 2-oxoarginine and 4-guanidinobutanoate (Figure S5I).

The majority of significant acylcarnitines (18/22) and carnitine were correlated with nLAD (FDR<0.05, Table S4). Carnitine and acylcarnitines were correlated with one another.

Metabolic Pathways

Five KEGG pathways, including arginine biosynthesis, synthesis, and degradation of ketone bodies, nicotinate and nicotinamide metabolism, histidine metabolism,

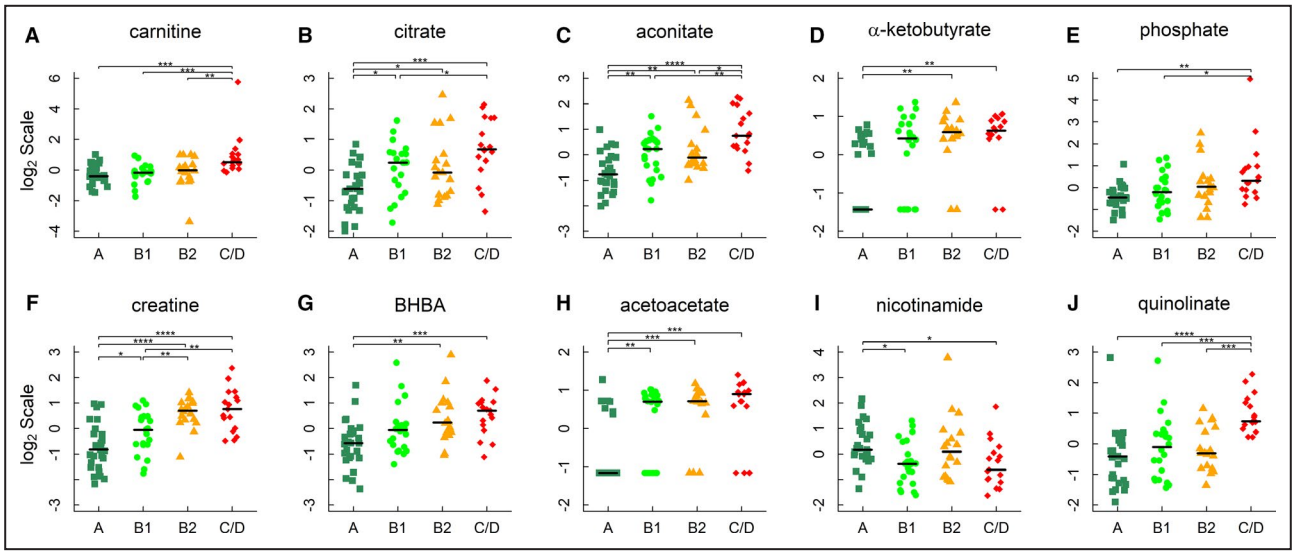

Figure 3. Metabolites in energy metabolism.
A through **F**, Carnitine, citrate, aconitate, α-ketobutyrate, phosphate, and creatine; (**G** and **H**) 3-hydroxybutyrate/β-hydroxybutyrate (BHBA) and acetoacetate; and (**I** and **J**) nicotinamide and quinolinate. Horizontal lines denote medians. The metabolites were identified by linear regression analyses and subject to post hoc comparisons with corrections for multiple testing. Adjusted *P* values: **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001.

and branched chain amino acid biosynthesis, were overrepresented (*P*<0.05, Figure 7A and Table S5). In addition, qMSEA identified 33 metabolic pathways enriched in group C/D over group A (Figure 7B and Table S6). The overrepresented KEGG pathways except branched chain amino acid biosynthesis pathway were

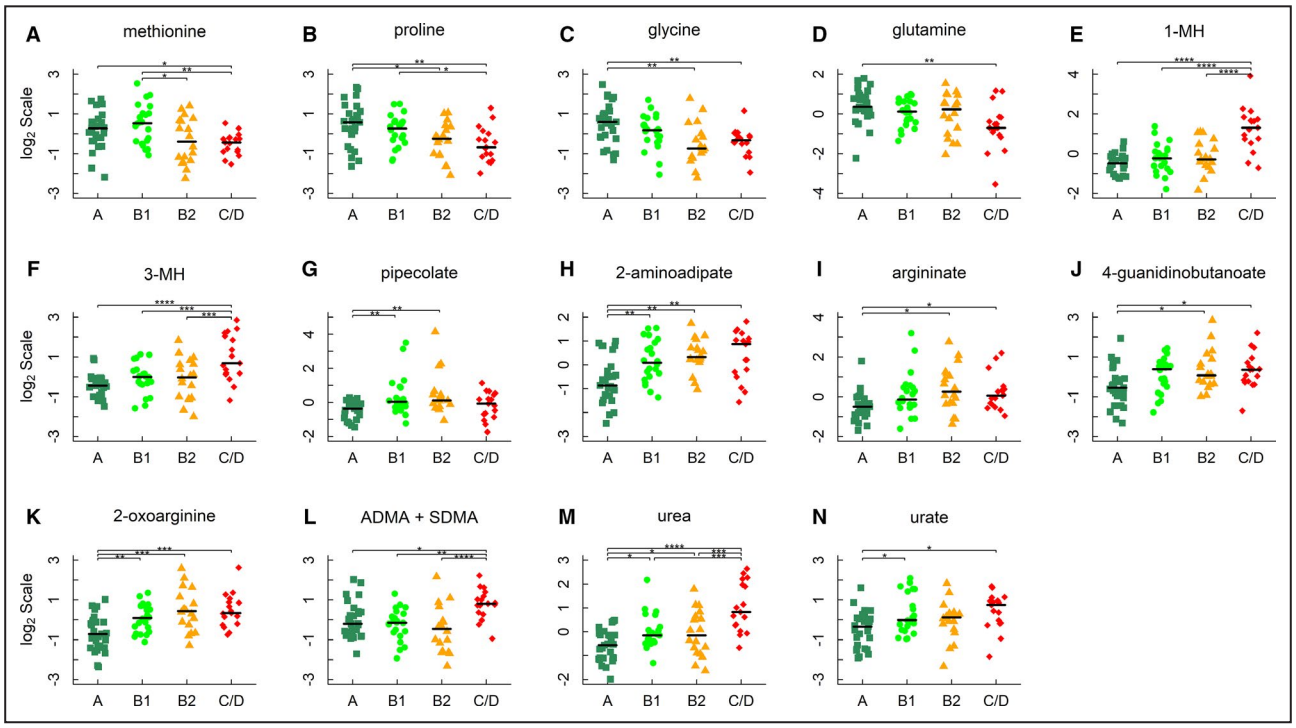

Figure 4. Amino acids, their derivatives, and uremic toxins.
A through **D**, Methionine, proline, glycine, and glutamine; (**E** through **H**) 1-methylhistidine (1-MH), 3-methylhistidine (3-MH), pipecolate, and 2-aminoadipate; and (**I** through **N**) argininate, 4-guanidinobutanoate, 2-oxoarginine, asymmetric and symmetric dimethylarginine (ADMA+SDMA), urea, and urate. Horizontal lines denote medians. The metabolites were identified by linear regression analyses and subject to post hoc comparisons with corrections for multiple testing. Adjusted *P* values: **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001.

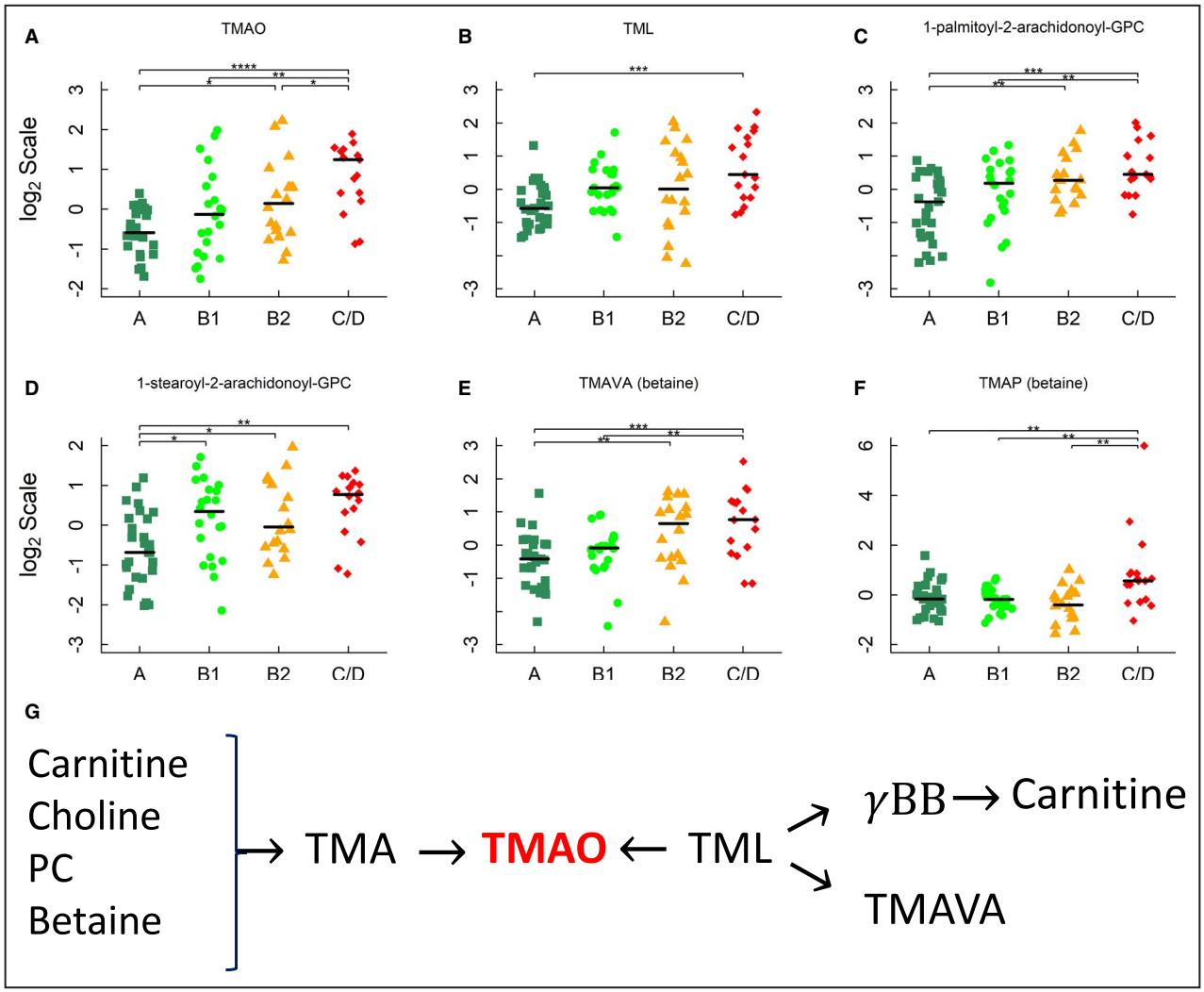

Figure 5. Trimethylamine N-oxide (TMAO) and TMAO-producing precursors.
A, TMAO, (**B**) trimethyllysine, (**C** and **D**) 1-palmitoyl-arachidonoyl-GPC and 1-stearoyl-2-arachidonoyl-GPC, and (**E** and **F**) N,N,N-trimethyl-5-aminovalerate (also known as 5-aminovaleric acid betaine) (TMAVA) or N,N,N-trimethyl-L-alanyl-L-proline betaine (TMAP). **G**, Carnitine, choline, phosphatidylcholine, and betaine can be converted to trimethylamine by certain gut bacteria. Trimethylamine is released into circulation and converted to TMAO in the liver. Trimethyllysine is another precursor for microbial TMAO synthesis, as well as the substrate for γ-butyrobetaine (γBB) and TMAVA synthesis. Horizontal lines denote medians. The metabolites were identified by linear regression analyses and subject to post hoc comparisons with corrections for multiple testing. Adjusted *P* values: **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001.

captured by both MetPA and qMSEA. Methionine metabolism, betaine metabolism, and homocysteine degradation pathways were associated with sulfur amino acid metabolism and methylation. Methylhistidine and histidine pathways were overrepresented in dogs with CHF. Many of the enriched pathways, including transfer of acetyl groups into mitochondria, citric acid cycle, lactose synthesis and degradation, ketone body metabolism, oxidation of branched chain FAs, butyrate metabolism, pantothenate (vitamin B5) and coenzyme A biosynthesis, nicotinate and nicotinamide metabolism, lysine degradation, threonine, and 2-oxobutyric acid degradation were associated with or led to energy productions. Tryptophan metabolism and bile acid biosynthesis pathways were also overrepresented in group C/D.

DISCUSSION

Our analysis demonstrated robust changes in the metabolome of dogs with different stages of MMVD that affected several key metabolic pathways. Although the majority of these changes occurred at the stage of CHF, 41% of the significant metabolites had changes at the very early B1 stage, providing an opportunity to understand this early transition during the disease progression. In addition, a small

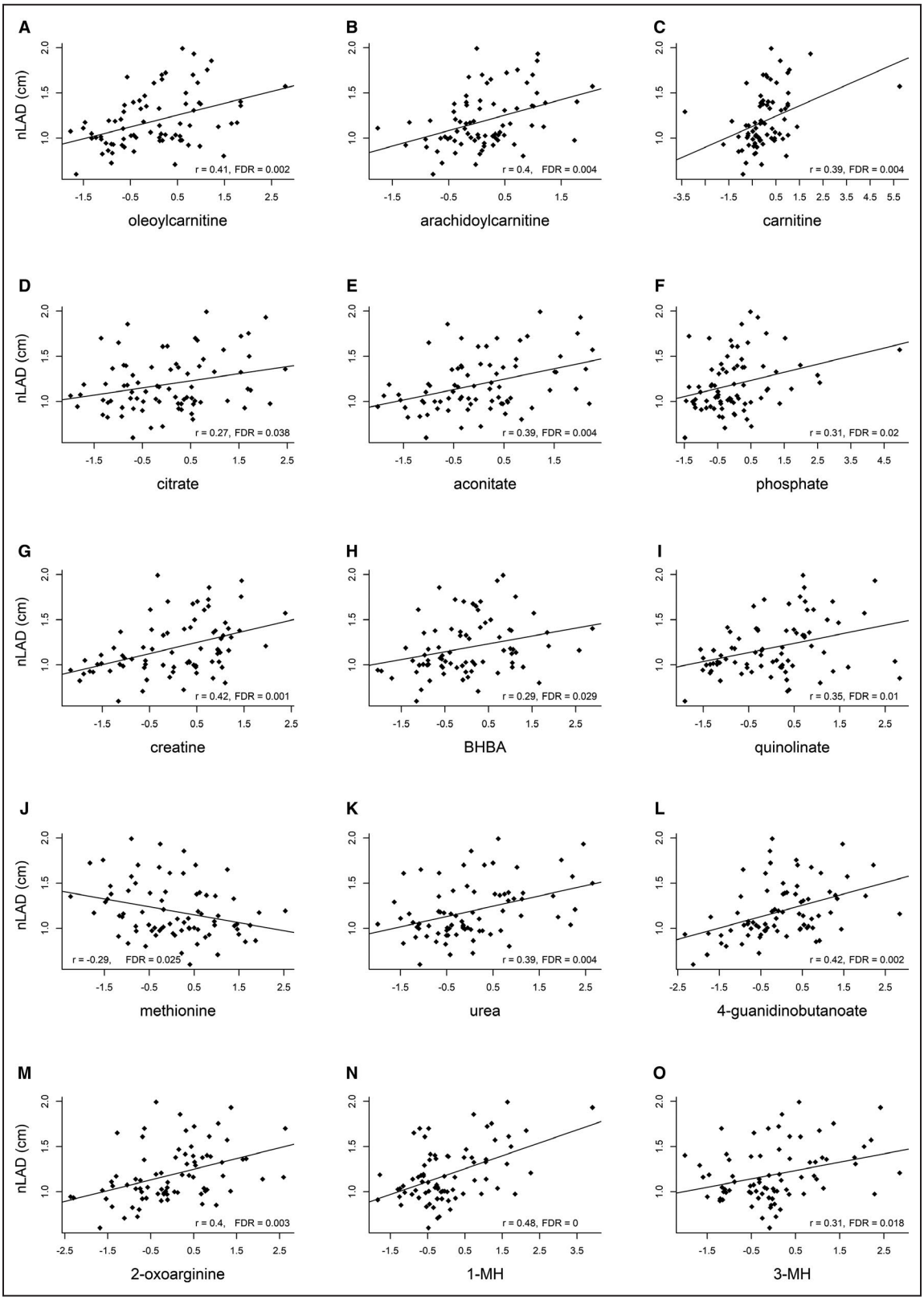

Figure 6. Pearson's correlations between normalized left atrial diameter (nLAD) and metabolites.
A and **B**, Oleoylcarnitine and arachidoylcarnitine; (**C** through **J**) carnitine, citrate, aconitate, phosphate, creatine, 3-hydroxybutyrate/β-hydroxybutyrate (BHBA), quinolinate, and methionine, (**K** through **M**) urea, 4-guanidinobutanoate, and 2-oxoarginine, (**N** and **O**) 1-methylhistidine (1-MH) and 3-methylhistidine (3-MH). A fitted linear regression line, correlation coefficient (r), and adjusted *P* value (FDR) were included in each graph.

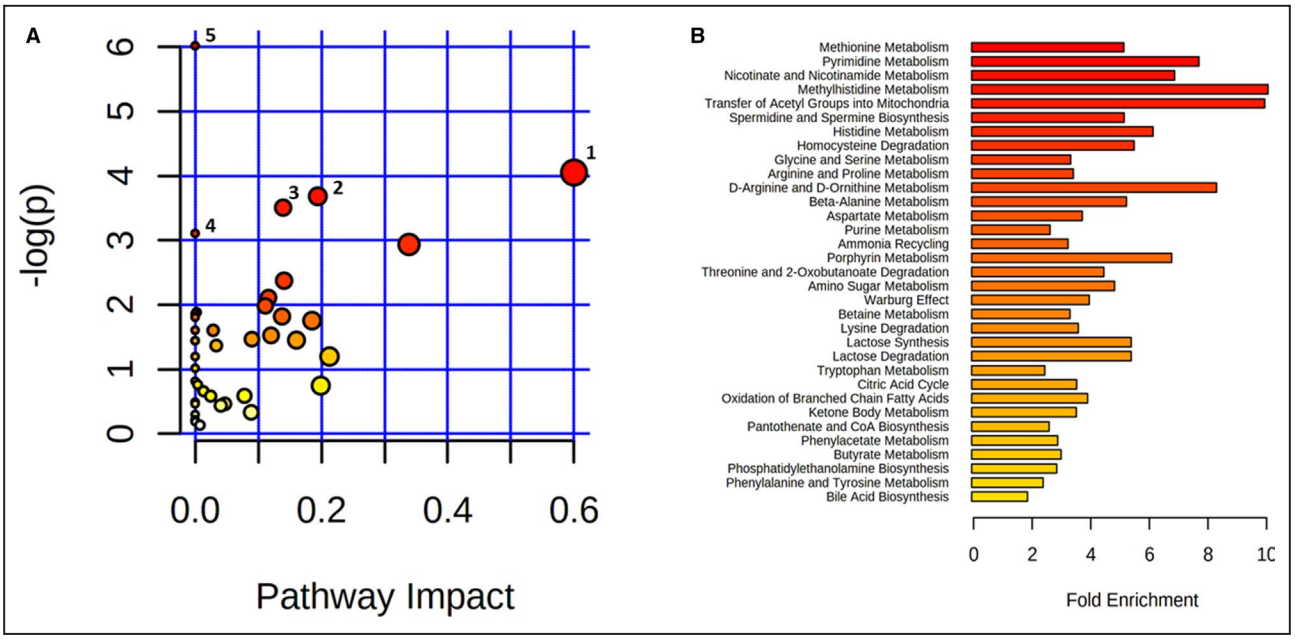

Figure 7. Metabolomic pathway analysis (A) and quantitative metabolite set enrichment analysis (B). **A**, The *y* axis represents the negative of natural logarithm of the *P* values. The significance of each pathway is represented by moving upwards on the *y* axis and by the color scale from white (low significance) to red (high significance). The impact of each pathway is represented by moving rightward on the *x* axis and by the diameter of each circle. The 5 pathways with $P<0.05$ are: (1) synthesis and degradation of ketone bodies; (2) nicotinate and nicotinamide metabolism; (3) histidine metabolism; (4) valine, leucine, and isoleucine biosynthesis; and (5) arginine biosynthesis. **B**, The significance of each enriched metabolite set is represented by the color scale from white (low significance) to red (high significance) as shown in the key. Fold of enrichment is indicated in the *x* axis.

number of metabolites had changes that occurred in the absence of overt congestion or any evidence of volume overload. Could they be early compensatory responses? Derangements in cardiac bioenergetics contribute to the pathogenesis of CHF in humans and animal models.[15,34,35] Previous transcriptomics analysis of mitral valve and left ventricular tissues, as well as metabolomics analysis of serum samples, have suggested compromised long-chain FA oxidation and increased reliance on glycolysis in dogs with preclinical MMVD compared with healthy dogs.[21,22] Recent studies demonstrated increased ketone body utilization in well-defined mouse models of HF and in the failing human heart.[11,12] A higher ketone body uptake was reported by canine failing hearts in vivo.[36] Therapeutic ketosis holds significant promise in HF.[37] Supplement of ketogenic medium-chain triglycerides in diets ameliorated cardiac remodeling in spontaneously hypertensive rats and reduced left atrial enlargements in dogs with early-stage MMVD.[38,39] In this study, the concentrations of both BHBA and acetoacetate, which account for >90% of ketone bodies in man, were increased in B1, B2, and C/D MMVD dogs compared with healthy dogs. Our result was consistent with the observation that blood ketone bodies were elevated in human patients with CHF as compared with those free of CHF, and were in proportion to the severity of cardiac dysfunction.[40,41]

Catabolism of threonine, serine, and methionine converges to α-ketobutyrate, which enters mitochondrial matrix to produce succinyl-coenzyme A via the propionate catabolic pathway. Accumulations of α-ketobutyrate, TCA cycle intermediates, and inorganic phosphate signified perturbations in oxidative phosphorylation. In sarcolemma, creatine, which is produced in the liver and kidneys, is taken up by a membrane creatine transporter from the bloodstream.[42] Creatine kinase catalyzes the transfer of high-energy phosphate bond in ATP to creatine to form phosphocreatine, a small molecule that rapidly diffuses from mitochondria to myofibrils.[9] Increased level of circulating creatine may suggest impaired creatine transporter or uncoupling of energy production and transfer. Acylcarnitines are key intermediates of long-chain FA transport and oxidation. Circulating acylcarnitines accumulate as a result of incomplete or inefficient FA oxidation and have been used as diagnostic markers for disorders in peroxisomal or mitochondrial oxidation processes.[43–45] Elevated plasma long-chain acylcarnitines were documented in human patients with HF compared with normal controls.[46,47] Accumulation of long-chain acylcarnitines may contribute to the HF by stimulating reactive oxygen species production and releasing circulating inflammatory mediators.[47] Twenty-two acylcarnitines were associated with increased MMVD severity in

Table 1. Physical Characteristics, Echocardiography, and Common Cardiac Medications of the Dogs

ACVIM Stage	A	B1	B2	C/D	*P* Value
Sample size	27	22	18	17	
Male/female	14/13	12/10	11/7	10/7	0.93
Age, y	8.3±0.6	10.2±0.5	10.4±0.6	12.4±0.5	<0.0001
Body weight, kg	14.9±1.6	10.5±1.1	8.1±0.9	7.8±1.0	0.0004
BCS (1–9)	5.1±0.2	5.6±0.2	5.2±0.3	4.8±0.3	0.14
Cardiac medications					
Pimobendan	0	4	15	15	
Lasix	0	1	2	15	
ACEI	1	0	5	10	
Spironolactone	0	0	1	8	
Echocardiography*					
nLVIDd, cm	1.47±0.04	1.47±0.04	1.80±0.05	2.03±0.09	<0.0001
nLVIDs, cm	0.98±0.04	0.88±0.04	0.89±0.05	1.00±0.07	0.18
nLAD, cm	0.97±0.03	1.04±0.03	1.30±0.06	1.51±0.08	<0.0001
nAoD, cm	0.76±0.02	0.76±0.02	0.80±0.04	0.72±0.03	0.23
LA/Ao	1.30±0.05	1.40±0.05	1.70±0.10	2.17±0.12	<0.0001

Continuous variables are reported as mean±standard error. ACEI indicates angiotensin-converting enzyme inhibitor; ACVIM, American College of Veterinary Internal Medicine; BCS, body condition score; LA/Ao, left atrial to aortic root diameter ratio; nAoD, normalized aortic root diameter; nLAD, normalized left atrial diameter; nLVIDd, normalized left ventricular internal diameter end-diastole; and nLVIDs, normalized left ventricular internal diameter end-systole.

*Eight group A dogs had no echocardiography.

dogs. Some of these acylcarnitines were decreased in response to a diet intervention with demonstrated clinical benefits in MMVD dogs.[22] Taken together, our data suggest perturbed energy metabolic machinery in dogs with MMVD.

Carnitine level was also significantly increased in dogs with CHF. Carnitine plays a key role in the transport of long-chain FAs into mitochondrial matrix for oxidation, and its deficiency was associated with dilated cardiomyopathy in dogs and humans.[48,49] However, elevations in circulating carnitine concentration have also been reported in human patients with dilated cardiomyopathy and CHF.[50,51] It is possible that the increase of carnitine concentration in circulation was caused by reduced capacity of FA oxidation in dogs with CHF or signifies a compensatory effort for the failing heart to increase FA oxidation. It is also possible that the elevated level of circulating carnitine contributed to the TMAO production.

In mammalian cells, NAD^+ is an essential cofactor for mitochondrial bioenergetics and important for TCA cycle, glycolysis, and FA oxidation. It is synthesized by 3 pathways, the main salvage pathway that recycles nicotinamide or nicotinamide riboside, the de novo biosynthesis through the kynurenine pathway from L-tryptophan, and the Preiss-Handler pathway from nicotinic acid.[52] The concentration of nicotinamide was reduced in groups B1, B2, and C/D, compared with group A. In contrast, quinolinate, a key intermediate of the kynurenine pathway, was increased in dogs with CHF compared with dogs in other groups. Higher circulating level of quinolinate was reported in human patients with CHF versus healthy patients and was associated with a higher mortality rate.[53] Thus, it is possible that the predominant salvage pathway is compromised in MMVD dogs, and the de novo NAD^+ biosynthesis is activated.

The "gut hypothesis" in cardiovascular disease and HF has gained considerable attention in recent years.[16,17] Evidence for the causal association has begun to emerge.[54] Several notable gut microbiota-mediated metabolic pathways and metabolites, including short-chain FAs, bile acids, and TMA/TMAO, were associated with cardiovascular disease and HF.[16] Gut microbiota metabolize dietary nutrients such as L-carnitine, phosphatidylcholine, choline, and betaine to produce trimethylamine, which is oxidized to TMAO in the liver and released into circulation (Figure 5G). Although less efficient than trimethylamine, trimethyllysine is another source of TMAO, and can also serve as the substrate for the synthesis of γ-butyrobetaine and TMAVA.[55] Circulating TMAO levels were higher in patients with HF versus healthy humans and dogs.[20,56] Both B2 preclinical dogs and CHF dogs had higher TMAO levels than healthy dogs, suggesting that the change began before the onset of CHF. However, the causal relationship between the TMAO-mediated microbial pathway and canine MMVD has yet to be established. Integrational analysis with microbiome changes from these dogs may provide additional insights.

Several uremic toxins were identified in this study. Deficiency in arginase, the enzyme that catalyzes the

conversion from arginine to urea in the urea cycle, results in accumulations of guanidino compounds. The levels of 3 guanidino compounds, argininate, 2-oxoarginine, and 4-guanidinobutanoate, were elevated in groups B1, B2, and C/D versus group A. TMAP, a novel marker for kidney function, was also increased in CHF dogs versus non-CHF dogs. The changes in blood uremic toxins, including TMAO, guanidino compounds, and urea and uric acid, as well as TMAP, provide yet another example of the complex interplay along the cardiorenal axis.[57] It will be interesting to sort out which of these uremic toxins are relevant to mitral regurgitation or MMVD, and those that are associated with CHF. Nonetheless, pharmaceutical or nutritional therapies to reduce these circulating uremic toxins and to improve renal functions may provide clinical benefits to patients with MMVD or CHF.

A majority of the significant metabolites were correlated with both nLAD and ratio of the left atrial diameter to the aortic root diameter, but not left ventricular internal dimensions in end-diastole. Left atrial enlargement was considered to be the most reliable independent indicator for increased risk of progression of MMVD in dogs.[58] This may provide opportunities to explore potential markers for progression or risk prediction. The significance of this, if any, remains to be determined.

The MetPA approach depends on a list of significant metabolites that meet the stringent threshold to discern telltale biological clues, while the qMSEA approach uses predefined pathways based on prior biological knowledge to determine whether and how many members of a metabolic pathway act in concert to exert cellular functions. Degradations of homocysteine, methionine, or threonine lead to the production of α-ketobutyrate, and lysine catabolism generates acetyl-coenzyme A in the mitochondria. In our study, >30% of metabolic pathways identified by qMSEA were associated with or led to energy productions. Perturbations in arginine and proline metabolism, ammonia recycling, and urea cycle were evidenced with changes in several uremic toxins in the blood. The 3-MH is the methylation product of histidine residues on the main myofibrillar proteins actin and myosin. Muscle atrophy or cachexia is common in dogs with CHF.[59,60] Myofibrillar protein overdegradation was reported in human patients with clinically stable CHF, resulting in a higher circulating level of 3-MH when compared with healthy patients.[61] The level of 1-methylhistidine, which derives from dietary anserine (β-alanyl-3-methyl-L-histidine), was inversely associated with left ventricular diastolic function in humans.[62,63] Finally, tryptophan serves as a substrate for the generation of several important compounds, such as conversion to serotonin and degradation through kynurenine pathway leading to the de novo NAD^+ biosynthesis.[64] In summary, our data revealed amino acid metabolic reprogramming and renal insufficiency in the pathogenesis of canine MMVD. Changes in numerous circulating metabolites raised the opportunity for new therapeutic targets and biomarkers that could be used for diagnosis, prognosis, or interventional studies.

Our study underscored the challenge of matching age and body weight between healthy dogs and dogs with MMVD. MMVD is prevalent in small-breed geriatric dogs, and dogs with CHF generally experience body weight loss attributable to cachexia.[60] Several attempts were made to reduce age and body weight differences without success. The bootstrap resampling experiment supported the hypothesis that the observed difference was independent of age or body weight. Despite this effort, we cannot completely rule out the possibility of small confounding effects from age or body weight. Our analysis also showed no difference between dogs in stable cardiac medications and those that were not. This study is one of our initial efforts towards the understanding of canine MMVD progression and pathogenesis. A targeted metabolomics study on a different cohort of dogs can be used to confirm the findings. A multilayered approach that integrates metabolomics and other "omics" data will allow us to delve further into the mechanisms[8] Last, many pet dogs are often given treats and human food in addition to their base dog food diets, making it difficult to accurately assess diet effects. In addition, we were unable to rule out potential confounding effects from breed or fasting state in this study. A study with research colony dogs may address these limitations. However, the challenge would be to enroll a large number of research dogs with naturally occurring MMVD of different stages.

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Supplementary Material

Supplementary Methods
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Data S1-S2

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SUPPLEMENTAL MATERIAL

Supplemental Methods

Metabolon Platform

Sample Accessioning: Following receipt, samples were inventoried and immediately stored at -80°C. Each sample received was accessioned into the Metabolon LIMS system and was assigned by the LIMS a unique identifier that was associated with the original source identifier only. This identifier was used to track all sample handling, tasks, results, etc. The samples (and all derived aliquots) were tracked by the LIMS system. All portions of any sample were automatically assigned their own unique identifiers by the LIMS when a new task was created; the relationship of these samples was also tracked. All samples were maintained at -80°C until processed.

Sample Preparation: Samples were prepared using the automated MicroLab STAR® system from Hamilton Company. Several recovery standards were added prior to the first step in the extraction process for QC purposes. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation. The resulting extract was divided into five fractions: two for analysis by two separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC-MS/MS with negative ion mode ESI, one for analysis by HILIC/UPLC-MS/MS with negative ion mode ESI, and one sample was reserved for backup. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis.

QA/QC: Several types of controls were analyzed in concert with the experimental samples: a pooled matrix sample generated by taking a small volume of each experimental sample (or alternatively, use of a pool of well-characterized human plasma) served as a technical replicate throughout the data set; extracted water samples served as process blanks; and a cocktail of QC standards that were carefully chosen not to interfere with the measurement of endogenous compounds were spiked into every analyzed sample, allowed instrument performance monitoring and aided chromatographic alignment. Tables 1 and 2 describe these QC samples and standards. Instrument variability was determined by calculating the median relative standard deviation (RSD) for the standards that were added to each sample prior to injection into the mass spectrometers. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrument standards) present in 100% of the pooled matrix samples. Experimental samples were randomized across the platform run with QC samples spaced evenly among the injections, as outlined in Figure 1.

Table 1: Description of Metabolon QC Samples

Type	Description	Purpose
MTRX	Large pool of human plasma maintained by Metabolon that has been characterized extensively.	Assure that all aspects of the Metabolon process are operating within specifications.
CMTRX	Pool created by taking a small aliquot from every customer sample.	Assess the effect of a non-plasma matrix on the Metabolon process and distinguish biological variability from process variability.
PRCS	Aliquot of ultra-pure water	Process Blank used to assess the contribution to compound signals from the process.
SOLV	Aliquot of solvents used in extraction.	Solvent Blank used to segregate contamination sources in the extraction.

Table 2: Metabolon QC Standards

Type	Description	Purpose
RS	Recovery Standard	Assess variability and verify performance of extraction and instrumentation.
IS	Internal Standard	Assess variability and performance of instrument.

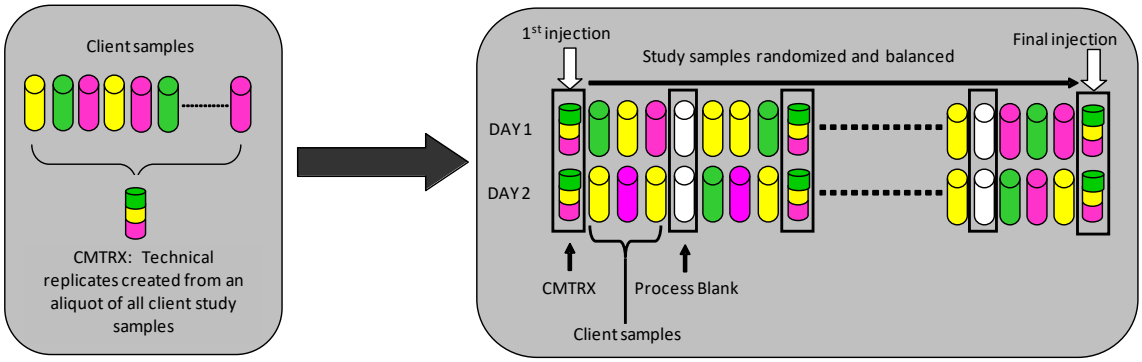


Figure 1. Preparation of client-specific technical replicates. A small aliquot of each client sample (colored cylinders) is pooled to create a CMTRX technical replicate sample (multi-colored cylinder), which is then injected periodically throughout the platform run. Variability among consistently detected biochemicals can be used to calculate an estimate of overall process and platform variability.

Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS): All methods utilized a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. The sample extract was dried then reconstituted in solvents compatible to each of the four methods. Each reconstitution solvent contained a series of standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion conditions, chromatographically optimized for more hydrophilic compounds. In this method, the extract was gradient eluted from a C18 column (Waters UPLC BEH C18-2.1x100 mm, 1.7 µm) using water and methanol, containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% formic acid (FA). Another aliquot was also analyzed using acidic positive ion conditions, however it was chromatographically optimized for more hydrophobic compounds. In this method, the extract was gradient eluted from the same afore mentioned C18 column using methanol, acetonitrile, water, 0.05% PFPA and 0.01% FA and was operated at an overall higher organic content. Another aliquot was analyzed using basic negative ion optimized conditions using a separate dedicated C18 column. The basic extracts were gradient eluted from the column using methanol and water, however with 6.5mM Ammonium Bicarbonate at pH 8. The fourth aliquot was analyzed via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1x150 mm, 1.7 µm) using a gradient consisting of water and acetonitrile with 10mM Ammonium Formate, pH 10.8. The MS analysis alternated between MS and data-dependent MS^n scans using dynamic exclusion. The scan range varied slighted between methods but covered 70-1000 m/z. Raw data files are archived and extracted as described below.

Bioinformatics: The informatics system consisted of four major components, the Laboratory Information Management System (LIMS), the data extraction and peak-identification software, data processing tools for QC and compound identification, and a collection of information interpretation and visualization tools for use by data analysts. The hardware and software foundations for these informatics components were the LAN backbone, and a database server running Oracle 10.2.0.1 Enterprise Edition.

LIMS: The purpose of the Metabolon LIMS system was to enable fully auditable laboratory automation through a secure, easy to use, and highly specialized system. The scope of the Metabolon LIMS system encompasses sample accessioning, sample preparation and instrumental analysis and reporting and advanced data analysis. All of the subsequent software systems are grounded in the LIMS data structures. It has been modified to leverage and interface with the in-house information extraction and data visualization systems, as well as third party instrumentation and data analysis software.

Data Extraction and Compound Identification: Raw data was extracted, peak-identified and QC processed using Metabolon's hardware and software. These systems are built on a web-service platform utilizing Microsoft's .NET technologies, which run on high-performance application servers and fiber-channel storage arrays in clusters to provide active failover and load-balancing. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Metabolon maintains a library based on authenticated standards that contains the retention time/index (RI), mass to charge ratio (*m/z)*, and chromatographic data (including MS/MS spectral data) on all molecules present in the library. Furthermore, biochemical identifications are based on three criteria: retention index within a narrow RI window of the proposed identification, accurate mass match to the library +/- 10 ppm, and the

MS/MS forward and reverse scores between the experimental data and authentic standards. The MS/MS scores are based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum. While there may be similarities between these molecules based on one of these factors, the use of all three data points can be utilized to distinguish and differentiate biochemicals. More than 3300 commercially available purified standard compounds have been acquired and registered into LIMS for analysis on all platforms for determination of their analytical characteristics. Additional mass spectral entries have been created for structurally unnamed biochemicals, which have been identified by virtue of their recurrent nature (both chromatographic and mass spectral). These compounds have the potential to be identified by future acquisition of a matching purified standard or by classical structural analysis.

Curation: A variety of curation procedures were carried out to ensure that a high quality data set was made available for statistical analysis and data interpretation. The QC and curation processes were designed to ensure accurate and consistent identification of true chemical entities, and to remove those representing system artifacts, mis-assignments, and background noise. Metabolon data analysts use proprietary visualization and interpretation software to confirm the consistency of peak identification among the various samples. Library matches for each compound were checked for each sample and corrected if necessary.

Metabolite Quantification and Data Normalization: Peaks were quantified using area-under-the-curve. For studies spanning multiple days, a data normalization step was performed to correct variation resulting from instrument inter-day tuning differences. Essentially, each compound was corrected in run-day blocks by registering the medians to equal one (1.00) and normalizing each data point proportionately (termed the "block correction"; Figure 2). For studies that did not require more than one day of analysis, no normalization is necessary, other than for purposes of data visualization. In certain instances, biochemical data may have been normalized to an additional factor (e.g., cell counts, total protein as determined by Bradford assay, osmolality, etc.) to account for differences in metabolite levels due to differences in the amount of material present in each sample.

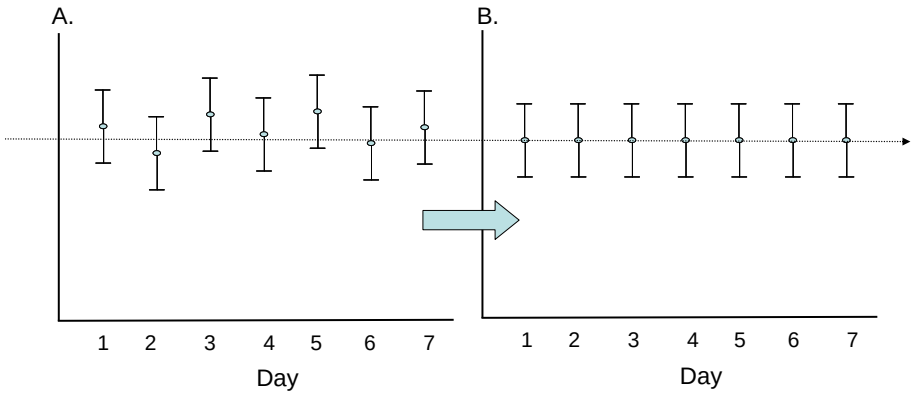


Figure 2: Visualization of data normalization steps for a multiday platform run.

Table S1. Metabolites that were significantly different among the four ACVIM groups.

	Linear model		Adjusted P values from pairwise comparisons						Mean, log2 transformed				Fold changes						Pathways				
BIOCHEMICAL	P value	FDR	A v B1	A v B2	A v C/D	B1 v B2	B1 v C/D	B2 v C/D	A	B1	B2	C/D	B1/A	B2/A	CD/A	CD/B1	CD/B2	B2/B1	SUPER_PATHW	SUB_PATHWAY	PUBCHEM	CAS	KEGG	HMDB
hydroxyasparagine	0	0	0.9158	0.5446	0	0.5446	0	0	-0.356	-0.33	-0.132	1.132	1.018	1.168	2.804	2.756	2.401	1.148	Amino Acid	Alanine and Aspartate Metabolism	97663		C03124	HMDB32332
N-acetylalanine	0	0.0001	0.0816	0.1206	0	0.8626	0.001	0.001	-0.523	-0.039	-0.087	0.973	1.398	1.353	2.819	2.017	2.084	0.968	Amino Acid	Alanine and Aspartate Metabolism	88064	97-69-8	C02847	HMDB00766
N-acetylasparagine	0	0.0001	0.0031	0.0015	0	0.6188	0.0891	0.2146	-0.696	0.126	0.264	0.664	1.768	1.946	2.567	1.452	1.32	1.101	Amino Acid	Alanine and Aspartate Metabolism	99715	4033-40-3		HMDB06028
N-carbamoylalanine	0.0052	0.024	0.0992	0.3867	0.0236	0.0236	0.4185	0.01	-0.222	0.28	-0.507	0.527	1.417	0.821	1.681	1.187	2.048	0.579	Amino Acid	Alanine and Aspartate Metabolism	426409	77340-50-2		
N,N-dimethylalanine	0.0082	0.0349	0.1143	0.7388	0.1099	0.2355	0.0051	0.1099	0.033	-0.457	-0.064	0.606	0.712	0.935	1.487	2.088	1.59	1.313	Amino Acid	Alanine and Aspartate Metabolism	5488191	2812-31-9		
creatine	0	0	0.0151	0	0	0.0073	0.0027	0.6561	-0.738	-0.145	0.601	0.723	1.509	2.53	2.754	1.825	1.089	1.677	Amino Acid	Creatine Metabolism	586	57-00-1	C00300	HMDB00064
alpha-ketoglutaramate*	0.0001	0.0009	0.1807	0.585	0.0001	0.4491	0.0047	0.0007	-0.402	4E-04	-0.253	0.905	1.321	1.109	2.473	1.872	2.231	0.839	Amino Acid	Glutamate Metabolism	48	18465-19-5		
carboxyethyl-GABA	0.0009	0.0055	0.3716	0.5805	0.008	0.7059	0.0013	0.0037	-0.048	-0.357	-0.246	0.799	0.807	0.872	1.799	2.229	2.064	1.08	Amino Acid	Glutamate Metabolism	2572	3/2/4386		HMDB02201
glutamine	0.0032	0.0157	0.2137	0.1762	0.0024	0.7746	0.0659	0.1068	0.411	0.044	-0.042	-0.666	0.775	0.73	0.474	0.611	0.649	0.942	Amino Acid	Glutamate Metabolism	5961	56-85-9	C00064	HMDB00641
N-acetylglutamate	0.0102	0.0418	0.3284	0.2489	0.0059	0.7329	0.0718	0.1285	-0.369	-0.068	0.035	0.638	1.232	1.324	2.01	1.632	1.519	1.074	Amino Acid	Glutamate Metabolism	70914	8/3/5817	C00624	HMDB01138
N-acetylglutamine	0	0.0006	0.0484	0.0383	0	0.6898	0.0205	0.0383	-0.551	-0.015	0.099	0.788	1.45	1.569	2.529	1.745	1.612	1.082	Amino Acid	Glutamate Metabolism	182230	2490-97-3	C02716	HMDB06029
2-hydroxybutyrate/2-hydroxyisobut	0	0	0.0004	0	0	0.4142	0.1358	0.4594	-0.787	0.155	0.408	0.619	1.921	2.289	2.65	1.379	1.157	1.192	Amino Acid	Glutathione Metabolism				
glycine	0.0005	0.0035	0.1499	0.0038	0.0081	0.0976	0.1499	0.7184	0.504	0.09	-0.5	-0.387	0.751	0.499	0.54	0.719	1.082	0.664	Amino Acid	Glycine, Serine and Threonine Metabolism	750	56-40-6	C00037	HMDB00123
N-acetylserine	0.0004	0.003	0.1216	0.0716	0.0002	0.6703	0.0215	0.0565	-0.486	-0.052	0.072	0.762	1.351	1.473	2.375	1.757	1.613	1.09	Amino Acid	Glycine, Serine and Threonine Metabolism	65249	97-14-3		HMDB02931
N-acetylthreonine	0	0	0.0037	0.0019	0	0.6182	0.0103	0.0385	-0.711	0.069	0.203	0.825	1.717	1.884	2.9	1.689	1.539	1.097	Amino Acid	Glycine, Serine and Threonine Metabolism	152204	17093-74-2		HMDB62557
sarcosine	0.011	0.0437	0.0208	0.9025	0.5837	0.0387	0.0144	0.5837	-0.164	0.591	-0.128	-0.369	1.688	1.025	0.867	0.514	0.846	0.607	Amino Acid	Glycine, Serine and Threonine Metabolism	1088	107-97-1	C00213	HMDB00271
4-guanidinobutanoate	0.0124	0.0478	0.0743	0.0203	0.0203	0.5246	0.5246	0.9753	-0.495	0.084	0.334	0.324	1.494	1.777	1.764	1.181	0.993	1.189	Amino Acid	Guanidino and Acetamido Metabolism	500	463-003;463-(	C01035	HMDB03464
1-methyl-5-imidazoleacetate	0.0003	0.0022	0.6647	0.9478	0.0005	0.6647	0.0024	0.001	-0.291	-0.101	-0.273	0.882	1.141	1.013	2.255	1.978	2.227	0.888	Amino Acid	Histidine Metabolism	6451814	4200-48-0		HMDB04988
1-methylhistidine	0	0	0.3465	0.2562	0	0.7211	0	0	-0.483	-0.244	-0.155	1.246	1.18	1.255	3.313	2.807	2.64	1.063	Amino Acid	Histidine Metabolism	92105	332-80-9	C01152	HMDB00001
3-methylhistidine	0	0.0002	0.2057	0.2495	0	0.8879	0.0008	0.0008	-0.459	-0.083	-0.122	0.966	1.298	1.264	2.686	2.069	2.126	0.973	Amino Acid	Histidine Metabolism	64969	368-16-1	C01152	HMDB00479
carnosine	0.0007	0.0045	0.6641	0.2386	0.0007	0.4037	0.0022	0.0332	-0.349	-0.234	0.048	0.807	1.083	1.317	2.229	2.059	1.693	1.216	Amino Acid	Histidine Metabolism	439224	305-84-0	C00386	HMDB00033
formiminoglutamate	0	0.0001	0.0259	0.0149	0	0.6106	0.0103	0.0259	-0.602	-0.014	0.128	0.837	1.503	1.658	2.711	1.804	1.635	1.103	Amino Acid	Histidine Metabolism	439233	816-90-0	C00439	HMDB00854
imidazole lactate	0.0002	0.0018	0.0077	0.0077	0.0002	0.8901	0.21	0.2397	-0.625	0.154	0.194	0.589	1.716	1.764	2.32	1.352	1.315	1.028	Amino Acid	Histidine Metabolism	793	14403-45-3	C05568	HMDB02320
N-acetyl-1-methylhistidine*	0	0	0.0342	0.0727	0	0.7835	0	0	-0.588	-0.053	-0.123	1.134	1.449	1.38	3.298	2.276	2.39	0.952	Amino Acid	Histidine Metabolism	53859791			
N-acetyl-3-methylhistidine*	0	0.0004	0.0892	0.0892	0	0.9362	0.0088	0.011	-0.502	-0.033	-0.011	0.852	1.384	1.406	2.556	1.847	1.818	1.016	Amino Acid	Histidine Metabolism	193270	37841-04-6		
2-hydroxy-3-methylvalerate	0.0003	0.0025	0.0042	0.0063	0.004	0.9372	0.7875	0.7875	-0.616	0.259	0.236	0.392	1.834	1.805	2.011	1.096	1.114	0.984	Amino Acid	Leucine, Isoleucine and Valine Metabolism	164623	488-15-3		HMDB00317
3-hydroxy-2-ethylpropionate	0	0	0.021	0.0028	0	0.3129	0.0117	0.1009	-0.646	-0.019	0.261	0.775	1.544	1.875	2.677	1.734	1.428	1.214	Amino Acid	Leucine, Isoleucine and Valine Metabolism	188979	4374-62-3		HMDB00396
3-hydroxyisobutyrate	0.002	0.0107	0.0784	0.0784	0.0014	0.8728	0.1042	0.1347	-0.503	0.058	0.106	0.612	1.476	1.525	2.166	1.468	1.42	1.034	Amino Acid	Leucine, Isoleucine and Valine Metabolism	87	2068-83-9	C06001	HMDB00336
3-methyl-2-oxobutyrate	0.0004	0.0029	0.051	0.0061	0.0024	0.3864	0.2012	0.6124	-0.564	0.04	0.333	0.492	1.52	1.862	2.079	1.368	1.117	1.225	Amino Acid	Leucine, Isoleucine and Valine Metabolism	49	3715-29-5	C00141	HMDB00019
3-methylglutaconate	0	0.0005	0.0223	0.2197	0	0.2916	0.0239	0.0037	-0.527	0.134	-0.165	0.838	1.581	1.285	2.575	1.629	2.004	0.813	Amino Acid	Leucine, Isoleucine and Valine Metabolism	1551553	5746-90-7		HMDB00522
ethylmalonate	0	0	0.1001	0.1041	0	0.9844	0.0005	0.0006	-0.527	-0.073	-0.079	1.015	1.37	1.365	2.912	2.126	2.134	0.996	Amino Acid	Leucine, Isoleucine and Valine Metabolism	11756	601-75-2		HMDB00622
isobutyrylcarnitine (C4)	0	0	0.0194	0.0057	0	0.5295	0.0008	0.0057	-0.643	-0.055	0.112	0.973	1.503	1.687	3.065	2.039	1.816	1.123	Amino Acid	Leucine, Isoleucine and Valine Metabolism	168379	25518-49-4		HMDB00736
isovalerylcarnitine (C5)	0.0002	0.0017	0.0417	0.1712	0.0001	0.545	0.0417	0.0191	-0.497	0.087	-0.089	0.772	1.499	1.328	2.41	1.608	1.815	0.886	Amino Acid	Leucine, Isoleucine and Valine Metabolism	6426851	31023-24-2		HMDB00688
N-acetylisoleucine	0	0	0.0022	0.0022	0	0.875	0.0395	0.057	-0.706	0.134	0.177	0.76	1.79	1.844	2.763	1.544	1.499	1.03	Amino Acid	Leucine, Isoleucine and Valine Metabolism	306109	3077-46-1		HMDB61684
N-acetylleucine	0.0002	0.0017	0.0235	0.066	0.0001	0.557	0.066	0.0362	-0.559	0.149	-0.021	0.717	1.634	1.453	2.422	1.483	1.667	0.889	Amino Acid	Leucine, Isoleucine and Valine Metabolism	70912	1188-21-2	C02710	HMDB11756
N-acetylvaline	0	0	0.0084	0.003	0	0.5689	0.0026	0.0084	-0.682	-0.002	0.149	0.928	1.603	1.78	3.054	1.906	1.716	1.111	Amino Acid	Leucine, Isoleucine and Valine Metabolism	66789	96-81-1		HMDB11757
tiglyl carnitine (C5)	0.0001	0.0012	0.171	0.0967	0	0.6493	0.0048	0.0173	-0.478	-0.097	0.033	0.85	1.303	1.426	2.511	1.928	1.761	1.094	Amino Acid	Leucine, Isoleucine and Valine Metabolism	22833596	64191-86-2		HMDB02366
2-aminoadipate	0.0001	0.0007	0.0049	0.001	0.001	0.5175	0.5175	0.9756	-0.649	0.165	0.392	0.402	1.757	2.057	2.071	1.179	1.007	1.171	Amino Acid	Lysine Metabolism	469	542-32-5;111(	C00956	HMDB00510
5-(galactosylhydroxy)-L-lysine	0	0.0001	0.248	0.248	0	0.939	0.0009	0.0013	-0.44	-0.119	-0.098	0.957	1.249	1.268	2.634	2.108	2.077	1.015	Amino Acid	Lysine Metabolism	123986	32448-36-5		
6-oxopiperidine-2-carboxylate	0.0003	0.0025	0.0097	0.1683	0.0003	0.1683	0.1683	0.0166	-0.534	0.255	-0.151	0.678	1.729	1.304	2.318	1.341	1.777	0.754	Amino Acid	Lysine Metabolism	3014237	34622-39-4		HMDB61705
N,N,N-trimethyl-5-aminovalerate	0.0005	0.0033	0.3693	0.0095	0.0008	0.079	0.0093	0.3483	-0.464	-0.228	0.342	0.669	1.178	1.748	2.194	1.862	1.255	1.484	Amino Acid	Lysine Metabolism	14274897			
N2-acetyl,N6-methyllysine	0.0011	0.0066	0.1059	0.6158	0.0024	0.2929	0.1059	0.0132	-0.376	0.116	-0.233	0.693	1.406	1.104	2.098	1.493	1.9	0.786	Amino Acid	Lysine Metabolism				
N6-acetyllysine	0.0015	0.0083	0.0081	0.0671	0.0026	0.4684	0.4684	0.2402	-0.56	0.265	0.05	0.495	1.771	1.527	2.077	1.173	1.361	0.862	Amino Acid	Lysine Metabolism	92832	692-04-6	C02727	HMDB00206
N6-carboxyethyllysine	0.0076	0.0333	0.2212	0.5862	0.0046	0.5123	0.0866	0.0246	-0.344	0.052	-0.188	0.678	1.316	1.115	2.032	1.543	1.822	0.847	Amino Acid	Lysine Metabolism	22283604	3/2/5746		
N6,N6-dimethyllysine	0.0015	0.0085	0.1343	0.4349	0.0009	0.4349	0.0477	0.013	-0.4	0.057	-0.174	0.746	1.373	1.17	2.213	1.612	1.892	0.852	Amino Acid	Lysine Metabolism	193344	2259-86-1	C05545	HMDB13287
N6,N6,N6-trimethyllysine	0.0011	0.0067	0.0651	0.0759	0.0006	0.933	0.0651	0.0651	-0.496	0.059	0.034	0.676	1.469	1.444	2.254	1.534	1.561	0.983	Amino Acid	Lysine Metabolism	440120	23284-33-5	C03793	HMDB01325
pipecolate	0.0021	0.0113	0.0073	0.007	0.3414	0.696	0.125	0.0887	-0.487	0.351	0.467	-0.176	1.788	1.938	1.24	0.694	0.64	1.084	Amino Acid	Lysine Metabolism	849	4043-87-2	C00408	HMDB00070
2,3-dihydroxy-5-methylthio-4-pente	0.0001	0.0009	0.3584	0.6352	0.0001	0.6415	0.0019	0.0011	-0.379	-0.075	-0.207	0.918	1.235	1.127	2.458	1.99	2.182	0.912	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism				
alpha-ketobutyrate	0.0016	0.0091	0.0661	0.004	0.0064	0.2339	0.3226	0.7781	-0.551	0.028	0.45	0.362	1.493	2.001	1.882	1.26	0.94	1.34	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	58	600-18-0	C00109	HMDB00005
methionine	0.003	0.0153	0.2908	0.1146	0.0453	0.0201	0.0094	0.6098	0.163	0.481	-0.35	-0.512	1.246	0.701	0.626	0.502	0.893	0.562	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	6137	63-68-3	C00073	HMDB00696
S-carboxyethylcysteine	0.0002	0.0019	0.0225	0.4849	0.0002	0.1179	0.0838	0.0029	-0.467	0.204	-0.275	0.768	1.592	1.143	2.354	1.478	2.06	0.718	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism		4033-46-9		
S-methylmethionine	0.0071	0.0314	0.638	0.0214	0.0741	0.0497	0.166	0.5392	0.347	0.218	-0.523	-0.278	0.914	0.547	0.648	0.709	1.185	0.599	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	458	12/7/3493	C05319	HMDB38670
N-acetylphenylalanine	0.0001	0.0006	0.0328	0.1943	0	0.4487	0.0199	0.0053	-0.513	0.085	-0.131	0.843	1.513	1.303	2.56	1.692	1.964	0.861	Amino Acid	Phenylalanine Metabolism	74839	2018-61-3	C03519	HMDB00512
5-methylthioadenosine (MTA)	0.0005	0.0035	0.1924	0.2657	0.0004	0.835	0.0198	0.0198	-0.422	-0.015	-0.077	0.771	1.326	1.271	2.287	1.725	1.8	0.958	Amino Acid	Polyamine Metabolism	439176	2457-80-9	C00170	HMDB01173
N-acetyl-isoputreanine	0.0008	0.0051	0.9244	0.7019	0.0012	0.7019	0.0013	0.0076	-0.271	-0.246	-0.086	0.841	1.018	1.137	2.161	2.124	1.901	1.117	Amino Acid	Polyamine Metabolism	131802901			
N-acetyltryptophan	0.0124	0.0478	0.2399	0.6895	0.0121	0.4463	0.1517	0.0369	-0.318	0.071	-0.201	0.625	1.309	1.084	1.922	1.468	1.773	0.828	Amino Acid	Tryptophan Metabolism	700653	1218-34-4	C03137	HMDB13713
2-oxoarginine*	0	0.0004	0.0062	0.0002	0.0002	0.2183	0.2183	0.9901	-0.685	0.088	0.475	0.472	1.709	2.236	2.23	1.305	0.997	1.308	Amino Acid	Urea cycle; Arginine and Proline Metabolism	558	10/4/3715	C03771	HMDB04225
argininate*	0.0088	0.0364	0.0505	0.0116	0.0475	0.4738	0.7376	0.6386	-0.507	0.114	0.418	0.216	1.538	1.898	1.651	1.074	0.87	1.235	Amino Acid	Urea cycle; Arginine and Proline Metabolism	160437	157-07-3		HMDB03148
dimethylarginine (ADMA + SDMA)	0.0007	0.0044	0.3902	0.1279	0.0125	0.3902	0.0026	0.0005	0.003	-0.224	-0.482	0.797	0.854	0.715	1.735	2.03	2.428	0.836	Amino Acid	Urea cycle; Arginine and Proline Metabolism	123831	102783-24-4	C03626	HMDB01539
homocitrulline	0	0.0001	0.1729	0.2746	0	0.0291	0.0031	0	-0.29	0.072	-0.576	0.977	1.285	0.82	2.407	1.873	2.934	0.638	Amino Acid	Urea cycle; Arginine and Proline Metabolism	65072	1190-49-4	C02427	HMDB00679
N-acetylcitrulline	0.0059	0.0267	0.0094	0.0807	0.0524	0.506	0.671	0.7295	-0.507	0.385	0.095	0.206	1.856	1.517	1.639	0.883	1.08	0.817	Amino Acid	Urea cycle; Arginine and Proline Metabolism	656979	33965-42-3	C15532	HMDB00856
N-acetylhomocitrulline	0	0	0.0015	0.4155	0	0.0295	0.0569	0.0002	-0.556	0.301	-0.343	0.856	1.811	1.159	2.659	1.468	2.296	0.64	Amino Acid	Urea cycle; Arginine and Proline Metabolism				
N-acetylproline	0.0027	0.0137	0.933	0.0045	0.933	0.0046	0.933	0.0079	0.231	0.208	-0.764	0.172	0.984	0.502	0.96	0.975	1.914	0.51	Amino Acid	Urea cycle; Arginine and Proline Metabolism	322640	1074-79-9		
N-delta-acetylornithine	0.0112	0.0442	0.2055	0.0814	0.0086	0.4276	0.1418	0.3853	-0.443	-0.034	0.207	0.528	1.329	1.57	1.96	1.476	1.249	1.182	Amino Acid	Urea cycle; Arginine and Proline Metabolism	9920500			

N-methylproline	0.0129	0.0488	0.243	0.0326	0.0211	0.243	0.1898	0.6858	-0.437	-0.071	0.318	0.449	1.289	1.688	1.848	1.433	1.095	1.309	Amino Acid	Urea cycle; Arginine and Proline Metabolism	557	475-11-6		
N,N,N-trimethyl-alanylproline betai	0.0006	0.0043	0.6429	0.5268	0.002	0.6429	0.0012	0.001	-0.106	-0.232	-0.367	0.857	0.917	0.835	1.95	2.127	2.335	0.911	Amino Acid	Urea cycle; Arginine and Proline Metabolism				
N2,N5-diacetylornithine	0	0.0004	0.0831	0.0093	0	0.2775	0.0093	0.0831	-0.565	-0.087	0.222	0.775	1.393	1.726	2.532	1.817	1.467	1.239	Amino Acid	Urea cycle; Arginine and Proline Metabolism	65977	39825-23-5		
proline	0.0008	0.0054	0.3172	0.0177	0.0057	0.1414	0.0491	0.5524	0.452	0.151	-0.352	-0.541	0.811	0.573	0.503	0.619	0.878	0.706	Amino Acid	Urea cycle; Arginine and Proline Metabolism	145742	147-85-3	C00148	HMDB00162
urea	0	0	0.0204	0.0479	0	0.7796	0.0005	0.0005	-0.601	0.001	-0.073	1.03	1.518	1.442	3.095	2.04	2.147	0.95	Amino Acid	Urea cycle; Arginine and Proline Metabolism	1176	57-13-6	C00086	HMDB00294
erythronate*	0	0	0.0354	0.0739	0	0.7875	0.0001	0.0001	-0.582	-0.042	-0.112	1.098	1.454	1.385	3.204	2.204	2.313	0.953	Carbohydrate	Aminosugar Metabolism	2781043	88759-55-1		HMDB00613
N-acetylglucosaminylasparagine	0.0002	0.0021	0.043	0.043	0.0003	0.8834	0.0757	0.099	-0.547	0.07	0.113	0.658	1.534	1.58	2.306	1.503	1.459	1.03	Carbohydrate	Aminosugar Metabolism	123826	2776-93-4	C04540	HMDB00489
N-acetylneuraminate	0.0014	0.008	0.1304	0.1304	0.0011	0.9773	0.0709	0.0709	-0.447	0.005	0.014	0.688	1.368	1.376	2.196	1.606	1.596	1.006	Carbohydrate	Aminosugar Metabolism	439197	131-48-6	C00270	HMDB00230
arabonate/xylonate	0	0	0.2125	0.8574	0	0.2125	0	0	-0.381	-0.061	-0.426	1.135	1.249	0.969	2.861	2.291	2.952	0.776	Carbohydrate	Pentose Metabolism				
ribonate	0	0	0.0235	0.2766	0	0.2766	0.0003	0	-0.54	0.04	-0.268	1.09	1.496	1.208	3.095	2.07	2.562	0.808	Carbohydrate	Pentose Metabolism	5460677	8/3/5336	C01685	HMDB00867
ribulonate/xylulonate/lyxonate*	0.0003	0.0023	0.0403	0.0597	0.0001	0.8967	0.0348	0.0348	-0.533	0.053	0.015	0.762	1.501	1.462	2.453	1.635	1.678	0.974	Carbohydrate	Pentose Metabolism				
2-O-methylascorbic acid	0	0.0002	0.0168	0.1788	0	0.3446	0.0167	0.0025	-0.541	0.112	-0.153	0.876	1.572	1.309	2.671	1.699	2.041	0.832	Cofactors and	Ascorbate and Aldarate Metabolism	99779	17860-87-6		
ascorbic acid 3-sulfate*	0.0009	0.0055	0.6895	0.6895	0.0011	0.9931	0.0052	0.0056	-0.302	-0.146	-0.144	0.821	1.114	1.116	2.178	1.955	1.951	1.002	Cofactors and	Ascorbate and Aldarate Metabolism	11425365			
gulonate*	0	0.0001	0.1979	0.0379	0	0.3624	0.0002	0.004	-0.523	-0.18	0.069	0.99	1.269	1.507	2.854	2.25	1.894	1.188	Cofactors and	Ascorbate and Aldarate Metabolism	152304	20246-53-1	C00257	HMDB03290
N1-Methyl-2-pyridone-5-carboxami	0	0.0001	0.6394	0.6808	0	0.5089	0	0.0004	-0.235	-0.391	-0.126	1.013	0.897	1.079	2.376	2.647	2.202	1.202	Cofactors and	Nicotinate and Nicotinamide Metabolism	69698	701-44-0	C05842	HMDB04193
nicotinamide	0.013	0.0488	0.0462	0.892	0.0462	0.0783	0.9475	0.0783	0.355	-0.357	0.26	-0.378	0.61	0.936	0.602	0.986	0.643	1.534	Cofactors and	Nicotinate and Nicotinamide Metabolism	936	98-92-0	C00153	HMDB01406
nicotinamide N-oxide	0.0018	0.0096	0.0055	0.846	0.0334	0.0135	0.5703	0.0636	0.395	-0.524	0.34	-0.309	0.529	0.963	0.614	1.161	0.638	1.819	Cofactors and	Nicotinate and Nicotinamide Metabolism	72661	1986-81-8		HMDB02730
quinolinate	0	0.0002	0.3112	0.3771	0	0.8584	0.0005	0.0005	-0.426	-0.107	-0.157	0.982	1.247	1.205	2.654	2.128	2.203	0.966	Cofactors and	Nicotinate and Nicotinamide Metabolism	1066	89-00-9	C03722	HMDB00232
pterin	0.0049	0.0231	0.1111	0.1111	0.0032	0.9734	0.1473	0.1473	-0.481	0.086	0.076	0.573	1.481	1.471	2.075	1.401	1.411	0.993	Cofactors and	Pterin Metabolism	73000	22363-60-4	C00715	HMDB00802
alpha-CEHC sulfate	0.0015	0.0083	0.0057	0.0147	0.0147	0.829	0.829	0.9818	0.574	-0.347	-0.228	-0.221	0.528	0.573	0.576	1.091	1.005	1.086	Cofactors and	Tocopherol Metabolism				
carotene diol (1)	0.0003	0.0025	0.026	0.0022	0.0049	0.3164	0.4674	0.7206	0.587	-0.085	-0.454	-0.342	0.628	0.486	0.525	0.837	1.081	0.774	Cofactors and	Vitamin A Metabolism				
pyridoxal	0.0109	0.0437	0.0191	0.0372	0.2952	0.7661	0.266	0.2952	0.451	-0.382	-0.291	0.086	0.562	0.598	0.776	1.382	1.298	1.065	Cofactors and	Vitamin B6 Metabolism	1050	65-22-5	C00250	HMDB01545
phosphate	0.0029	0.0151	0.4399	0.1188	0.0022	0.3645	0.0153	0.1188	-0.383	-0.175	0.133	0.694	1.155	1.43	2.111	1.827	1.476	1.238	Energy	Oxidative Phosphorylation	1061	7664-38-2	C00009	HMDB01429
aconitate [cis or trans]	0	0	0.006	0.0018	0	0.4842	0.0018	0.0124	-0.703	6E-04	0.185	0.92	1.629	1.851	3.079	1.891	1.664	1.137	Energy	TCA Cycle				
citrate	0	0.0005	0.0211	0.0211	0.0001	0.8663	0.0494	0.0711	-0.596	0.079	0.128	0.709	1.597	1.651	2.47	1.547	1.496	1.034	Energy	TCA Cycle	311	77-92-9	C00158	HMDB00094
carnitine	0.0004	0.0031	0.9682	0.772	0.0006	0.772	0.0006	0.0032	-0.267	-0.256	-0.122	0.883	1.007	1.106	2.219	2.203	2.007	1.098	Lipid	Carnitine Metabolism	10917	461-05-2	C00318	HMDB00062
adipoylcarnitine (C6-DC)	0.0003	0.0025	0.7965	0.9621	0.0006	0.7965	0.0017	0.0011	-0.274	-0.135	-0.261	0.885	1.101	1.009	2.232	2.027	2.212	0.916	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	71296139			HMDB61677
(R)-3-hydroxybutyrylcarnitine	0.0001	0.0009	0.3628	0.0218	0.0004	0.1489	0.0059	0.1679	-0.456	-0.217	0.265	0.724	1.18	1.649	2.266	1.92	1.374	1.397	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	53481617			HMDB13127
(S)-3-hydroxybutyrylcarnitine	0	0.0001	0.2131	0.1283	0	0.6627	0.001	0.0044	-0.474	-0.131	-0.009	0.932	1.269	1.381	2.651	2.09	1.92	1.088	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)				
3-hydroxyhexanoylcarnitine (1)	0.0023	0.0121	0.02	0.1737	0.0038	0.3524	0.3524	0.1424	-0.502	0.247	-0.049	0.53	1.68	1.369	2.044	1.216	1.493	0.815	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)				
3-hydroxypalmitoylcarnitine	0.0043	0.0206	0.0317	0.0483	0.0039	0.8687	0.3441	0.3441	-0.533	0.171	0.122	0.496	1.63	1.575	2.041	1.252	1.296	0.966	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)				
arachidoylcarnitine (C20)*	0	0.0005	0.0152	0.0035	0	0.454	0.0364	0.1696	-0.648	0.045	0.256	0.699	1.617	1.871	2.544	1.573	1.359	1.157	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)				HMDB06460
lignoceroylcarnitine (C24)*	0.0002	0.0017	0.0181	0.0181	0.0001	0.7856	0.0672	0.1139	-0.605	0.086	0.164	0.677	1.614	1.704	2.433	1.507	1.428	1.056	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)				
margaroylcarnitine (C17)*	0.0062	0.0273	0.0405	0.0104	0.0104	0.55	0.55	0.9483	-0.532	0.109	0.332	0.353	1.559	1.82	1.847	1.184	1.015	1.167	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)		106182-29-0		HMDB06210
palmitoylcarnitine (C16)	0.001	0.0063	0.128	0.0151	0.0009	0.2866	0.0517	0.2954	-0.523	-0.063	0.284	0.612	1.376	1.75	2.196	1.596	1.255	1.272	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Sat	461	6865-14-1	C02990	HMDB00222
stearoylcarnitine (C18)	0.0001	0.001	0.0334	0.0069	0.0001	0.366	0.0334	0.1812	-0.602	-0.004	0.254	0.693	1.514	1.811	2.453	1.621	1.355	1.196	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Sat	6426855	18822-91-8		HMDB00848
eicosenoylcarnitine (C20:1)*	0	0.0005	0.0176	0.0176	0	0.9403	0.0262	0.0329	-0.619	0.081	0.102	0.772	1.624	1.648	2.622	1.614	1.591	1.015	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)				
oleoylcarnitine (C18:1)	0.0001	0.001	0.042	0.0284	0.0002	0.636	0.042	0.1138	-0.567	0.03	0.167	0.685	1.513	1.663	2.382	1.575	1.433	1.1	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Monounsatura	6441392	38677-66-6		HMDB05065
arachidonoylcarnitine (C20:4)	0.0001	0.0006	0.0285	0.024	0	0.7398	0.0226	0.0285	-0.585	0.014	0.108	0.798	1.515	1.616	2.608	1.722	1.614	1.067	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)				
dihomo-linolenoylcarnitine (C20:3n	0.0035	0.017	0.2875	0.252	0.0018	0.7945	0.0354	0.0593	-0.391	-0.072	0.006	0.708	1.247	1.317	2.142	1.717	1.627	1.055	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)				
dihomo-linoleoylcarnitine (C20:2)*	0.0031	0.0156	0.283	0.1077	0.0019	0.4294	0.0381	0.1429	-0.424	-0.103	0.133	0.667	1.249	1.471	2.131	1.706	1.448	1.178	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)				
linolenoylcarnitine (C18:3)*	0.0117	0.0458	0.0431	0.0656	0.0198	0.8564	0.5991	0.5991	-0.495	0.19	0.135	0.398	1.607	1.547	1.857	1.156	1.201	0.963	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)				
acetylcarnitine (C2)	0	0	0.2218	0.0399	0	0.343	0.0002	0.0055	-0.514	-0.184	0.076	0.973	1.257	1.505	2.802	2.23	1.862	1.198	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Short Chain)	1	5080-50-2	C02571	HMDB00201
butyrylcarnitine (C4)	0.0002	0.0021	0.2735	0.2735	0.0002	0.8522	0.0078	0.0131	-0.413	-0.095	-0.04	0.822	1.247	1.295	2.354	1.887	1.818	1.038	Lipid	Fatty Acid Metabolism (also BCAA Metabolism)	439829	25576-40-3	C02862	HMDB02013
methylmalonate (MMA)	0.0022	0.0113	0.8078	0.2056	0.0034	0.2724	0.0055	0.1132	-0.319	-0.253	0.108	0.719	1.047	1.344	2.054	1.963	1.528	1.285	Lipid	Fatty Acid Metabolism (also BCAA Metabolism)	487	516-05-2	C02170	HMDB00202
propionylcarnitine (C3)	0	0	0.016	0.0013	0	0.3049	0.0013	0.0176	-0.679	-0.055	0.218	0.919	1.542	1.862	3.028	1.964	1.626	1.208	Lipid	Fatty Acid Metabolism (also BCAA Metabolism)	107738	17298-37-2	C03017	HMDB00824
malonate	0.0004	0.003	0.0871	0.1334	0.0004	0.8455	0.0483	0.0483	-0.472	0.037	-0.02	0.722	1.424	1.368	2.288	1.607	1.673	0.961	Lipid	Fatty Acid Synthesis	867	141-82-2;265	C00383	HMDB00691
2-aminoheptanoate	0.013	0.0488	0.2141	0.0388	0.0204	0.3865	0.2141	0.6345	0.453	0.017	-0.285	-0.439	0.739	0.6	0.539	0.729	0.899	0.811	Lipid	Fatty Acid, Amino	227939	1115-90-8		
azelate (C9-DC)	0.0105	0.0424	0.3457	0.0327	0.0265	0.1953	0.1338	0.737	0.413	0.121	-0.342	-0.45	0.817	0.593	0.55	0.673	0.928	0.726	Lipid	Fatty Acid, Dicarboxylate	2266	123-99-9	C08261	HMDB00784
maleate	0	0.0003	0.1357	0.0425	0	0.492	0.0021	0.0158	-0.519	-0.114	0.079	0.889	1.324	1.513	2.654	2.005	1.754	1.143	Lipid	Fatty Acid, Dicarboxylate	444266	110-16-7	C01384	HMDB00176
2R,3R-dihydroxybutyrate	0	0.0001	0.0469	0.0644	0	0.9158	0.0008	0.0008	-0.565	-0.032	-0.06	1.002	1.447	1.418	2.962	2.047	2.088	0.98	Lipid	Fatty Acid, Dihydroxy	13120901	759-06-8		HMDB00498
2S,3R-dihydroxybutyrate	0.0002	0.002	0.0527	0.0527	0.0001	0.89	0.0288	0.0372	-0.525	0.006	0.045	0.779	1.445	1.485	2.469	1.709	1.663	1.028	Lipid	Fatty Acid, Dihydroxy	10964471	5057-93-2		HMDB02453
3,4-dihydroxybutyrate	0	0.0004	0.0026	0.3436	0.0001	0.0505	0.2114	0.0026	-0.535	0.329	-0.279	0.719	1.821	1.195	2.386	1.311	1.997	0.656	Lipid	Fatty Acid, Dihydroxy		51267-44-8		
2-hydroxynervonate*	0.0082	0.0349	0.3324	0.0949	0.0113	0.3324	0.0968	0.3324	-0.414	-0.083	0.219	0.533	1.258	1.552	1.929	1.533	1.243	1.233	Lipid	Fatty Acid, Monohydroxy	5312783			
3-hydroxystearate	0.0041	0.0196	0.1712	0.0153	0.0153	0.2874	0.2748	0.8669	-0.481	-0.007	0.349	0.403	1.389	1.778	1.846	1.329	1.038	1.281	Lipid	Fatty Acid, Monohydroxy	5282907	45261-96-9		
chiro-inositol	0.0004	0.0031	0.4298	0.2154	0.0002	0.5643	0.003	0.016	-0.402	-0.161	0.006	0.841	1.182	1.327	2.368	2.004	1.785	1.123	Lipid	Inositol Metabolism		643-12-9	C19891	HMDB34220
3-hydroxybutyrate (BHBA)	0.0001	0.0009	0.0614	0.0016	0.0009	0.1747	0.1009	0.6975	-0.59	-0.017	0.407	0.527	1.488	1.996	2.169	1.458	1.087	1.342	Lipid	Ketone Bodies	441	625-72-9	C01089	HMDB00357
acetoacetate	0	0.0006	0.0061	0.0003	0.0003	0.2616	0.2655	0.9122	-0.681	0.096	0.481	0.448	1.713	2.238	2.188	1.277	0.977	1.306	Lipid	Ketone Bodies	96	11/2/3483	C00164	HMDB00060
docosatrienoate (22:3n3)	0.004	0.0195	0.1596	0.0638	0.0044	0.4414	0.1189	0.3303	-0.467	-0.025	0.206	0.557	1.359	1.595	2.034	1.497	1.275	1.174	Lipid	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	5312556	59708-86-0	C16534	HMDB02823
hexadecatrienoate (16:3n3)	0.0099	0.0408	0.7634	0.5277	0.0082	0.6416	0.0139	0.062	0.277	0.194	0.006	-0.697	0.945	0.829	0.509	0.539	0.614	0.878	Lipid	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	5312428	7561-64-0		
1-palmitoyl-2-arachidonoyl-GPC (16	0.0004	0.0029	0.1453	0.0069	0.0004	0.1658	0.0244	0.3008	-0.541	-0.104	0.328	0.647	1.354	1.827	2.279	1.683	1.248	1.349	Lipid	Phosphatidylcholine (PC)	10747814	35418-58-7		HMDB07982
1-stearoyl-2-arachidonoyl-GPC (18:(	0.0012	0.0069	0.0146	0.0146	0.0018	0.8354	0.3611	0.4246	-0.58	0.15	0.212	0.503	1.659	1.731	2.119	1.277	1.224	1.043	Lipid	Phosphatidylcholine (PC)	16219824	35418-59-8		HMDB08048
1-palmitoyl-2-oleoyl-GPI (16:0/18:1	0.0008	0.0049	0.0221	0.0168	0.0006	0.7086	0.16	0.2779	-0.585	0.099	0.208	0.581	1.607	1.733	2.245	1.397	1.295	1.079	Lipid	Phosphatidylinositol (PI)				HMDB09783
1-stearoyl-2-arachidonoyl-GPI (18:0	0	0.0001	0.0001	0	0.0001	0.6339	0.7781	0.7527	-0.772	0.273	0.493	0.351	2.064	2.403	2.179	1.056	0.907	1.164	Lipid	Phosphatidylinositol (PI)		383907-33-3		HMDB09815
1-stearoyl-2-oleoyl-GPI (18:0/18:1)*	0.0004	0.0031	0.0033	0.0129	0.0007	0.7039	0.4584	0.3518	-0.628	0.255	0.145	0.513	1.845	1.709	2.206	1.196	1.291	0.927	Lipid	Phosphatidylinositol (PI)				
trimethylamine N-oxide	0	0.0003	0.1084	0.0165	0	0.2992	0.0039	0.0462	-0.549	-0.115	0.178	0.833	1.351	1.655	2.607	1.93	1.575	1.225	Lipid	Phospholipid Metabolism	1145	1184-78-7	C01104	HMDB00925
1-(1-enyl-palmitoyl)-2-arachidonoyl	0.002	0.0109	0.2298	0.0173	0.0035	0.1962	0.0532	0.4721	-0.473	-0.121	0.331	0.558	1.277	1.746	2.044	1.601	1.171	1.368	Lipid	Plasmalogen				HMDB11352

1-(1-enyl-palmitoyl)-2-linoleoyl-GP(	0.0078	0.0339	0.0235	0.0235	0.0601	0.9168	0.8727	0.8727	0.501	-0.259	-0.291	-0.152	0.59	0.578	0.636	1.078	1.102	0.978	Lipid	Plasmalogen				HMDB11211
1-(1-enyl-palmitoyl)-2-palmitoyl-GP	0.0005	0.0035	0.008	0.0145	0.0043	0.8707	0.6454	0.6454	0.587	-0.238	-0.19	-0.423	0.564	0.583	0.496	0.88	0.851	1.034	Lipid	Plasmalogen	11146967			HMDB11206
1-(1-enyl-stearoyl)-2-arachidonoyl-(	0.0004	0.0029	0.6243	0.007	0.0016	0.0256	0.0055	0.5187	-0.429	-0.301	0.402	0.645	1.093	1.78	2.106	1.926	1.183	1.628	Lipid	Plasmalogen	9547058			HMDB05779
1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-	0.0051	0.0238	0.1158	0.0235	0.0056	0.4621	0.1755	0.4907	-0.505	0.014	0.274	0.494	1.433	1.717	2	1.395	1.165	1.198	Lipid	Plasmalogen		144371-68-6		HMDB11375
sphingomyelin (d18:1/18:1, d18:2/1	0.0051	0.0238	0.0031	0.1125	0.1232	0.2619	0.2494	0.8672	0.498	-0.48	-0.108	-0.055	0.508	0.657	0.682	1.343	1.038	1.294	Lipid	Sphingomyelins	6443882	108392-10-5		HMDB12101
sphingomyelin (d18:1/20:1, d18:2/2	0	0.0001	0.002	0.002	0.0002	0.7692	0.3749	0.4909	0.678	-0.195	-0.279	-0.529	0.546	0.515	0.433	0.794	0.841	0.944	Lipid	Sphingomyelins		222403-67-0		
sphingomyelin (d18:1/20:2, d18:2/2	0.0079	0.0339	0.011	0.011	0.2486	0.8256	0.293	0.2819	0.493	-0.32	-0.386	0.039	0.569	0.544	0.73	1.282	1.342	0.955	Lipid	Sphingomyelins				
sphingomyelin (d18:1/22:2, d18:2/2	0.0015	0.0085	0.0322	0.009	0.009	0.5751	0.5127	0.807	0.552	-0.109	-0.32	-0.397	0.633	0.547	0.518	0.819	0.948	0.864	Lipid	Sphingomyelins				
sphingomyelin (d18:2/24:2)*	0.0017	0.0094	0.0352	0.0103	0.0103	0.5573	0.5573	0.9734	0.54	-0.115	-0.349	-0.338	0.635	0.54	0.544	0.857	1.007	0.85	Lipid	Sphingomyelins				
stearoyl sphingomyelin (d18:1/18:0	0.0036	0.0177	0.003	0.0903	0.0903	0.2883	0.2883	0.917	0.511	-0.469	-0.115	-0.082	0.507	0.648	0.663	1.308	1.023	1.278	Lipid	Sphingomyelins	6453725	85187-10-6;8	C00550	HMDB01348
1-methylhypoxanthine	0.0001	0.0009	0.1015	0.1015	0.0118	0.9323	0.0002	0.0002	0.062	-0.391	-0.415	0.847	0.731	0.719	1.724	2.358	2.398	0.983	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containin	70765	1125-39-9		HMDB13141
allantoin	0	0	0.0404	0.1099	0	0.6889	0	0	-0.572	-0.053	-0.155	1.141	1.434	1.335	3.278	2.287	2.455	0.931	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containin	204	97-59-6	C02350	HMDB00462
inosine	0.0126	0.0482	0.131	0.3152	0.0846	0.0296	0.6619	0.0286	-0.177	0.296	-0.504	0.431	1.388	0.797	1.524	1.098	1.912	0.574	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containin	6021	58-63-9	C00294	HMDB00195
N1-methylinosine	0.0019	0.0103	0.0878	0.0878	0.0923	0.9897	0.0035	0.0035	0.153	-0.385	-0.389	0.667	0.689	0.687	1.429	2.074	2.079	0.997	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containin	65095	20245-33-4		HMDB02721
urate	0.008	0.0341	0.0194	0.2205	0.0158	0.3171	0.6254	0.2205	-0.474	0.287	-0.052	0.437	1.695	1.34	1.88	1.11	1.403	0.791	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containin	1175	69-93-2;120K	C00366	HMDB00289
7-methylguanine	0.0005	0.0035	0.0535	0.1622	0.0535	0.6204	0.001	0.0042	0.116	-0.451	-0.306	0.724	0.675	0.746	1.524	2.258	2.042	1.106	Nucleotide	Purine Metabolism, Guanine containing	11361	578-76-7	C02242	HMDB00897
N2,N2-dimethylguanosine	0	0	0.1082	0.1014	0.0004	0.8234	0	0	0.006	-0.412	-0.472	1.024	0.749	0.718	2.025	2.705	2.821	0.959	Nucleotide	Purine Metabolism, Guanine containing	92919	2140-67-2		HMDB04824
5-hydroxymethylcytidine	0.0104	0.0419	0.5052	0.0545	0.0545	0.0209	0.0209	0.9665	-0.201	-0.421	0.413	0.427	0.858	1.531	1.545	1.8	1.009	1.783	Nucleotide	Pyrimidine Metabolism, Cytidine containing		19235-17-7		
5-methyl-2'-deoxycytidine	0.0046	0.0219	0.2715	0.0429	0.0098	0.2715	0.1053	0.4663	0.441	0.086	-0.281	-0.515	0.782	0.607	0.516	0.659	0.85	0.775	Nucleotide	Pyrimidine Metabolism, Cytidine containing	440055	838-07-3	C03592	HMDB02224
N4-acetylcytidine	0.0001	0.0013	0.5441	0.9035	0.0008	0.5752	0.0002	0.0011	-0.145	-0.381	-0.179	0.914	0.849	0.977	2.084	2.454	2.132	1.151	Nucleotide	Pyrimidine Metabolism, Cytidine containing	107461	3768-18-1		HMDB05923
3-ureidopropionate	0.0007	0.0045	0.76	0.239	0.0006	0.3385	0.0014	0.0321	-0.34	-0.259	0.056	0.817	1.057	1.316	2.23	2.109	1.695	1.244	Nucleotide	Pyrimidine Metabolism, Uracil containing	111	462-88-4	C02642	HMDB00026
5,6-dihydrouridine	0	0.0001	0.9153	0.7941	0	0.7941	0	0.0002	-0.313	-0.286	-0.166	1.042	1.019	1.107	2.558	2.512	2.31	1.087	Nucleotide	Pyrimidine Metabolism, Uracil containing	94312	5/4/5627		
pseudouridine	0	0	0.9467	0.9467	0	0.9467	0	0	-0.31	-0.294	-0.232	1.118	1.011	1.055	2.689	2.659	2.549	1.043	Nucleotide	Pyrimidine Metabolism, Uracil containing	15047	1445-07-4	C02067	HMDB00767
bilirubin degradation product, C17H	0.0061	0.0271	0.0085	0.1986	0.0149	0.252	0.8652	0.3069	0.507	-0.387	0.029	-0.335	0.538	0.718	0.558	1.037	0.777	1.334	Partially Chara	Partially Characterized Molecules				
gamma-glutamyl-alpha-lysine	0.0129	0.0488	0.2492	0.1855	0.0175	0.6878	0.1855	0.2599	-0.395	-0.009	0.114	0.519	1.307	1.423	1.885	1.442	1.324	1.089	Peptide	Gamma-glutamyl Amino Acid	65254			
gamma-glutamylglutamate	0.0011	0.0065	0.0495	0.0088	0.0016	0.4632	0.1628	0.4672	-0.564	0.042	0.298	0.526	1.522	1.817	2.129	1.398	1.171	1.194	Peptide	Gamma-glutamyl Amino Acid	92865	1116-22-9	C05282	HMDB11737
gamma-glutamylisoleucine*	0.0011	0.0066	0.1629	0.0838	0.0024	0.5384	0.0838	0.1629	-0.451	-0.044	0.14	0.625	1.325	1.506	2.107	1.59	1.399	1.136	Peptide	Gamma-glutamyl Amino Acid	14253342			HMDB11170
gamma-glutamylphenylalanine	0.0053	0.0241	0.5712	0.6723	0.008	0.8137	0.037	0.037	-0.298	-0.057	-0.128	0.682	1.182	1.125	1.971	1.669	1.753	0.952	Peptide	Gamma-glutamyl Amino Acid	111299	7432-24-8		HMDB00594
gamma-glutamyltyrosine	0.0103	0.0419	0.69	0.0537	0.4532	0.1087	0.3467	0.0224	0.127	0.017	-0.58	0.391	0.926	0.613	1.201	1.296	1.96	0.661	Peptide	Gamma-glutamyl Amino Acid	94340	7432-23-7		HMDB11741
N,N-dimethyl-pro-pro	0.0001	0.0008	0.9006	0.9006	0.0001	0.9006	0.0003	0.0003	-0.279	-0.2	-0.245	0.961	1.056	1.024	2.362	2.237	2.307	0.97	Peptide	Modified Peptides				
2-methoxyhydroquinone sulfate (1)	0.0048	0.0225	0.0696	0.0051	0.0807	0.2741	0.9521	0.2741	0.501	-0.126	-0.495	-0.108	0.647	0.501	0.656	1.013	1.308	0.774	Xenobiotics	Benzoate Metabolism				
4-vinylcatechol sulfate	0.0008	0.005	0.0489	0.0055	0.0055	0.3775	0.3775	0.9059	0.55	-0.065	-0.365	-0.402	0.653	0.531	0.517	0.791	0.974	0.812	Xenobiotics	Benzoate Metabolism				
4-vinylphenol sulfate	0.0003	0.0024	0.214	0.0044	0.0018	0.0855	0.0353	0.619	0.508	0.151	-0.411	-0.566	0.781	0.529	0.475	0.609	0.898	0.677	Xenobiotics	Benzoate Metabolism	6426766	2628-17-3	C05627	HMDB62775
hippurate	0.0021	0.0112	0.9024	0.9024	0.0032	0.9024	0.0032	0.0092	-0.207	-0.24	-0.137	0.785	0.977	1.05	1.989	2.035	1.894	1.075	Xenobiotics	Benzoate Metabolism	464	495-69-2	C01586	HMDB00714
S-(3-hydroxypropyl)mercapturic aci	0	0	0.0808	0.0603	0	0.7491	0.0001	0.0003	-0.558	-0.118	-0.034	1.075	1.357	1.438	3.102	2.286	2.157	1.06	Xenobiotics	Chemical	3371179	23127-40-4		
sulfate*	0	0	0.017	0.0053	0	0.5407	0	0.0005	-0.661	-0.088	0.067	1.092	1.488	1.657	3.372	2.266	2.035	1.114	Xenobiotics	Chemical	1118	14808-79-8	C00059	HMDB01448
triethanolamine	0.0123	0.0478	0.1	0.0109	0.0634	0.3007	0.6291	0.5465	-0.482	0.061	0.451	0.21	1.457	1.909	1.615	1.109	0.846	1.31	Xenobiotics	Chemical	7618	102-71-6	C06771	HMDB32538
2-aminophenol sulfate	0.003	0.0153	0.0863	0.0016	0.1489	0.1489	0.625	0.0943	0.48	-0.117	-0.606	0.031	0.661	0.471	0.732	1.108	1.555	0.713	Xenobiotics	Food Component/Plant	181670			HMDB61116
ferulic acid 4-sulfate	0.0052	0.024	0.2756	0.0093	0.0692	0.1156	0.368	0.411	0.436	0.071	-0.51	-0.245	0.776	0.519	0.624	0.804	1.202	0.669	Xenobiotics	Food Component/Plant	6305574	86321-29-1		HMDB29200
gluconate	0	0.0006	0.025	0.346	0	0.1977	0.0344	0.0021	-0.493	0.16	-0.236	0.826	1.572	1.195	2.495	1.587	2.089	0.76	Xenobiotics	Food Component/Plant	10690	527-07-1	C00257	HMDB00625
homostachydrine*	0.0056	0.0251	0.166	0.0026	0.0847	0.0847	0.5743	0.1984	-0.462	-0.027	0.589	0.144	1.352	2.072	1.523	1.126	0.735	1.533	Xenobiotics	Food Component/Plant	441447	1195-94-4	C08283	HMDB33433
mannonate*	0	0.0001	0.2345	0.2273	0	0.837	0.0003	0.0007	-0.463	-0.139	-0.083	1.003	1.252	1.302	2.761	2.206	2.122	1.04	Xenobiotics	Food Component/Plant	3246006	642-99-9		
X - 11381	0.0001	0.0009	0.0774	0.0008	0.0002	0.0774	0.0272	0.5713	-0.591	-0.112	0.443	0.614	1.394	2.047	2.305	1.653	1.126	1.469						
X - 12097	0.0011	0.0066	0.4356	0.4356	0.0031	0.1833	0.0239	0.0011	-0.198	0.01	-0.448	0.776	1.155	0.841	1.964	1.701	2.336	0.728						
X - 12100	0.0076	0.0331	0.1789	0.1789	0.0072	0.9922	0.1626	0.1626	-0.401	0.018	0.021	0.591	1.338	1.34	1.989	1.487	1.484	1.002						
X - 12101	0	0	0.0904	0.0639	0	0.7311	0.0002	0.0007	-0.548	-0.114	-0.022	1.042	1.351	1.439	3.009	2.228	2.091	1.066						
X - 12117	0.0012	0.0067	0.0368	0.0584	0.0014	0.85	0.1953	0.1953	-0.538	0.146	0.09	0.569	1.607	1.545	2.154	1.341	1.394	0.962						
X - 12127	0.0001	0.0008	0.5841	0.2304	0.0001	0.4561	0.0007	0.0113	-0.377	-0.235	0.018	0.883	1.103	1.314	2.393	2.169	1.821	1.191						
X - 12680	0.0002	0.002	0.0101	0.4344	0.0002	0.101	0.1355	0.0047	-0.487	0.259	-0.272	0.725	1.677	1.161	2.317	1.381	1.995	0.692						
X - 12689	0.0003	0.0023	0.0023	0.0089	0.0011	0.7233	0.6142	0.5077	-0.636	0.276	0.174	0.47	1.882	1.753	2.152	1.144	1.228	0.931						
X - 13684	0.0123	0.0478	0.1239	0.5606	0.0107	0.3036	0.2412	0.0515	-0.364	0.153	-0.195	0.588	1.431	1.124	1.934	1.352	1.72	0.786						
X - 13695	0.0038	0.0183	0.0196	0.0164	0.0152	0.777	0.777	0.777	0.546	-0.169	-0.272	-0.362	0.609	0.567	0.533	0.875	0.939	0.931						
X - 15461	0	0.0005	0.1038	0.0456	0	0.5896	0.0042	0.0194	-0.524	-0.082	0.07	0.864	1.358	1.509	2.615	1.926	1.733	1.111						
X - 16580	0.0084	0.0353	0.6211	0.4246	0.0075	0.6211	0.0269	0.0793	-0.309	-0.146	0.004	0.676	1.119	1.242	1.978	1.768	1.593	1.109						
X - 18345	0.0011	0.0067	0.0299	0.2727	0.0006	0.2727	0.1397	0.0283	-0.479	0.178	-0.145	0.684	1.578	1.261	2.239	1.419	1.776	0.799						
X - 22162	0	0	0.589	0.589	0	0.8635	0	0	-0.399	-0.235	-0.189	1.138	1.121	1.157	2.902	2.589	2.509	1.032						
X - 23536	0.0009	0.0057	0.6397	0.7285	0.0011	0.7285	0.006	0.0045	-0.304	-0.092	-0.206	0.82	1.158	1.07	2.18	1.882	2.037	0.924						
X - 23593	0.0085	0.0357	0.6381	0.6381	0.0071	0.9433	0.028	0.028	-0.292	-0.089	-0.111	0.696	1.151	1.134	1.984	1.723	1.749	0.985						
X - 23659	0	0.0005	0.9814	0.9814	0.0001	0.9814	0.0002	0.0002	-0.276	-0.228	-0.235	0.983	1.034	1.029	2.393	2.315	2.325	0.995						
X - 23665	0.003	0.0151	0.0478	0.0046	0.0463	0.3112	0.7459	0.4554	-0.523	0.098	0.478	0.197	1.538	2.001	1.647	1.071	0.823	1.301						
X - 23739	0	0.0003	0.0538	0.2489	0	0.4786	0.0033	0.0009	-0.505	0.028	-0.168	0.943	1.447	1.263	2.728	1.885	2.161	0.873						
X - 24408	0.0134	0.0498	0.0631	0.0931	0.022	0.8484	0.5271	0.5271	-0.475	0.172	0.113	0.412	1.565	1.503	1.849	1.181	1.23	0.96						
X - 24623	0	0	0.0002	0	0	0.2945	0.5033	0.6543	-0.801	0.204	0.551	0.424	2.006	2.552	2.336	1.165	0.915	1.272						
X - 24728	0	0	0.0392	0.04	0	0.9794	0.0006	0.0008	-0.585	-0.04	-0.033	1.017	1.459	1.466	3.035	2.08	2.07	1.005						
X - 25247	0.0003	0.0022	0.0659	0.0006	0.0082	0.0782	0.3639	0.3905	0.571	0.002	-0.571	-0.304	0.674	0.453	0.545	0.809	1.203	0.672						
X - 25419	0.0089	0.0369	0.016	0.016	0.0854	0.7266	0.6823	0.5751	-0.516	0.263	0.369	0.088	1.716	1.846	1.52	0.886	0.823	1.076						
X - 25422	0	0	0.4007	0.4684	0	0.8553	0	0	-0.453	-0.201	-0.246	1.24	1.191	1.154	3.233	2.715	2.802	0.969						

X - 25794	0.0112	0.0442	0.0447	0.0447	0.0225	0.8551	0.6471	0.6739	-0.494	0.144	0.199	0.387	1.556	1.617	1.842	1.184	1.139	1.039						
X - 25948	0	0	0.061	0.009	0	0.365	0	0.0012	-0.611	-0.148	0.088	1.069	1.378	1.623	3.203	2.324	1.974	1.177						
X - 26035	0	0	0.3293	0.0569	0	0.3124	0	0.0006	-0.496	-0.263	0.034	1.093	1.175	1.444	3.008	2.56	2.083	1.229						

Table S2. P value distributions from a bootstrap subsampling study

	Resampling size				Variation Explained		P values					
Iteration	A	B1	B2	C/D	PC1 (%)	PC2 (%)	PC1	PC2	Age	BCS	Body weight	Sex
1	10	10	10	10	15.2	8.6	0.001183	0.070508	0.06754	0.744694	0.0542404	0.392559
2	10	10	10	10	15.1	8.2	3.15E-06	0.146352	0.084529	0.098693	0.05361359	0.410112
3	10	10	10	10	12.4	9.3	0.00028	0.531539	0.223515	0.573529	0.07686274	0.969057
4	10	10	10	10	13.7	8.8	0.007622	0.21972	0.090911	0.505312	0.13676947	1
5	10	10	10	10	15.7	9	0.000325	0.24474	0.128654	0.566646	0.26767972	0.969057
6	10	10	10	10	14.5	9.1	0.003234	0.720436	0.208693	0.595038	0.10319423	0.642298
7	10	10	10	10	15.6	8.8	6.39E-05	0.033048	0.154502	0.142478	0.34166331	1
8	10	10	10	10	13.5	9.7	1.43E-05	0.681202	0.087986	0.033059	0.06187466	0.726949
9	10	10	10	10	16	8.3	6.89E-05	0.915567	0.086226	0.370308	0.47209743	0.600571
10	10	10	10	10	11.9	9.4	4.07E-05	0.884057	0.145653	0.141674	0.16059284	0.684285
11	10	10	10	10	14.5	9.6	0.006612	0.315633	0.113612	0.477426	0.08467992	1
12	10	10	10	10	14	9.5	0.000205	0.133699	0.093926	0.810124	0.10714826	1
13	10	10	10	10	12	9.4	4.06E-05	0.037046	0.184981	0.008503	0.05588512	0.783186
14	10	10	10	10	15.1	8.4	0.000394	0.061978	0.055667	0.047621	0.39344308	1
15	10	10	10	10	12.6	8.6	0.000257	0.096358	0.113514	0.227209	0.15256719	0.897606
16	10	10	10	10	12.4	8.4	0.001286	0.025318	0.101169	0.229236	0.07250139	0.726949
17	10	10	10	10	14.6	9	0.000156	0.203072	0.090936	0.128633	0.113736	1
18	10	10	10	10	15.5	9.4	0.001402	0.155934	0.10813	0.096057	0.12303757	1
19	10	10	10	10	14.5	9.4	0.000689	0.49528	0.181322	0.470677	0.38799428	0.969057
20	10	10	10	10	14.7	9.1	0.000772	0.240639	0.229444	0.907359	0.05166925	1
21	10	10	10	10	12.5	8.2	1.77E-05	0.419127	0.23555	0.282169	0.28843598	0.684285
22	10	10	10	10	13.2	7.9	1.38E-05	0.614165	0.075208	0.981894	0.0938096	0.684285
23	10	10	10	10	15.9	8.8	0.00156	0.523986	0.149735	0.255732	0.06590854	1
24	10	10	10	10	15.9	8.6	0.002623	0.141546	0.209909	0.661177	0.08030861	0.851798
25	10	10	10	10	15.1	8.6	0.000265	0.877152	0.05727	0.4875	0.0525548	0.970745
26	10	10	10	10	13	10.6	0.001401	0.582323	0.075549	0.011838	0.37898745	0.851798
27	10	10	10	10	16.1	9.9	0.000344	0.620809	0.221061	0.340364	0.14046016	1
28	10	10	10	10	14.1	9.6	0.000177	0.087658	0.111755	0.08738	0.26388862	0.627147
29	10	10	10	10	15.1	7.9	3.95E-05	0.074181	0.060841	0.063928	0.21598928	0.969057
30	10	10	10	10	14.6	9.3	0.000116	0.233741	0.054195	0.252148	0.22918415	0.715437
31	10	10	10	10	15.3	8.8	0.000138	0.029782	0.064164	0.442496	0.47058824	0.865456

32	10	10	10	10	13.5	9.7	0.000242	0.095229	0.182337	0.183773	0.30995848	0.897606
33	10	10	10	10	13.9	8.8	0.000154	0.425447	0.145248	0.221992	0.09694064	0.31176
34	10	10	10	10	13.4	11	0.000156	0.254732	0.267763	0.22301	0.31390304	0.894795
35	10	10	10	10	15.3	7.8	4.2E-05	0.879759	0.062503	0.377616	0.20689479	0.688927
36	10	10	10	10	16.5	7.7	0.001003	0.450305	0.057179	0.361604	0.7555242	0.341394
37	10	10	10	10	15.1	9.1	0.000409	0.465285	0.065419	0.330447	0.74337872	0.641891
38	10	10	10	10	12.7	9.7	0.000126	0.085036	0.112987	0.683971	0.38250436	1
39	10	10	10	10	12.6	9.4	0.001613	0.14771	0.139354	0.137491	0.08632695	0.715437
40	10	10	10	10	13.5	9.4	0.002035	0.191266	0.077283	0.340364	0.07671969	0.889491
41	10	10	10	10	16	8.8	0.000328	0.167299	0.330831	0.37295	0.08435731	1
42	10	10	10	10	15.2	9.4	0.000193	0.642325	0.109656	0.809324	0.22263356	0.528081
43	10	10	10	10	15.1	8.8	0.000254	0.434908	0.053729	0.173974	0.48959048	0.678373
44	10	10	10	10	15.6	8.4	0.000154	0.327034	0.063962	0.334678	0.44338568	1
45	10	10	10	10	13.2	9.8	0.000406	0.539191	0.213572	0.240978	0.16933386	0.715437
46	10	10	10	10	15.2	8.4	0.000254	0.805446	0.104361	0.924353	0.19599014	0.894795
47	10	10	10	10	13.6	8.7	0.000418	0.021968	0.284257	0.386108	0.05787573	1
48	10	10	10	10	15.5	8.3	0.000305	0.86906	0.11038	0.52511	0.22808267	0.402566
49	10	10	10	10	14.6	9.4	0.000394	0.256649	0.175034	0.395091	0.07936651	0.410112
50	10	10	10	10	13.8	9.5	0.000261	0.038818	0.413948	0.259724	0.1325843	0.894795
51	10	10	10	10	14.4	9.3	0.000654	0.281038	0.257104	0.511467	0.42120478	0.684285
52	10	10	10	10	14.7	9	0.00017	0.605826	0.095164	0.063903	0.1220913	0.894795
53	10	10	10	10	14.3	9.6	0.000442	0.560091	0.1234	0.962178	0.58359931	0.662157
54	10	10	10	10	13.8	9.8	0.000849	0.136716	0.206222	0.221538	0.11333071	0.889491
55	10	10	10	10	14.5	9.6	7.21E-06	0.287108	0.057829	0.008774	0.32083612	0.897606
56	10	10	10	10	14.1	9.1	1.74E-07	0.428548	0.070605	0.091221	0.1405942	0.642298
57	10	10	10	10	15.8	9.9	3.07E-05	0.45433	0.190637	0.663969	0.35966529	1
58	10	10	10	10	15.8	8.9	0.002521	0.197514	0.074013	0.056241	0.82599288	0.969057
59	10	10	10	10	14	9.5	3.06E-05	0.487298	0.099617	0.104569	0.09521663	1
60	10	10	10	10	15.5	8.4	0.000363	0.457847	0.104035	0.37862	0.24428358	1
61	10	10	10	10	15	8.8	0.000475	0.334217	0.080959	0.528141	0.36510877	0.783186
62	10	10	10	10	15.4	8.7	0.000564	0.316927	0.062043	0.492867	0.05887165	0.678373
63	10	10	10	10	17.3	7.6	9.46E-05	0.001811	0.348	0.648411	0.58771005	1
64	10	10	10	10	14.4	9.6	1.14E-05	0.073108	0.36307	0.819518	0.301322	0.538008
65	10	10	10	10	14.5	10.1	0.000117	0.078575	0.159807	0.917639	0.07796497	0.538008

66	10	10	10	10	14.5	9.4	1.71E-07	0.293448	0.081376	0.017478	0.06093505	0.897606
67	10	10	10	10	14.1	8.9	0.002456	0.902474	0.123927	0.640149	0.15885904	0.969057
68	10	10	10	10	16.3	8.4	0.000224	0.153466	0.168878	0.095337	0.07261736	0.433148
69	10	10	10	10	14.1	8.2	0.000105	0.546562	0.593803	0.12315	0.20313042	0.970745
70	10	10	10	10	15.7	9.6	0.000254	0.581101	0.073972	0.358373	0.08900392	0.688927
71	10	10	10	10	15.5	9.4	2.14E-05	0.353216	0.081706	0.980579	0.05896502	0.715437
72	10	10	10	10	14	10	8.92E-05	0.113399	0.142311	0.475564	0.215377	0.889491
73	10	10	10	10	14	10.5	0.003044	0.775273	0.620981	0.535467	0.08665017	1
74	10	10	10	10	12.9	9	0.006832	0.094136	0.25126	0.20492	0.4452356	1
75	10	10	10	10	15.2	8.8	0.00158	0.958492	0.310557	0.975152	0.08521172	0.688927
76	10	10	10	10	17.2	9.4	0.000473	0.33756	0.070077	0.408795	0.12750215	1
77	10	10	10	10	14	9.5	0.020279	0.326104	0.139802	0.09557	0.61004381	1
78	10	10	10	10	14.4	9.2	0.00048	0.422001	0.052036	0.738764	0.15729539	0.684285
79	10	10	10	10	14.2	8	0.00048	0.508843	0.327702	0.236181	0.05579793	1
80	10	10	10	10	14.4	9.1	0.000237	0.464298	0.118501	0.47211	0.172807	0.688927
81	10	10	10	10	15.3	8.7	0.000119	0.314442	0.05842	0.188548	0.07249767	0.897606
82	10	10	10	10	14.1	9.1	4.37E-06	0.981317	0.073567	0.00651	0.30216675	1
83	10	10	10	10	14.6	9.3	0.00041	0.174179	0.41236	0.251083	0.43565649	0.894795
84	10	10	10	10	15.6	9.1	5.35E-05	0.439883	0.053241	0.838255	0.0749002	0.894795
85	10	10	10	10	16.9	9.2	2.19E-06	0.087995	0.092855	0.08585	0.06939476	0.476884
86	10	10	10	10	15.2	8.7	0.00064	0.296322	0.118572	0.637462	0.46922479	1
87	10	10	10	10	13.8	9.8	0.00558	0.088212	0.165974	0.118585	0.37273213	0.897606
88	10	10	10	10	14.3	8.6	0.000148	0.319417	0.076358	0.562747	0.06396015	1
89	10	10	10	10	14.8	9.2	4.06E-05	0.244381	0.066454	0.545455	0.10862026	0.263255
90	10	10	10	10	15.3	9.7	1.64E-05	0.473905	0.06168	0.270666	0.07483052	0.970745
91	10	10	10	10	15.3	8.5	2.35E-05	0.507405	0.071624	0.089346	0.14105494	0.479046
92	10	10	10	10	13.2	9.1	3.81E-05	0.220973	0.121585	0.913344	0.18565285	0.969057
93	10	10	10	10	13.2	9.5	0.000153	0.471931	0.233728	0.574434	0.5997494	1
94	10	10	10	10	15.2	9.1	7.25E-05	0.143725	0.118875	0.408491	0.0852853	0.715437
95	10	10	10	10	14	9.6	0.000372	0.22024	0.213461	0.076022	0.05756272	1
96	10	10	10	10	14.3	9.2	4.86E-05	0.068715	0.065379	0.142319	0.36611249	0.969057
97	10	10	10	10	14.6	9.6	0.001489	0.405458	0.284746	0.237545	0.28653984	0.969057
98	10	10	10	10	16	7.8	0.002198	0.688818	0.08227	0.306553	0.05294746	0.969057
99	10	10	10	10	13.6	8.6	8.16E-05	0.987409	0.455925	0.139163	0.14350678	0.969057

100	10	10	10	10	15.1	9.5	0.003581	0.320797	0.4829	0.223621	0.06390499	0.865456
101	10	10	10	10	13.6	9.6	2.69E-05	0.664547	0.097649	0.774976	0.25580205	0.889491
102	10	10	10	10	15.9	8.5	0.000243	0.191024	0.083957	0.02569	0.22614173	0.31176
103	10	10	10	10	14.5	8.6	0.018878	0.443702	0.301467	0.448153	0.07691323	0.970745
104	10	10	10	10	13.4	8.2	1.06E-05	0.419548	0.089897	0.213152	0.07341288	0.969057
105	10	10	10	10	13.2	10.1	0.000289	0.065963	0.097564	0.630291	0.09526896	0.512969
106	10	10	10	10	16.2	8.5	6.53E-05	0.161891	0.085991	0.039021	0.16733769	0.392559
107	10	10	10	10	17.8	8.1	5.86E-05	0.070801	0.105814	0.676201	0.12424418	0.257296
108	10	10	10	10	15.1	9.2	0.000107	0.415658	0.235545	0.621019	0.15173325	0.688927
109	10	10	10	10	13.1	9.1	0.000162	0.310398	0.217101	0.713144	0.23555273	0.715437
110	10	10	10	10	15.9	9	0.004717	0.217724	0.120508	0.42088	0.19938548	0.969057
111	10	10	10	10	14.8	8.8	0.010558	0.579524	0.964871	0.44433	0.06656957	0.969057
112	10	10	10	10	14.9	9.2	0.00268	0.515505	0.141353	0.097721	0.31738884	0.715437
113	10	10	10	10	14.5	8.7	0.000312	0.496242	0.071241	0.077339	0.20759038	0.268815
114	10	10	10	10	16.8	8	7.3E-05	0.398824	0.059538	0.355868	0.16679195	1
115	10	10	10	10	15	9.1	5.82E-06	0.495541	0.054133	0.706159	0.101234	0.402566
116	10	10	10	10	13.9	9.1	0.001948	0.442849	0.12918	0.097516	0.12708777	0.969057
117	10	10	10	10	15.9	8.1	2.93E-05	0.646848	0.123174	0.338554	0.27514907	0.969057
118	10	10	10	10	14	9.1	0.001575	0.744077	0.261605	0.402183	0.47970205	0.662157
119	10	10	10	10	14.8	9.7	0.000137	0.305081	0.071689	0.0816	0.13170836	1
120	10	10	10	10	15.1	8.9	0.000892	0.520254	0.055641	0.722092	0.50134984	0.257296
121	10	10	10	10	12.1	9.4	7.7E-05	0.719244	0.132065	0.091963	0.13498011	0.970745
122	10	10	10	10	14.4	9.7	0.000733	0.485155	0.20321	0.225503	0.15632289	0.969057
123	10	10	10	10	15.9	9.5	4.33E-05	0.518932	0.050702	0.075641	0.1709285	0.433148
124	10	10	10	10	15.5	9.2	0.002367	0.109291	0.420117	0.468951	0.31566016	0.714444
125	10	10	10	10	14.8	7.6	0.001559	0.439432	0.109375	0.25135	0.13003736	0.835815
126	10	10	10	10	14.9	8.9	0.000464	0.404778	0.498675	0.975082	0.51133069	0.851798
127	10	10	10	10	14.6	10	0.002789	0.31662	0.235992	0.693804	0.11910832	0.894795
128	10	10	10	10	16.4	9.2	0.001918	0.444899	0.15178	0.45796	0.33128582	0.894795
129	10	10	10	10	13.3	8.6	7.19E-05	0.622149	0.229204	0.120184	0.34144364	1
130	10	10	10	10	14	9.4	0.000132	0.120847	0.197498	0.041169	0.39857238	0.33233
131	10	10	10	10	13.6	10	2.46E-06	0.27344	0.084509	0.065968	0.10362677	1
132	10	10	10	10	14.8	8.5	0.001209	0.164173	0.066371	0.452263	0.09354636	0.894795
133	10	10	10	10	13.6	9.5	0.006461	0.390771	0.254966	0.75516	0.05080845	1

134	10	10	10	10	15	9.2	4.31E-05	0.167471	0.345398	0.370308	0.09081927	1
135	10	10	10	10	15.7	8.9	0.000685	0.516563	0.35946	0.34924	0.10834362	0.865456
136	10	10	10	10	15.3	8.7	0.003133	0.200024	0.0989	0.331615	0.09966473	0.715437
137	10	10	10	10	14.9	9.3	0.000427	0.197227	0.259996	0.779627	0.05413502	0.851798
138	10	10	10	10	14.6	8.5	0.002906	0.132122	0.088052	0.219674	0.38322332	0.783186
139	10	10	10	10	14.5	9.6	6.08E-05	0.354171	0.209144	0.053576	0.26338875	0.760758
140	10	10	10	10	16.8	9.4	7.35E-05	0.845769	0.078527	0.020836	0.09577757	1
141	10	10	10	10	13.8	8.7	1.32E-05	0.143165	0.124442	0.573579	0.50322351	1
142	10	10	10	10	14.6	9.8	0.000178	0.648473	0.156947	0.135034	0.46998433	0.851798
143	10	10	10	10	13.7	10	0.000405	0.630027	0.18562	0.390143	0.32291028	0.889491
144	10	10	10	10	13.9	9.2	1.37E-06	0.975025	0.085953	0.110633	0.11039127	0.969057
145	10	10	10	10	15.1	9	0.000117	0.221549	0.198389	0.043178	0.2562647	0.962884
146	10	10	10	10	14.7	7.9	9.4E-05	0.250029	0.18403	0.020797	0.53453113	0.894795
147	10	10	10	10	15.5	8.7	4.21E-05	0.119231	0.077658	0.699787	0.30758495	0.894795
148	10	10	10	10	15.7	9.5	0.005257	0.374197	0.179036	0.335657	0.12949277	0.715437
149	10	10	10	10	15	9.6	0.001478	0.42683	0.497232	0.146525	0.06685971	0.783186
150	10	10	10	10	12.9	9.5	4.13E-05	0.571919	0.116864	0.457238	0.16797602	0.528081
151	10	10	10	10	14.9	9.2	1.79E-05	0.460601	0.058254	0.470759	0.17656756	0.970745
152	10	10	10	10	13.7	9	1.57E-06	0.456605	0.078993	0.299433	0.0619239	0.969057
153	10	10	10	10	12.9	9.5	6.14E-05	0.49592	0.293273	0.399742	0.12967042	0.684285
154	10	10	10	10	13.5	10.4	0.000916	0.435152	0.061308	0.47211	0.18151395	1
155	10	10	10	10	14	9.3	0.000371	0.713523	0.075484	0.610703	0.25787576	0.715437
156	10	10	10	10	14.6	7.9	3.15E-05	0.172813	0.124056	0.235502	0.36213941	0.894795
157	10	10	10	10	14.8	8	0.003226	0.980795	0.207501	0.125296	0.49221026	0.715437
158	10	10	10	10	12.2	9.5	0.000267	0.723231	0.05655	0.681378	0.88597133	0.688927
159	10	10	10	10	16.7	9.5	0.000426	0.430747	0.080287	0.571388	0.20883689	0.627147
160	10	10	10	10	14.1	9.4	0.000853	0.493464	0.059358	0.517602	0.29057636	1
161	10	10	10	10	13.5	9.6	0.00197	0.546341	0.078239	0.04449	0.06610737	0.970745
162	10	10	10	10	13.6	9.5	0.00289	0.584132	0.064365	0.666215	0.35912909	1
163	10	10	10	10	14.2	9.5	0.000909	0.037671	0.271952	0.091144	0.14107279	0.662157
164	10	10	10	10	16.3	8.2	0.000219	0.019041	0.05523	0.230186	0.11773847	0.889491
165	10	10	10	10	15.3	10.5	1.8E-05	0.381673	0.075263	0.467145	0.3145502	0.894795
166	10	10	10	10	13.2	9.7	4.2E-06	0.984944	0.109485	0.00469	0.09022141	1
167	10	10	10	10	14	9.3	4.93E-06	0.235206	0.052064	0.317581	0.31297719	0.505903

168	10	10	10	10	16.4	9.3	0.000335	0.145398	0.132363	0.010306	0.55693887	0.889491
169	10	10	10	10	14.6	9.5	6.58E-05	0.554826	0.061821	0.05972	0.06209674	0.391327
170	10	10	10	10	15.3	9.2	3.66E-05	0.536667	0.077176	0.175528	0.06406548	0.715437
171	10	10	10	10	15.2	8.8	1.41E-05	0.444034	0.081399	0.970642	0.41467559	0.894795
172	10	10	10	10	14.5	10.2	0.001262	0.189075	0.252783	0.198741	0.09562496	0.715437
173	10	10	10	10	12.8	8.1	4.76E-05	0.428104	0.335353	0.668028	0.16309763	1
174	10	10	10	10	13.7	10.7	0.001744	0.503042	0.156466	0.67134	0.06850823	0.476884
175	10	10	10	10	16.1	8.5	0.000531	0.068911	0.413662	0.651096	0.30585145	1
176	10	10	10	10	15.6	8.8	0.000375	0.218655	0.07021	0.364427	0.10402496	0.715437
177	10	10	10	10	15.6	8.7	0.00213	0.432228	0.052005	0.037878	0.14459604	0.715437

Table S3. Pearson's correlation analysis between significant metabolites and key echo variables.

	P values			FDR			Correlation coefficients		
BIOCHEMICAL	**nLVIDd**	**nLAD**	**LA/Ao**	**nLVIDd**	**nLAD**	**LA/Ao**	**nLVIDd**	**nLAD**	**LA/Ao**
hydroxyasparagine	0.034912	0.000333	0.000129	0.143833	0.00308	0.002098	0.242359	0.40087	0.425254
N,N-dimethylalanine	0.197889	0.027048	0.116845	0.302856	0.048555	0.142811	0.149342	0.253639	0.18139
N-acetylalanine	0.221365	0.009516	0.0083	0.316871	0.024272	0.020344	0.141918	0.295651	0.300724
N-acetylasparagine	0.365624	0.152091	0.253786	0.446874	0.183343	0.291937	0.105232	0.165891	0.132523
N-carbamoylalanine	0.271966	0.985715	0.95614	0.35721	0.985715	0.961604	0.127611	-0.00209	-0.00641
creatine	0.002969	0.000139	0.001394	0.052205	0.002036	0.005706	0.336369	0.423345	0.360172
alpha-ketoglutaramate*	0.281813	0.005324	0.000491	0.367401	0.017064	0.003307	0.125042	0.316639	0.390294
carboxyethyl-GABA	0.203971	0.038676	0.015363	0.306828	0.060388	0.031078	0.147361	0.237701	0.277143
glutamine	0.051926	0.005333	0.037242	0.166163	0.017064	0.060691	-0.22384	-0.31658	-0.23943
N-acetylglutamate	0.234305	0.045105	0.073372	0.319671	0.067275	0.098576	0.138061	0.230552	0.206588
N-acetylglutamine	0.417994	0.05001	0.009107	0.493738	0.072742	0.020823	0.094258	0.225645	0.29729
2-hydroxybutyrate/2-hydroxyisobutyrat	0.231709	0.012126	0.011939	0.3186	0.02907	0.025315	0.138822	0.286431	0.287032
glycine	0.370873	0.008706	0.007557	0.450163	0.022532	0.019277	-0.10409	-0.29896	-0.30416
N-acetylserine	0.483024	0.038892	0.065287	0.549395	0.060388	0.090476	0.081681	0.237444	0.21254
N-acetylthreonine	0.152862	0.001757	0.005674	0.258689	0.007587	0.016108	0.165584	0.353086	0.314407
sarcosine	0.038679	0.007617	0.029745	0.151278	0.020624	0.049859	-0.2377	-0.30387	-0.24949
4-guanidinobutanoate	0.01348	0.000188	0.000449	0.105949	0.002407	0.003307	0.282311	0.4157	0.39276
1-methyl-5-imidazoleacetate	0.159687	0.014347	0.015564	0.264185	0.032372	0.031127	0.162914	0.279859	0.276624
1-methylhistidine	0.00242	1.06E-05	6.31E-06	0.052205	0.000467	0.000278	0.342997	0.481579	0.492184
3-methylhistidine	0.109993	0.005884	0.008309	0.238135	0.017551	0.020344	0.184809	0.31313	0.300687
carnosine	0.110949	0.012553	0.010546	0.238135	0.02907	0.023796	0.184322	0.285091	0.291776
formiminoglutamate	0.303264	0.034035	0.037864	0.389594	0.056511	0.061138	0.119645	0.243504	0.238672
imidazole lactate	0.387221	0.015425	0.012933	0.466787	0.032709	0.026468	0.1006	0.27698	0.283929
N-acetyl-1-methylhistidine*	0.013803	4.97E-05	4.57E-05	0.105949	0.001248	0.001006	0.281381	0.447917	0.449811
N-acetyl-3-methylhistidine*	0.225051	0.00364	0.003809	0.316871	0.013135	0.012036	0.140803	0.32963	0.32811
2-hydroxy-3-methylvalerate	0.754811	0.572708	0.253197	0.772364	0.59834	0.291937	-0.03642	0.065722	0.132686
3-hydroxy-2-ethylpropionate	0.148748	0.015266	0.043699	0.254224	0.032709	0.068063	0.167237	0.277395	0.232039
3-hydroxyisobutyrate	0.558203	0.277571	0.622313	0.602723	0.311162	0.651947	0.068215	0.126141	0.057407
3-methyl-2-oxobutyrate	0.360137	0.164301	0.064631	0.443245	0.196714	0.090279	0.106435	0.161156	0.213048
3-methylglutaconate	0.46797	0.030896	0.021788	0.539661	0.052285	0.03913	0.084505	0.247818	0.262855
ethylmalonate	0.129447	0.000517	0.000696	0.25033	0.003793	0.003952	0.175486	0.388879	0.380546

isobutyrylcarnitine (C4)	0.03057	0.001443	0.012872	0.141586	0.006513	0.026468	0.248287	0.359122	0.284114
isovalerylcarnitine (C5)	0.05525	0.004643	0.007555	0.170596	0.016023	0.019277	0.220821	0.321381	0.304169
N-acetylisoleucine	0.261995	0.061462	0.090872	0.349326	0.08451	0.11674	0.130276	0.215565	0.195314
N-acetylleucine	0.510527	0.450697	0.438147	0.565112	0.480743	0.476011	0.076639	0.087807	0.09025
N-acetylvaline	0.131139	0.019495	0.025161	0.25033	0.038796	0.043845	0.174727	0.26748	0.256755
tiglyl carnitine (C5)	0.116455	0.003402	0.024717	0.244	0.01274	0.043502	0.181581	0.331884	0.257517
2-aminoadipate	0.205785	0.051292	0.40253	0.306933	0.073086	0.440033	0.146778	0.224427	0.097409
5-(galactosylhydroxy)-L-lysine	0.042624	0.000568	0.001268	0.15337	0.003947	0.005441	0.233204	0.386296	0.363046
6-oxopiperidine-2-carboxylate	0.12485	0.036109	0.316814	0.246895	0.057254	0.355154	0.177586	0.240834	0.116364
N,N,N-trimethyl-5-aminovalerate	0.004438	0.001044	0.00714	0.060081	0.005104	0.018757	0.32293	0.368818	0.306215
N2-acetyl,N6-methyllysine	0.087614	0.048259	0.050258	0.213507	0.071374	0.075602	0.197274	0.227349	0.225407
N6,N6,N6-trimethyllysine	0.663378	0.086233	0.250221	0.699129	0.111595	0.291937	0.050736	0.198122	0.133514
N6,N6-dimethyllysine	0.119815	0.081195	0.258388	0.246895	0.105854	0.295301	0.179956	0.20131	0.131257
N6-acetyllysine	0.30603	0.262403	0.45607	0.390299	0.297955	0.492444	0.118968	0.130166	0.086772
N6-carboxyethyllysine	0.021475	0.004927	0.003298	0.118896	0.016676	0.010952	0.263461	0.319333	0.332915
pipecolate	0.657513	0.574542	0.927073	0.697122	0.59834	0.937729	0.051678	0.065409	0.010676
2,3-dihydroxy-5-methylthio-4-pentenoa	0.139257	0.000747	0.000747	0.253017	0.004405	0.004092	0.171185	0.378544	0.37854
alpha-ketobutyrate	0.231212	0.092058	0.082853	0.3186	0.11573	0.10964	0.138969	0.194615	0.200244
methionine	0.064928	0.010215	0.017519	0.181388	0.025323	0.033883	-0.21282	-0.29298	-0.27186
S-carboxyethylcysteine	0.195963	0.02367	0.075103	0.302539	0.042948	0.100138	0.149978	0.25936	0.205382
S-methylmethionine	0.148779	0.005544	0.011932	0.254224	0.017118	0.025315	-0.16722	-0.31522	-0.28705
N-acetylphenylalanine	0.14219	0.034994	0.045225	0.253017	0.056548	0.069821	0.169944	0.242253	0.230427
5-methylthioadenosine (MTA)	0.2619	0.118116	0.067662	0.349326	0.144364	0.092314	0.130302	0.180773	0.210732
N-acetyl-isoputreanine	0.316991	0.006507	0.00383	0.398503	0.018775	0.012036	0.116321	0.309551	0.32793
N-acetyltryptophan	0.316533	0.111978	0.085372	0.398503	0.138789	0.112131	0.116431	0.183802	0.198656
2-oxoarginine*	0.016254	0.000298	0.000483	0.105949	0.00308	0.003307	0.274885	0.403739	0.390768
argininate*	0.056854	0.089002	0.206999	0.172522	0.113509	0.246162	0.21942	0.196432	0.14639
dimethylarginine (ADMA + SDMA)	0.429621	0.183738	0.059445	0.501839	0.2185	0.085059	0.091932	0.154126	0.217222
homocitrulline	0.035141	0.023034	0.061361	0.143833	0.042228	0.087093	0.242064	0.260515	0.215647
N,N,N-trimethyl-alanylproline betaine (T	0.469137	0.056075	0.040928	0.539661	0.07771	0.064895	0.084284	0.220096	0.235091
N2,N5-diacetylornithine	0.011237	0.002851	0.008323	0.102971	0.011406	0.020344	0.289359	0.337694	0.300625
N-acetylcitrulline	0.494273	0.702994	0.87052	0.55409	0.723549	0.890764	-0.0796	0.044451	0.019011
N-acetylhomocitrulline	0.132277	0.067916	0.063017	0.25033	0.090554	0.088728	0.17422	0.210542	0.214317
N-acetylproline	0.270269	0.076652	0.018252	0.35721	0.100677	0.034542	-0.12806	-0.20432	-0.27018

N-delta-acetylornithine	0.000865	0.016012	0.069114	0.030444	0.033549	0.093569	0.374319	0.275487	0.209653
N-methylproline	0.018976	0.069644	0.231979	0.115165	0.092161	0.274016	0.268591	0.209263	0.138743
proline	0.159648	0.001767	0.006469	0.264185	0.007587	0.017515	-0.16293	-0.3529	-0.30976
urea	0.01721	0.000515	0.011056	0.108176	0.003793	0.024323	0.272578	0.389002	0.289978
erythronate*	0.142322	0.002841	0.000894	0.253017	0.011406	0.004573	0.169889	0.337816	0.373374
N-acetylglucosaminylasparagine	0.392651	0.066052	0.05117	0.470113	0.089425	0.075833	0.099459	0.211951	0.224543
N-acetylneuraminate	0.039767	0.051907	0.041768	0.152151	0.073086	0.065635	0.236421	0.223852	0.234149
arabonate/xylonate	0.07999	0.011383	0.012376	0.207004	0.027826	0.025931	0.202096	0.288862	0.28564
ribonate	0.020687	0.001351	0.001197	0.118896	0.006259	0.005441	0.265022	0.361115	0.364748
ribulonate/xylulonate/lyxonate*	0.11089	0.029892	0.119663	0.238135	0.051078	0.145246	0.184352	0.249275	0.180029
2-O-methylascorbic acid	0.208607	0.029606	0.002941	0.308528	0.051078	0.010562	0.145879	0.249698	0.336688
ascorbic acid 3-sulfate*	0.100891	0.001101	0.000267	0.232394	0.005239	0.00298	0.189615	0.36724	0.406692
gulonate*	0.001996	1.01E-05	2.98E-06	0.050192	0.000467	0.000175	0.349098	0.482706	0.506847
N1-Methyl-2-pyridone-5-carboxamide	0.006218	0.000997	0.000767	0.072953	0.005104	0.004092	0.311174	0.370164	0.377773
nicotinamide	0.886829	0.211328	0.114435	0.891897	0.248032	0.140843	-0.0166	-0.14502	-0.18257
nicotinamide N-oxide	0.4916	0.863709	0.470743	0.55409	0.878687	0.502126	0.080093	-0.02002	-0.08398
quinolinate	0.015848	0.002254	0.000613	0.105949	0.009447	0.003718	0.275899	0.345254	0.384155
pterin	0.172486	0.211391	0.021045	0.275977	0.248032	0.038185	0.158125	0.145	0.264306
alpha-CEHC sulfate	0.680714	0.246267	0.48461	0.713129	0.283288	0.513803	-0.04797	-0.13462	-0.08139
carotene diol (1)	0.088557	0.09094	0.179138	0.213507	0.115146	0.214478	-0.1967	-0.19527	-0.15574
pyridoxal	0.558014	0.395883	0.733553	0.602723	0.427456	0.759444	-0.06825	-0.09879	0.039689
phosphate	0.010061	0.007183	0.053608	0.102971	0.020391	0.077975	0.293555	0.305999	0.222291
aconitate [cis or trans]	0.123472	0.000468	0.000348	0.246895	0.003746	0.003221	0.178228	0.391615	0.399681
citrate	0.556566	0.018899	0.006629	0.602723	0.038233	0.017678	0.068498	0.268757	0.308887
carnitine	0.033626	0.000583	0.003127	0.143833	0.003947	0.01079	0.244047	0.38554	0.334677
adipoylcarnitine (C6-DC)	0.104313	0.001009	0.00029	0.232394	0.005104	0.002999	0.18777	0.369811	0.404524
(R)-3-hydroxybutyrylcarnitine	0.104066	0.00774	0.010933	0.232394	0.02064	0.024323	0.187902	0.303285	0.290407
(S)-3-hydroxybutyrylcarnitine	0.029768	0.000326	0.000539	0.141586	0.00308	0.003389	0.249458	0.401369	0.387727
3-hydroxyhexanoylcarnitine (1)	0.344853	0.02888	0.004498	0.427423	0.050325	0.013649	0.109848	0.250787	0.322469
3-hydroxypalmitoylcarnitine	0.146751	0.051866	0.019992	0.254224	0.073086	0.036652	0.168052	0.223891	0.266439
arachidoylcarnitine (C20)*	0.000775	0.00037	3.2E-05	0.030444	0.003258	0.000806	0.377467	0.397994	0.457846
lignoceroylcarnitine (C24)*	0.023899	0.015375	0.004666	0.118896	0.032709	0.01392	0.258951	0.277111	0.321209
margaroylcarnitine (C17)*	0.082331	0.088182	0.103552	0.207004	0.113285	0.128346	0.200578	0.196928	0.188176
palmitoylcarnitine (C16)	0.048993	0.020082	0.005353	0.165262	0.038796	0.015445	0.226629	0.266254	0.31645

stearoylcarnitine (C18)	0.036854	0.021519	0.001982	0.147418	0.040291	0.007753	0.239905	0.263376	0.34932
eicosenoylcarnitine (C20:1)*	0.014802	0.008219	0.002346	0.105949	0.02159	0.008975	0.278623	0.301086	0.343988
oleoylcarnitine (C18:1)	0.005659	0.000223	3.21E-05	0.071137	0.002458	0.000806	0.314505	0.411289	0.45782
arachidonoylcarnitine (C20:4)	0.048226	0.000813	0.000271	0.165262	0.004519	0.00298	0.227381	0.376104	0.406287
dihomo-linolenoylcarnitine (C20:3n3 or	0.130694	0.001015	0.000909	0.25033	0.005104	0.004573	0.174926	0.36965	0.372861
dihomo-linoleoylcarnitine (C20:2)*	0.042178	0.034675	0.018129	0.15337	0.056548	0.034542	0.233694	0.242666	0.270461
linolenoylcarnitine (C18:3)*	0.16089	0.044012	0.053482	0.264185	0.067275	0.077975	0.162452	0.231705	0.222405
acetylcarnitine (C2)	0.003327	4.02E-05	9.75E-05	0.052205	0.001179	0.001906	0.33263	0.452743	0.432004
butyrylcarnitine (C4)	0.050651	0.000638	0.001054	0.165262	0.004162	0.005015	0.225033	0.382993	0.368531
methylmalonate (MMA)	0.0427	5.83E-05	0.000507	0.15337	0.001283	0.003307	0.233121	0.444197	0.389413
propionylcarnitine (C3)	0.00152	0.0002	0.005143	0.044585	0.002407	0.015086	0.357543	0.414163	0.317844
malonate	0.732932	0.020137	0.006371	0.754363	0.038796	0.017515	0.039785	0.266141	0.310307
2-aminoheptanoate	0.227598	0.016842	0.09201	0.317915	0.034873	0.117346	-0.14004	-0.27345	-0.19464
azelate (C9-DC)	0.113773	0.000751	0.000461	0.241254	0.004405	0.003307	-0.1829	-0.37839	-0.39207
maleate	0.219343	0.00334	0.000245	0.316871	0.01274	0.00298	0.142535	0.332499	0.408893
2R,3R-dihydroxybutyrate	0.223609	0.000205	0.001261	0.316871	0.002407	0.005441	0.141238	0.41349	0.363205
2S,3R-dihydroxybutyrate	0.239932	0.006075	0.00911	0.324831	0.01782	0.020823	0.13643	0.311998	0.297278
3,4-dihydroxybutyrate	0.501082	0.039115	0.100338	0.558167	0.060388	0.125244	0.078354	0.237183	0.189918
2-hydroxynervonate*	0.136674	0.049048	0.087348	0.253017	0.071937	0.113039	0.172295	0.226575	0.197436
3-hydroxystearate	0.184561	0.044751	0.051274	0.287458	0.067275	0.075833	0.153841	0.230922	0.224445
chiro-inositol	0.010586	0.003339	0.000695	0.102971	0.01274	0.003952	0.291631	0.332509	0.380603
3-hydroxybutyrate (BHBA)	0.103815	0.012483	0.03874	0.232394	0.02907	0.061983	0.188036	0.285309	0.237625
acetoacetate	0.031798	0.050527	0.375131	0.1435	0.072892	0.417025	0.246542	0.22515	0.103173
docosatrienoate (22:3n3)	0.064455	0.02028	0.018504	0.181388	0.038796	0.034645	0.213185	0.265847	0.269625
hexadecatrienoate (16:3n3)	0.053443	0.004574	0.001239	0.167964	0.016023	0.005441	-0.22244	-0.3219	-0.36373
1-palmitoyl-2-arachidonoyl-GPC (16:0/2	0.045181	0.052382	0.004392	0.159039	0.073168	0.01356	0.230472	0.223413	0.323287
1-stearoyl-2-arachidonoyl-GPC (18:0/20	0.176202	0.231356	0.016597	0.279383	0.267886	0.032821	0.156784	0.138926	0.274043
1-palmitoyl-2-oleoyl-GPI (16:0/18:1)*	0.003559	0.003657	0.00037	0.052205	0.013135	0.003252	0.330381	0.329477	0.398049
1-stearoyl-2-arachidonoyl-GPI (18:0/20:	0.102385	0.031504	0.003501	0.232394	0.052807	0.011412	0.188804	0.246954	0.330928
1-stearoyl-2-oleoyl-GPI (18:0/18:1)*	0.023565	0.015328	0.002923	0.118896	0.032709	0.010562	0.25955	0.277233	0.336884
trimethylamine N-oxide	0.483842	0.035342	0.048675	0.549395	0.056548	0.074494	0.081529	0.241806	0.226939
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPI	0.163975	0.249085	0.03352	0.264767	0.284668	0.055313	0.161279	0.133832	0.244188
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-	0.430555	0.306304	0.60755	0.501839	0.336935	0.640292	-0.09175	-0.1189	-0.05985
1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P	0.162113	0.014394	0.025891	0.264185	0.032372	0.044675	-0.16198	-0.27973	-0.25553

1-(1-enyl-stearoyl)-2-arachidonoyl-GPE	0.058957	0.035165	0.000409	0.175332	0.056548	0.003307	0.21763	0.242034	0.395323
1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0	0.009167	0.00733	0.001378	0.100839	0.020477	0.005706	0.297044	0.305266	0.360527
sphingomyelin (d18:1/18:1, d18:2/18:0)	0.655909	0.978139	0.967637	0.697122	0.983728	0.967637	-0.05194	0.003196	0.004732
sphingomyelin (d18:1/20:1, d18:2/20:0)	0.079406	0.009681	0.001	0.207004	0.02434	0.004887	-0.20248	-0.29501	-0.3701
sphingomyelin (d18:1/20:2, d18:2/20:1,	0.821416	0.949901	0.886364	0.83566	0.960819	0.901735	-0.02632	0.007329	-0.01667
sphingomyelin (d18:1/22:2, d18:2/22:1,	0.08524	0.027312	0.00848	0.211299	0.048555	0.020445	-0.19874	-0.25322	-0.29993
sphingomyelin (d18:2/24:2)*	0.134687	0.063757	0.017343	0.252181	0.086986	0.033883	-0.17316	-0.21373	-0.27227
stearoyl sphingomyelin (d18:1/18:0)	0.522859	0.690564	0.643704	0.575145	0.714937	0.670367	-0.07442	-0.04641	-0.05391
1-methylhypoxanthine	0.33906	0.022577	0.011645	0.423223	0.041827	0.025303	0.111166	0.26136	0.287989
allantoin	0.050705	0.000822	0.003092	0.165262	0.004519	0.01079	0.224981	0.3758	0.335043
inosine	0.411173	0.838581	0.277651	0.488962	0.858083	0.315269	0.095639	0.023757	0.12612
N1-methylinosine	0.297596	0.028681	0.006194	0.385124	0.050325	0.017305	0.121046	0.251089	0.311307
urate	0.880864	0.304036	0.050219	0.890989	0.336543	0.075602	-0.01748	0.119456	0.225444
7-methylguanine	0.073657	0.044392	0.055887	0.202557	0.067275	0.080624	0.206388	0.231301	0.220261
N2,N2-dimethylguanosine	0.059772	0.000693	0.000162	0.175332	0.004358	0.002375	0.21695	0.380664	0.419498
5-hydroxymethylcytidine	0.096946	0.113368	0.029516	0.230573	0.13953	0.049859	0.191804	0.183105	0.249832
5-methyl-2'-deoxycytidine	0.022595	0.018407	0.002405	0.118896	0.03767	0.009005	-0.26133	-0.26984	-0.3432
N4-acetylcytidine	0.015579	0.005293	0.001721	0.105949	0.017064	0.006883	0.276585	0.316846	0.353726
3-ureidopropionate	0.140595	0.005512	0.003296	0.253017	0.017118	0.010952	0.170617	0.315424	0.332936
5,6-dihydrouridine	0.06458	0.000112	0.000477	0.181388	0.001973	0.003307	0.213087	0.428613	0.391131
pseudouridine	0.034663	9.23E-05	0.000325	0.143833	0.001804	0.003179	0.242681	0.433332	0.401467
bilirubin degradation product, C17H18N	0.219978	0.224553	0.087322	0.316871	0.261731	0.113039	-0.14234	-0.14095	-0.19745
gamma-glutamyl-alpha-lysine	0.954182	0.328206	0.092838	0.954182	0.358784	0.11755	0.006702	0.113674	0.194158
gamma-glutamylglutamate	0.014769	0.007547	0.028637	0.105949	0.020624	0.048933	0.278711	0.304206	0.251156
gamma-glutamylisoleucine*	0.724806	0.486805	0.397606	0.750388	0.516131	0.437366	0.041045	0.080979	0.098427
gamma-glutamylphenylalanine	0.630797	0.268101	0.130104	0.676953	0.302472	0.156837	0.056014	0.128636	0.17519
gamma-glutamyltyrosine	0.180871	0.428901	0.818124	0.284226	0.460284	0.842046	-0.15513	-0.09208	0.026818
N,N-dimethyl-pro-pro	0.201517	0.012447	0.019395	0.30575	0.02907	0.035932	0.148155	0.285418	0.267691
2-methoxyhydroquinone sulfate (1)	0.12251	0.14205	0.376744	0.246895	0.17242	0.417025	-0.17868	-0.17	-0.10283
4-vinylcatechol sulfate	0.143927	0.066763	0.033628	0.253312	0.089697	0.055313	-0.16922	-0.21141	-0.24404
4-vinylphenol sulfate	0.079482	0.01976	0.009057	0.207004	0.038796	0.020823	-0.20243	-0.26692	-0.29749
hippurate	0.023544	0.005828	0.024017	0.118896	0.017551	0.042697	0.259587	0.313463	0.258742
S-(3-hydroxypropyl)mercapturic acid (H	0.011701	0.000126	0.000186	0.102971	0.002015	0.002523	0.287805	0.425756	0.415941
sulfate*	0.002729	1.94E-05	1.3E-05	0.052205	0.000682	0.000456	0.339116	0.468871	0.47741

triethanolamine	0.12108	0.548029	0.250928	0.246895	0.577563	0.291937	0.179354	0.069982	0.133317
2-aminophenol sulfate	0.224079	0.29955	0.296367	0.316871	0.333676	0.334363	-0.1411	-0.12056	-0.12135
ferulic acid 4-sulfate	0.081476	0.021157	0.008866	0.207004	0.040038	0.020823	-0.20113	-0.26409	-0.29829
gluconate	0.711676	0.094655	0.094266	0.741154	0.118151	0.118506	0.043091	0.193107	0.193331
homostachydrine*	0.081726	0.339542	0.470584	0.207004	0.368885	0.502126	0.200967	0.111055	0.084011
mannonate*	0.02432	0.000452	0.000131	0.118896	0.003746	0.002098	0.258209	0.392601	0.424758

Table S4. Pearson's correlation analysis on carnitine, significant acylcarnitines and echo variables

	P values (w/ echo variables)			FDR (w/ echo variables)		
Carnitine & acylcarnitines	nLVIDd	nLAD	LA/Ao	nLVIDd	nLAD	LA/Ao
adipoylcarnitine (C6-DC)	0.1043	0.001	0.0003	0.2324	0.0049	0.0028
carnitine	0.0336	0.0006	0.0031	0.1437	0.0039	0.0107
acetylcarnitine (C2)	0.0033	0	0.0001	0.0528	0	0.0016
butyrylcarnitine (C4)	0.0507	0.0006	0.0011	0.1652	0.0039	0.0052
tiglyl carnitine (C5)	0.1165	0.0034	0.0247	0.2441	0.0127	0.0435
(S)-3-hydroxybutyrylcarnitine	0.0298	0.0003	0.0005	0.1417	0.0028	0.0031
isovalerylcarnitine (C5)	0.0552	0.0046	0.0076	0.1704	0.0159	0.0194
dihomo-linoleoylcarnitine (C20:2)*	0.0422	0.0347	0.0181	0.1534	0.0565	0.0346
dihomo-linolenoylcarnitine (C20:3n3 or 6)*	0.1307	0.001	0.0009	0.2504	0.0049	0.0045
margaroylcarnitine (C17)*	0.0823	0.0882	0.1036	0.2069	0.1133	0.1284
3-hydroxyhexanoylcarnitine (1)	0.3449	0.0289	0.0045	0.4275	0.0504	0.0137
3-hydroxypalmitoylcarnitine	0.1468	0.0519	0.02	0.2543	0.0731	0.0367
isobutyrylcarnitine (C4)	0.0306	0.0014	0.0129	0.1417	0.0063	0.0264
propionylcarnitine (C3)	0.0015	0.0002	0.0051	0.044	0.0022	0.015
palmitoylcarnitine (C16)	0.049	0.0201	0.0054	0.1652	0.0388	0.0156
(R)-3-hydroxybutyrylcarnitine	0.1041	0.0077	0.0109	0.2324	0.0205	0.0243
eicosenoylcarnitine (C20:1)*	0.0148	0.0082	0.0023	0.1063	0.0215	0.0088
arachidonoylcarnitine (C20:4)	0.0482	0.0008	0.0003	0.1652	0.0044	0.0028
stearoylcarnitine (C18)	0.0369	0.0215	0.002	0.1476	0.0403	0.0078
linolenoylcarnitine (C18:3)*	0.1609	0.044	0.0535	0.2642	0.0673	0.078
arachidoylcarnitine (C20)*	0.0008	0.0004	0	0.0317	0.0035	0
lignoceroylcarnitine (C24)*	0.0239	0.0154	0.0047	0.1188	0.0327	0.014
oleoylcarnitine (C18:1)	0.0057	0.0002	0	0.0717	0.0022	0

	r (with echo variables)		
Carnitine & acylcarnitines	nLVIDd	nLAD	LA/Ao
adipoylcarnitine (C6-DC)	0.19	0.37	0.4
carnitine	0.24	0.39	0.33
acetylcarnitine (C2)	0.33	0.45	0.43
butyrylcarnitine (C4)	0.23	0.38	0.37

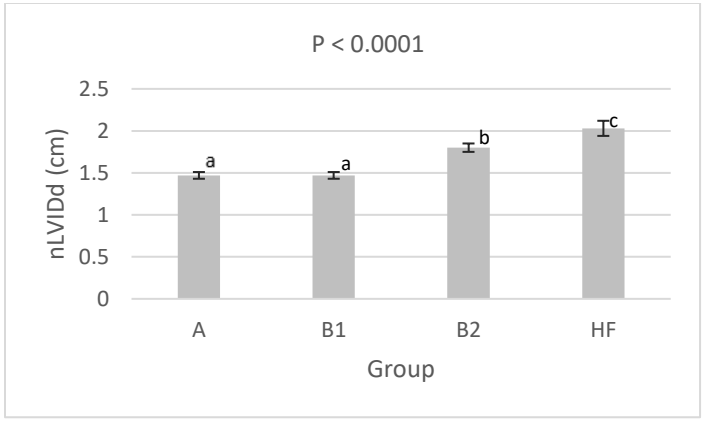
tiglyl carnitine (C5)	0.18	0.33	0.26
(S)-3-hydroxybutyrylcarnitine	0.25	0.4	0.39
isovalerylcarnitine (C5)	0.22	0.32	0.3
dihomo-linoleoylcarnitine (C20:2)*	0.23	0.24	0.27
dihomo-linolenoylcarnitine (C20:3n3 or 6)*	0.17	0.37	0.37
margaroylcarnitine (C17)*	0.2	0.2	0.19
3-hydroxyhexanoylcarnitine (1)	0.11	0.25	0.32
3-hydroxypalmitoylcarnitine	0.17	0.22	0.27
isobutyrylcarnitine (C4)	0.25	0.36	0.28
propionylcarnitine (C3)	0.36	0.41	0.32
palmitoylcarnitine (C16)	0.23	0.27	0.32
(R)-3-hydroxybutyrylcarnitine	0.19	0.3	0.29
eicosenoylcarnitine (C20:1)*	0.28	0.3	0.34
arachidonoylcarnitine (C20:4)	0.23	0.38	0.41
stearoylcarnitine (C18)	0.24	0.26	0.35
linolenoylcarnitine (C18:3)*	0.16	0.23	0.22
arachidoylcarnitine (C20)*	0.38	0.4	0.46
lignoceroylcarnitine (C24)*	0.26	0.28	0.32
oleoylcarnitine (C18:1)	0.31	0.41	0.46

Table S5. Significant KEGG Pathways

Pathways	Total	Hits	P val	FDR	Impact
Arginine Biosynthesis	14	4	0.0024	0.20	0
Synthesis and Degradation of Ketone Bodies	5	2	0.0174	0.63	0.6
Nicotinate and Nicotinamide Metabolism	15	3	0.0252	0.63	0.19
Histidine Metabolism	16	3	0.0300	0.63	0.14
Valine, Leucine and Isoleucine (BCAA) Biosynthesis	8	2	0.0448	0.74	0

Table S6. Quantitative metabolite set enrichment analysis (qMSEA)

Metabolite Sets	Total	Hits	P val	FDR
Methionine Metabolism	43	15	0.000	0.000
Pyrimidine Metabolism	59	8	0.000	0.000
Nicotinate and Nicotinamide Metabolism	37	6	0.000	0.000
Methylhistidine Metabolism	4	2	0.000	0.001
Transfer of Acetyl Groups into Mitochondria	22	3	0.000	0.001
Spermidine and Spermine Biosynthesis	18	5	0.000	0.001
Histidine Metabolism	43	12	0.000	0.003
Homocysteine Degradation	9	4	0.000	0.004
Glycine and Serine Metabolism	59	16	0.001	0.006
Arginine and Proline Metabolism	53	10	0.001	0.010
D-Arginine and D-Ornithine Metabolism	11	1	0.002	0.013
Beta-Alanine Metabolism	34	10	0.002	0.014
Aspartate Metabolism	35	10	0.003	0.016
Purine Metabolism	74	14	0.004	0.023
Ammonia Recycling	32	10	0.004	0.023
Porphyrin Metabolism	40	1	0.005	0.025
Threonine and 2-Oxobutanoate Degradation	20	2	0.006	0.025
Amino Sugar Metabolism	33	2	0.006	0.025
Warburg Effect	58	7	0.006	0.026
Betaine Metabolism	21	5	0.007	0.028
Lysine Degradation	30	4	0.012	0.045
Lactose Synthesis	20	1	0.014	0.047
Lactose Degradation	9	1	0.014	0.047
Tryptophan Metabolism	60	10	0.014	0.048
Citric Acid Cycle	32	5	0.016	0.050
Oxidation of Branched Chain Fatty Acids	26	3	0.020	0.061
Ketone Body Metabolism	13	2	0.025	0.075
Pantothenate and CoA Biosynthesis	21	4	0.028	0.079
Phenylacetate Metabolism	9	3	0.029	0.080
Butyrate Metabolism	19	3	0.03	0.08
Phosphatidylethanolamine Biosynthesis	12	3	0.038	0.098
Phenylalanine and Tyrosine Metabolism	28	6	0.042	0.106
Bile Acid Biosynthesis	65	10	0.050	0.123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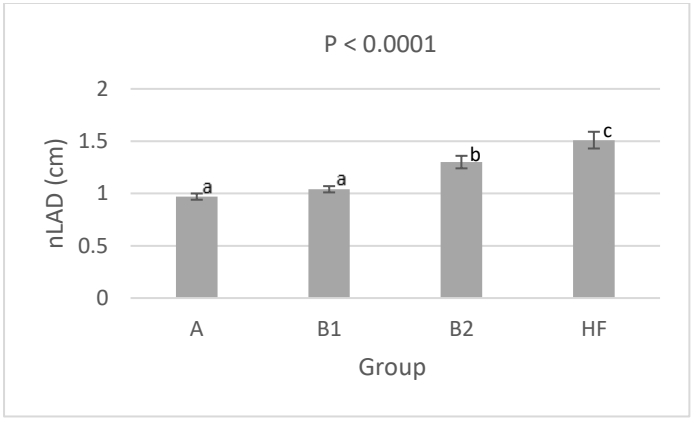
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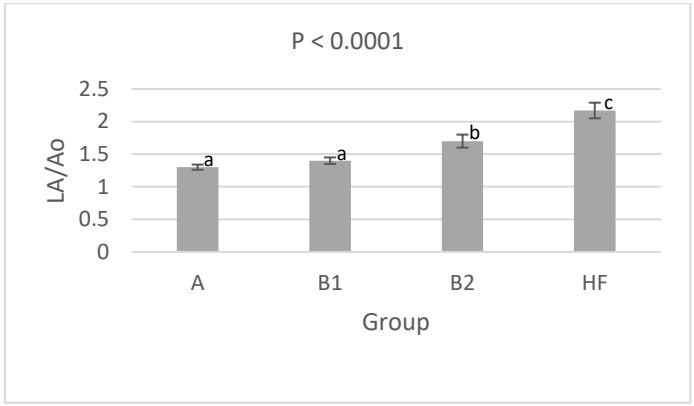
HF
Group


P < 0.0001
LA/Ao
2.5
2
1.5
1
0.5
0
a
a
b
c
A
B1
B2
HF
Group

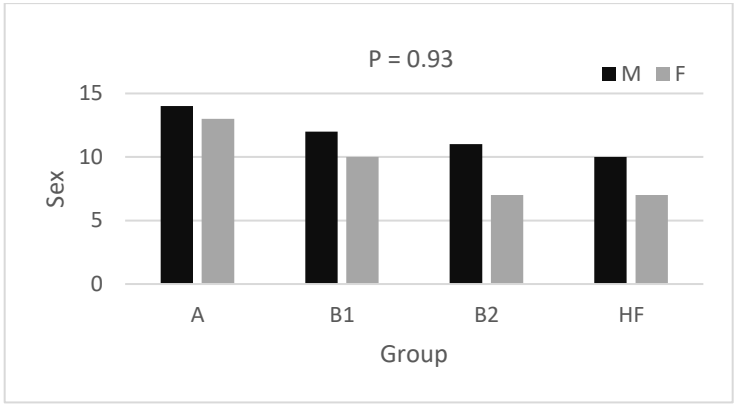
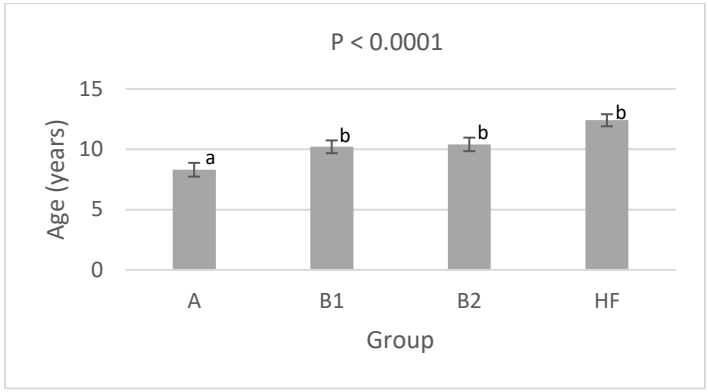
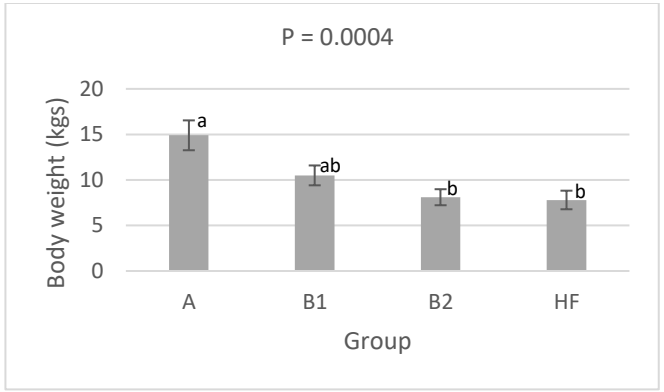

P = 0.93
M
F
Sex
15
10
5
0
A
B1
B2
HF
Group

Figure S1. Barplots of sex, age, body weights, BCS, and key echo variables. P value from ANOVA are displayed on the top of each barplot. Different letters indicate statistical difference.





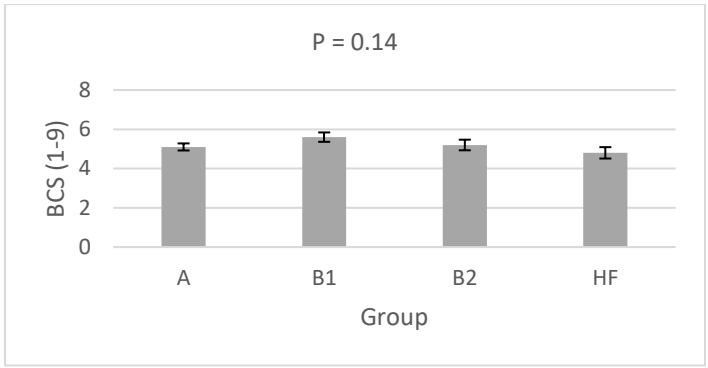


Figure S2. PLS-DA (top panel). The percentages of data variation explained by the first two components, are indicated on the x and y axes. Boxplots along component 1 (bottom panel). Tukey's test was performed to compare groups. *** P < 0.001; **** P < 0.0001.

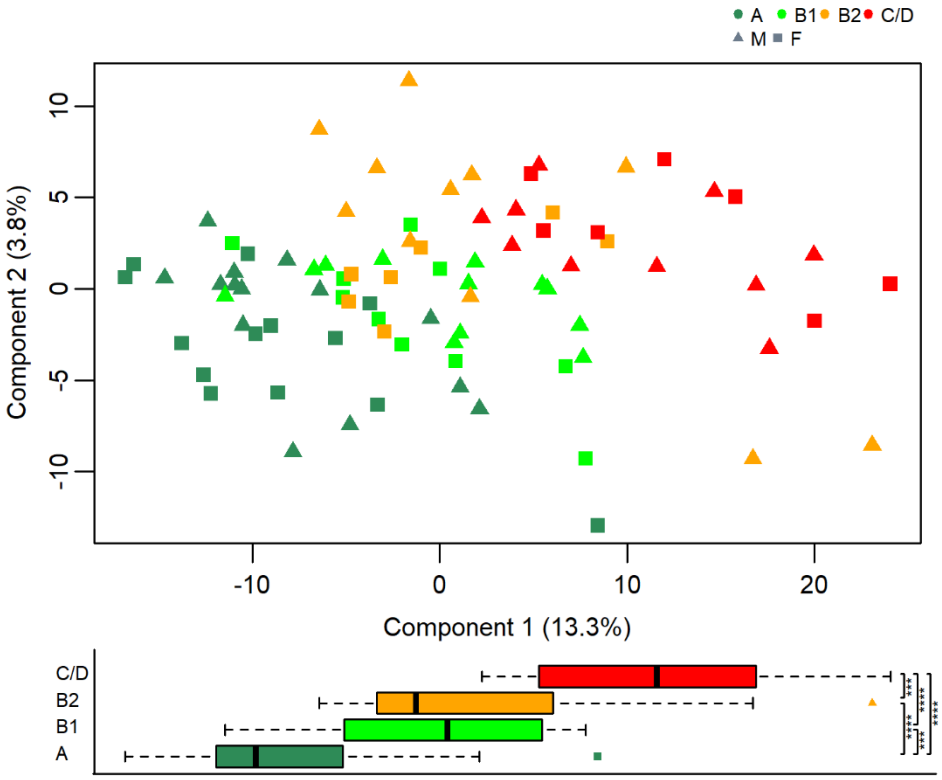


Figure S3. (A) Distribution of P values from bootstrap experiments. Only bootstrapped subsamples that showed no difference in age or body weight were analyzed. (Left) was plotted against each sample on PC1, PC1, age, body weight (BW), sex and BCS. (Right) The histogram of . The greyish dashed line denotes P = 0.05. (B) PCA analysis on cardiac medications. Only preclinical dogs in the B1 and B2 groups were included. Dogs were considered for two groups: those that were on stable cardiac medications for 2 weeks or longer (Y) and those that were not (N). Student's t-test was performed to compare the difference on PC1 or PC2 between groups.

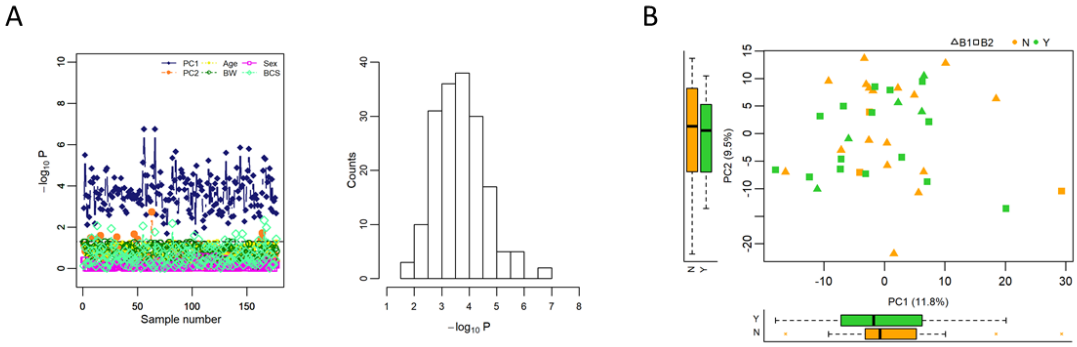


Figure S4. Heat map of 22 acylcarnitine concentrations from all samples. Hierarchical clustering was performed on the Euclidean distances between samples. The color scale corresponds to concentrations from low (deep blue) to high (maroon).

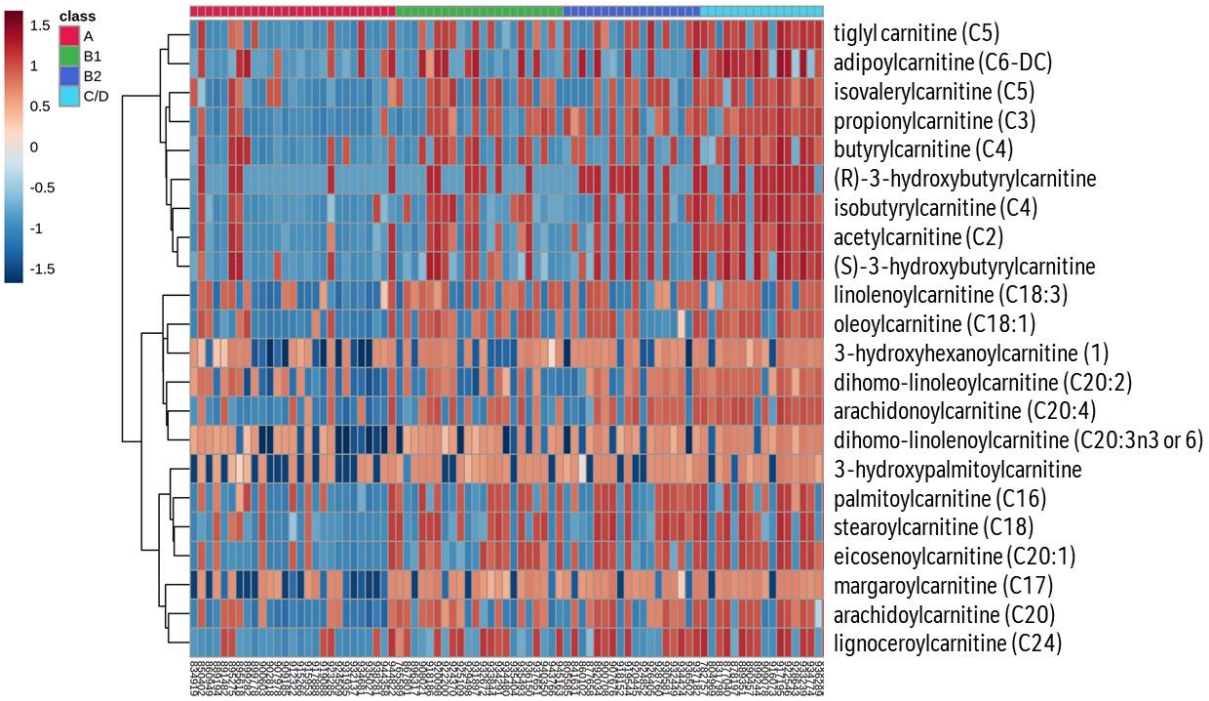


Figure S5. Pearson's correlations between aconitate and (A-G) citrate, quinolinate, urea, BHBA, TMAO, 3-MH, and carnosine, and between (H) 3-MH and urea, and (I) 4-guanidinobutanoate and 2-oxoarginine. FDR < 0.0001 in all cases.