**Microbial Metabolites Associated in Stool and Left Ventricle of Heart Failure Patients Revealed by Meta-Analysis**

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## Abstract: Heart Failure (HF) is a leading cause of death, impacting approximately 64 million people globally. While overall incidence of HF is relatively stable across countries, the overall number of HF patients is increasing due to aging populations. Many articles have been published on the role of the microbiome in recovery after HF, however, this research from humans has not been systematically combined for analysis. The aim of this meta-analysis is to bridge this gap by analyzing previously published research on human heart failure patients with untargeted metabolomics to understand whether microbially-mediated metabolites are important for heart failure development or recovery.

**Methods**: A systematic survey of the literature identified approximately 700 articles discussing HF, the microbiome, and metabolomics. Of these, 82 were primary studies of heart failure patients, 61 involved human adults, 23 included an untargeted metabolomics measure, and 3 studies had data that was usable and publicly accessible. These studies include a GCMS study from the stool of HF patients and controls, NMR of saliva and exhaled breath condensate from HF and control, and LCMS from left ventricle of HF patients undergoing transplantation therapy and unused donor hearts.

**Results**: Significant differences were observed from PCA between HF and control samples for stool and left ventricle, but not saliva or EBC samples. OPLS-DA was conducted for stool and ventricle samples, and further revealed significant group differences as well as the direction which metabolites contribute to variance. Univariate testing with FDR correction of p values revealed 8 significant microbially relevant metabolites (p < 0.005 after correction), most notably asparagine from left ventricle and 2-methylbutyryl carnitine from stool.

**Conclusion**: Overall, this meta-analysis found, though there is much discussion of the role of the microbiome in health outcomes in heart failure, limited research has been conducted in human populations. Overall, some microbial co-metabolites from both stool and heart were significantly associated with HF. Future work should dig deeper into the mechanisms behind these associations.

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## Background

Heart failure is a leading cause of death globally with rising incidence [(1)](https://www.zotero.org/google-docs/?OTIM4n). Heart failure is a disease in which the heart cannot successfully pump blood through the body, which can ultimately result in death. Though recent advances in novel therapies have increased survival for heart failure patients [(2)](https://www.zotero.org/google-docs/?68aLs1), the overall 3 year survival rate is still low [(3)](https://www.zotero.org/google-docs/?3i0TZp). Further, the improvements in therapies have increased the number of individuals living with chronic heart failure, which in turn increases the need for better therapies and prevention of heart failure alongside the increased demand from aging populations. One hopeful domain for new therapies has been the gut microbiome, as the microbes living in the gastrointestinal tract are thought to contribute towards disease etiology.

The human gut microbiome has been implicated in nearly every aspect of human health [(4–6)](https://www.zotero.org/google-docs/?AOz1A8). This is unsurprising as humans have evolved with microbes throughout our entire evolutionary history, which has allowed the human body to outsource certain key functions, like immune priming [(7)](https://www.zotero.org/google-docs/?3eqIeo) or digestion [(8)](https://www.zotero.org/google-docs/?Hkokzy), to these evolutionary partners. In the context of human health, microbiota, or the microbes living inside a person, present a potentially easily modifiable target for new disease therapies as the microbiota is functionally stable but malleable over the human lifespan [(9)](https://www.zotero.org/google-docs/?JwJU87). For heart failure specifically, the metabolites produced by gut microbes can be protective in the context of adverse cardiovascular events, such as butyrate [(10, 11)](https://www.zotero.org/google-docs/?TF3hmT), or risk factors, such as Trimethylamine, or TMA [(12)](https://www.zotero.org/google-docs/?2g8DXA).

As sequencing and metabolomics technology has advanced, much evidence has been generated from our group and others showing that the gut microbiome can indeed be a protective or risk factor for HF through modulation of metabolites [(12–14)](https://www.zotero.org/google-docs/?SUwYOF), though the evidence from human patients has not yet been systematically combined. In part, it is difficult to combine results across different metabolomics studies, as different studies will use different analytical methods, different heart failure categories, and different bioinformatics methods when analyzing their data. Further, though this topic is widely discussed with many review papers published on the topic, few empirical studies have actually been conducted with human patients with heart failure, as heart failure is still a relatively complex clinical event that is much simpler to study in animal models than in people.

The goal of this study is to examine all the evidence of microbially-mediated metabolites in heart failure patients from previously published studies which implement untargeted metabolomic methods of any biospecimen type. While certain microbially relevant metabolites (such as short chain fatty acids) are difficult to capture from untargeted methods, it is entirely possible that many important bacterially-mediated metabolites are missed from the targeted methods used for the most well-documented microbial metabolites. This study will apply the same analytical methods to previously published data with the same filtering parameters, which will make understanding the relationship between microbially-mediated metabolites in heart failure patients across studies and sample types easier, and in turn generate a more holistic understanding of the relevance of the microbiota in heart failure.

## Methods

### Systematic Survey of Literature

We conducted a systematic survey of all literature published prior to April 1, 2024 in PubMed and Web of Science and on metabolomics repositories MetaboLights and MASSIVE. The following search terms were used for PubMed and Web of Science search: ((metabolomics) OR (metabolite) OR (metabolic ) OR (LCMS) or (LC-MS) OR (liquid chromatography mass spectrometry) OR (GC-MS) OR (GCMS) OR (gas chromatography mass spectrometry) OR (NMR) OR (Nuclear magnetic resonance spectroscopy) ) AND ((Heart failure with preserved ejection fraction) OR (heart failure)) AND ((human) OR (person) OR (patient) OR (patients) OR (Homo sapiens)) AND ((microbe) OR (microbiome) OR (microbial) OR (microbially) OR (microbiota) OR (gut dysbiosis) OR (microbes) OR (holobiont)). For searching on metabolomics repositories, entries were searched with the following: Description="heart failure"; Sample Type = "Homo sapiens".

Papers were included if they met the following criteria: 1) an observational study of human adults (18 years of age or older) diagnosed with heart failure (not other cardiovascular diagnoses that puts one at risk for heart failure); 2) collected an untargeted metabolomics measure (e.g., GCMS, LCMS, NMR, etc.) from a biological sample (e.g. plasma or stool); 3) made their data publicly available without undue burden (e.g. “data available upon reasonable request”) and not in an unusable format (e.g. raw data with no internal controls data), and 4) the main body of the text is in English. Papers were excluded if they were not in English, not specifically examining heart failure, studied children or nonhuman mammals, conducted a targeted metabolomics method, or did not have publicly available data. We identified a total of 708 citations from PubMed (681), Web of Science (11), MassIVE (10), and Metabolomics Workbench (6). Of these 708 papers/studies in public repositories, 662 were in English with a PubMed ID and not duplicated in the citation plus repository search; 78 were studies of heart failure; 57 of those included human adults; 16 of those papers included an untargeted metabolomics measure; and only 3 of those 16 papers had publicly available data. A schematic is provided in **Supplemental Figure 1** highlighting the papers that passed each qualification check. All citations were screened by two reviewers for qualification criteria. Any discordance between screeners’ records was resolved through discussion between screeners, and overall discordance was low (~2-5% for reviewer pairs).

The resulting three studies which qualified for inclusion are as follows: **D1** is a study from Huang et al., 2023 [(14)](https://www.zotero.org/google-docs/?Odt7cA) examining stool metabolites with GCMS from 96 heart failure patients with/without depression and controls. Heart failure was diagnosed via the 2016 ESC guideline criteria for the diagnosis of heart failure, and integration of at least one of the following: elevated natriuretic peptide levels, objective evidence of cardiogenic pulmonary or body circulation stasis, including imaging (e.g., chest radiograph and echocardiogram), or resting or stress hemodynamic monitoring (e.g., right heart catheter and pulmonary artery catheter). **D2** comes from the Metabolites Repository and does not have an affiliated publication [(15)](https://www.zotero.org/google-docs/?OZIMJY). This cohort comprises of 11 stable heart failure patients and 12 control patients who provided exhaled breath condensate (EBC) and saliva sent for NMR. Heart failure was diagnosed as classic systolic HF who are admitted to St Mary’s Hospital with decompensated disease. Control subjects are age and gender matched controls. **D3** is a study from Li et al. 2020 [(16)](https://www.zotero.org/google-docs/?sKnUQR) of left ventricle (LV) metabolites from LCMS for 51 heart failure patients undergoing heart transplantation and donor hearts that could not be used due to patient-donor mismatch, transportation issues, and other reasons. Fifty-six donor hearts are age matched and represent even gender spread.

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### Meta-Analysis

Processed data files were downloaded from their respective repository. All samples had heart failure status available in metadata files, though only one study included patient sex. As such, all samples were included but only heart failure status is examined in subsequent analysis without correcting for sex (though all studies specify using sex- and age- matched controls). Abundance data on the repositories was already processed and normalized, so no further transformation (e.g. log transformation or uv scaling) was done. As each metabolomics study examines a different biospecimen tissue with a different metabolomics method, a late integration approach is used for analysis where studies are analyzed for significance separately and then their final predictions are combined [(17)](https://www.zotero.org/google-docs/?CNiQ0P).

For dimension reduction on already normalized abundance tables, metabolites that are present in fewer than 20% of samples per group (heart failure status) within their respective study were excluded from further analysis. Principal component analysis (PCA) was used to assess correlation structure of the data as a whole with R package PCAtest() (100 bootstraps 100 permutations) [(18)](https://www.zotero.org/google-docs/?l9TEDQ). Univariate testing is conducted with a two-tailed Mann-Whitney U-Test with function wicox\_test() [(19)](https://www.zotero.org/google-docs/?0OSCWd) and false discovery rate for multiple test corrections with p.adjust() [(20)](https://www.zotero.org/google-docs/?4aGPOe) (**Table 2)**. Metabolites significant after FDR correction were assigned to KEGG pathways with MetaboAnalyst [(21)](https://www.zotero.org/google-docs/?4H05J2) and underwent pathway analysis with FELLA() [(22)](https://www.zotero.org/google-docs/?qGtOer). Results of the top 5 KEGG pathways are in **Table 3**.

Ortholog partial least squares discriminant analysis (OPLS-DA) was conducted only for studies with significant group differences in PCA to examine what features contribute the most to group differences (D1 and D3) with R package ropls() [(23)](https://www.zotero.org/google-docs/?3WWhr1). OPLS-DA is inclined to overfit models to data, as discussed in [(24)](https://www.zotero.org/google-docs/?jNwaHZ), and the risk of overfitting results is reduced by only conducting OPLS-DA for data with significant group differences as revealed by PCA. VIPs from the OPLS-DA are not reported as VIPs are not useful for OPLS modeling of a single response (i.e. heart failure status). Further results of specific metabolites from OPLS-DA are not reported but included in figure [2] as univariate testing with stringent FDR correction and p-value filtering more appropriate for biomarker analysis [(25, 26)](https://www.zotero.org/google-docs/?SJgNGo).

## Results

Of 708 papers identified, only 3 papers had data properly uploaded to metabolomics repositories that were usable for further analysis (**Supplemental Figure 1**). A total of 162 patient samples were included for analysis in this study (HF = 111, control = 51). PCA plots and significance testing revealed that metabolites from stool (D1) and left ventricle (D3) of heart failure patients are significantly different than metabolites from the same tissues of controls (PCA p value < 0.01, high OPLS-DA R2Y and Q2Y; **Figure 1/Table 1**). While D2 EBC samples had a single significant principal component, this is likely due to confounding, such as from multicollinearity among metabolic pathways, rather than meaningful group differences, as significance was only in one dimension (PC1). D2 saliva samples had uneven group dispersion (within-group variance), so no conclusions should be drawn from D2 saliva PCA results.

OPLS-DA on D1 and D3 both revealed stable models, as indicated by high R2Y and Q2Y values, with high prediction (Q2Y) and low error (RMSEE). Overall, each model captured 22.9% and 31.2% of the variance in the data as indicated by R2X for D1 and D3, respectively. This, together with PCA results, indicates there are significant metabolite differences in the stool and left ventricle of heart failure patients compared to controls, but not in saliva or EBC.

Univariate testing with false discovery rate corrections show significant metabolites in D1 and D3 (**Figure 2)**, but no metabolites were significant in D2 saliva or EBC after FDR correction. Overall, a total of 66 metabolites were significant from univariate testing after FDR correction. Of these, 8 metabolites are either solely synthesized by bacteria or related to both bacterial and human metabolism (e.g. Asparagine from D1 stool). Two of these metabolites are related to ketone body synthesis through acetyl-CoA metabolism (Alpha-hydroxyisobutyric acid and 2-Methylbutyryl Carnitine), which is previously found to increase risk of thrombosis in a sick population [(27)](https://www.zotero.org/google-docs/?0DACKW). Other bacterial metabolites like asparagine have similarly been implicated in cardiovascular health previously [(28, 29)](https://www.zotero.org/google-docs/?OmXVOK), though it is not clear if this asparagine is from bacterial or human metabolism. Only three metabolites were significantly differentially abundant in both stool and left ventricle samples of heart failure patients–serotonin, N-carbamoyl-aspartate, and thymidine (serotonin and N-carbamoyl-aspartate adjusted p < 0.01; thymidine adjusted p < 0.05;  **Table 2**). Pathway analysis revealed that the top 5 KEGG pathways implicated in heart failure are more relevant to human and not bacterial metabolism, so they are not discussed here **(Table 3)**.

## Discussion

The human microbiome has been widely discussed as important for cardiovascular health and recovery after adverse cardiac events, though actual human studies of patients with heart failure are limited. This is potentially unsurprising, as the integration of microbiome and metabolomics measures into clinical studies is a relatively recent transition in research. However, the proportion of review studies (n=98) to empirically based studies (n=23) is quite surprising. Nonetheless, three empirical research studies (out of approximately 700 screened) were conducted that included a measure of untargeted metabolomics and have data properly deposited on public repositories. The objective of this study was to apply a unified data analysis approach to previously published data from heart failure patients in order to understand what microbially-relevant metabolites are consistently associated with heart failure.

We found that there were significant differences in metabolites from the stool and left ventricle of the heart from heart failure patients compared to controls, however, no significant differences from saliva or exhaled breath condensate (EBC) between HF and control. The study with saliva and EBC samples had a much smaller sample size (N = 23), so it is not clear if differences may exist in the saliva or EBC for heart failure patients in a larger sample. Nonetheless, of 66 metabolites identified from univariate testing, 8 of these metabolites are potential bacterial metabolites–most are human and bacterial metabolites. Most of these metabolites have been previously implicated in cardiovascular health outside of the original studies the datasets came from (Alpha-hydroxyisobutyric acid [(30, 31)](https://www.zotero.org/google-docs/?XKJPk4); 2-Methylbutyryl Carnitine [(27)](https://www.zotero.org/google-docs/?jTpKVx); Asparagine [(32)](https://www.zotero.org/google-docs/?0O5aqz); tetrahydrobiopterin [(33)](https://www.zotero.org/google-docs/?Lj7DUW); 7-methylguanine [(34)](https://www.zotero.org/google-docs/?RiW4l7); Cadaverine [(35)](https://www.zotero.org/google-docs/?4C16zl)), though minimal mechanistic work has been published with any of these metabolites, particularly in the context of the human microbiome.

Most original studies of the role of the microbiome in heart failure focus on the role of Trimethylamine N-oxide, or TMAO, 3-indolepropionic acid, or IPA, or short chain fatty acids as as they are clearly microbial metabolites with links to diet. However, these most discussed microbial metabolites require special experimental preparation, isotope labeling, or mass spectrometry settings due to their physical and biochemical properties, and are difficult to detect from untargetted metabolomic methods [(36–38)](https://www.zotero.org/google-docs/?t0g3sV). Little attention is paid to these other microbial metabolites that are more easily detectable by untargetted metabolomics. Future work trying to understand the influence of the microbiome on heart failure and cardiovascular health can follow up on these bacterial metabolites which have been repeatedly found to be up/downregulated in heart failure patients. Many of these metabolites are also implicated in metabolic syndrome [(35, 39, 40)](https://www.zotero.org/google-docs/?as6xCi). There is strong comorbidity between heart failure and metabolic syndrome [(41, 42)](https://www.zotero.org/google-docs/?IOuYRB), though not all patients with heart failure have metabolic syndrome and vice versa. It will be increasingly important to understand the role of metabolic disease in the generation of these metabolites and subsequent adverse cardiovascular events, particularly in the context of the microbiome, as the microbiota in the gastrointestinal tract process dietary foods into metabolites in a way that may be relevant to the development or progression of metabolic syndrome [(43, 44)](https://www.zotero.org/google-docs/?HAYeqD). As these microbially-mediated metabolites have been found to be significant for heart failure or cardiovascular events more broadly in multiple studies, it is worthwhile for future research to further the therapeutic potential of these metabolites.

The most significant KEGG pathway from D1 stool samples was the biosynthesis of unsaturated fatty acids. Previous research of human stool has found that gut microbiomes cluster by saturated fatty acid and polyunsaturated fatty acids and are relevant to steatosis [(45)](https://www.zotero.org/google-docs/?4NMfOK), and that dietary long chain unsaturated fatty acids can promote obesity in a microbe-dependent manner [(46)](https://www.zotero.org/google-docs/?Zmx5GF). Nucleotide metabolism was the second most significant pathway for both stool and left ventricle samples, possibly due to a shift from aerobic to anaerobic glycolysis as previously reported in congestive heart failure patients [(47)](https://www.zotero.org/google-docs/?yv9CM2). cGMP-PKG signaling pathway and cAMP signaling pathway are related to ligands that bind PKG and PKA respectively, and significant for both stool and left ventricle samples. This significance is attributed almost entirely to adenosine and serotonin, of which adenosine is upregulated in the left ventricle of heart failure patients, and serotonin is downregulated stool and left ventricle of heart failure patients. These broad signaling pathways don’t reveal much information beyond disrupted cell signaling without follow-up animal models, which may be an opportunity for future researchers.

One limitation of this study is the lack of control for sex. It is known that there can be sex differences in heart failure outcomes and relevant metabolites [(16, 48)](https://www.zotero.org/google-docs/?duEuRk), but as two of the three studies did not include patient sex in their publicly available metadata file, we are unable to control for it evenly in our analysis. An additional limitation of this meta-analysis is the relatively small sample size for a meta-analysis. This is largely due to inaccessible data accompanying publications. This also highlights a need for consistent, open-access data when participants consent to having their data publicly available. Nonetheless, this study highlights the relevance of the microbially produced metabolites in people living with heart failure. These metabolites present an opportunity for future work to confirm mechanistic pathways to identify potential disease mechanisms and test if modulation of the microbiota, and subsequently those metabolic pathways, can influence disease outcomes.

This study was able to implement a late-stage integration model for untargetted metabolomic data from heart failure patients with the same statistical methods across multiple datasets, as opposed to conducting an analysis on the statistics reported from multiple studies as is the traditional meta-analysis model. This is beneficial as the same statistical analysis and filtering methods when applied to different sets of data make the connections between significant results across studies more straightforward, whereas the original papers implemented different types of models and heart failure subgroupings that could make it difficult to understand the relevance of results between studies with the traditional meta-analysis approach. We found that, overall, the most reported on microbial metabolites in heart failure reviews (TMAO, IPA, SCFAs) are not detected in traditional untargetted metabolomics (and therefore not included in this study), but that 8 other bacterial metabolites are found to be significant across the stool and heart tissue of heart failure patients. These metabolites present the opportunity for future work to confirm mechanisms and create a more cohesive understanding of the role of the microbiome in people with heart failure.

**Conflicts of Interest**

Authors have no conflicts of interest to declare.

**Data Availability**

All data is previously published and publicly available. D1 data is available from MetaboLights under Project ID MTBLS8183. D2 saliva and EBC data are available from Metabolomics Workbench under Project ID PR000430. D3 data are available from Metabolomics Workbench under Project ID ST001364.

**Code Availability**

The code for all analyses accompanying this paper has been uploaded to github at <https://github.com/ewissel/HF_Metabolomics_MetaAnalysis>.

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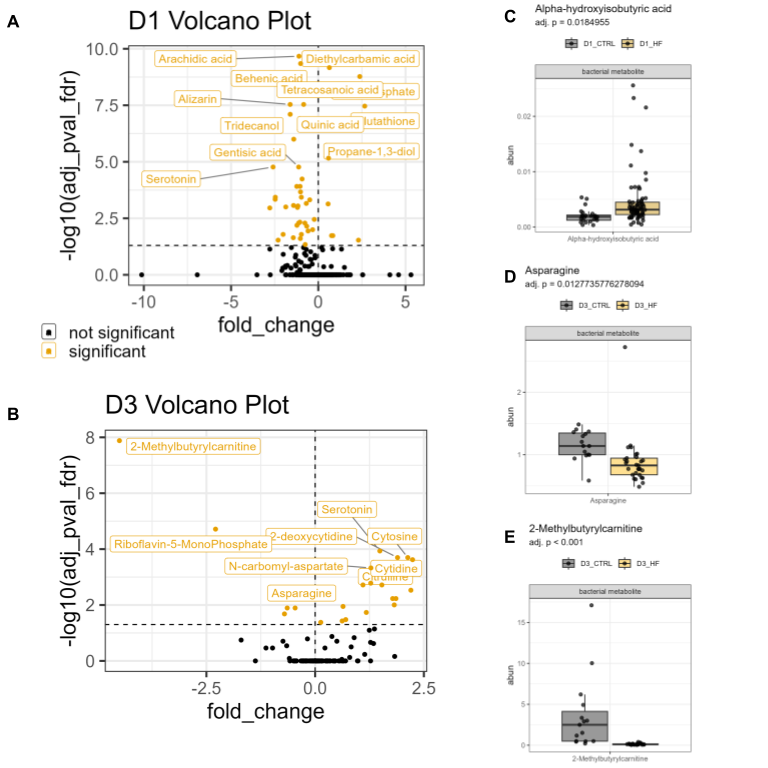
## Figure 1: Dimension Reduction: PCA and OPLS-DA Results

Principal component analysis for each study labeled by heart failure status. D1 stool (**A**) and D3 left ventricle (**B**) both display significant group differences as calculated by PCAtest(). D2 EBC (**C**) and saliva (**D**) do not display significant group differences, though PC1 is significant for D2 EBC (likely due to collinearity in metabolic pathways). D2 saliva PCA shows uneven group dispersion, so no further conclusions from D2 saliva PCA can be made. OPLS-DA plots for D1 (**E**) and D3 (**F**) show clear separation between heart failure conditions. R2X represents the fraction of variance explained by the metabolites, while R2Y and Q2Y represent the stability of the model. Q2Y is specifically the predictive performance of the model on all permutations of the model, with higher values indicating better model performance and stability across permutations. RMSEE is the root mean squared error of estimation where lower values show better model performance. OPLS-DA is not conducted for D2 samples as there are no significant group differences from the PCA analysis.

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## Figure 2: Differential Abundance of Significant Metabolites

Differential abundance volcano plots of the log fold change in metabolite abundance for D1 from stool (**A)** and D3 left ventricle (**B)**. Negative changes indicate metabolites that are higher in control samples than heart failure samples (left of the vertical dotted line), while positive fold changes indicate metabolites significantly more abundant in heart failure samples. Golden colored dots indicate which metabolites were significant after FDR correction (p < 0.05). Bacterially-relevant metabolites are shown in their reported abundance in heart failure versus control samples (**C, D, E** and **F**).



## Table 1: PCA Statistics

|  |  |  |  |
| --- | --- | --- | --- |
| **Study** | **P value** | **Number of significant axes** | **Percent variance explained by significant axes** |
| D1 | P < 0.001 | 20 | 62.1% |
| D2\_saliva | P < 0.001  (assumptions violated) | 3 | 66.6% |
| D2\_EBC | P < 0.001 (PC1 only) | 1 | 34.1% |
| D3 | P < 0.001 | 5 | 52.9% |

PCA revealed significant differences between group structure for D1 and D3 (observed psi and phi greater than null, p value < 0.01). While D2 EBC samples had a single significant principal component, this is likely due to confounding, such as from multicollinearity among metabolic pathways, rather than meaningful group differences, as significance was only in one dimension (PC1). Further, D2 saliva samples had uneven beta dispersion across groups (within-group variance), violating the PCA assumptions of multivariate homogeneity of group dispersions, so no conclusions of significance can be drawn from D2 PCA results.

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## Table 2: Univariate Statistics for Significant Metabolites with FDR Correction

|  |  |  |  |
| --- | --- | --- | --- |
| **metabolite** | **studyID** | **adj\_pval\_fdr** | **direction** |
| Kynurenine | D3 | 0.0112658640444824000000000000 | upregulated in D3 HF |
| **Serotonin** | D3 | 0.0001164007993275150000000000 | downregulated in D3 HF |
| **Serotonin** | D1 | 0.0000170307200000000000000000 | downregulated in D1 HF |
| Tryptamine | D1 | 0.0292728800000000000000000000 | downregulated in D1 HF |
| N-Acetyl-L-Alanine | D3 | 0.0127735776278094000000000000 | downregulated in D1 HF |
| Cystamine | D3 | 0.0019170586229779100000000000 | upregulated in D3 HF |
| Cysteamine | D3 | 0.0184662779826645000000000000 | upregulated in D3 HF |
| Gentisic acid | D1 | 0.0000170307200000000000000000 | looks like the relationship is driven by outliers |
| Glutathione | D1 | 0.0000000344211900000000000000 | upregulated in D1 HF |
| Catechin | D1 | 0.0115187700000000000000000000 | downregulated in D1 HF |
| 2-Methylbutyryl Carnitine (possible bacterial metabolite) | D3 | 0.0000000131528803925703000000 | downregulated in HF |
| 4a-carbinolamine tetrahydrobiopterin (possible bacterial metabolite) | D1 | 0.0456873600000000000000000000 | downregulated in D1 HF |
| 7-methylguanine (possible bacterial metabolite) | D1 | 0.0292728800000000000000000000 | upregulated in D1 HF |
| Alpha-hydroxyisobutyric acid (possible bacterial metabolite) | D1 | 0.0184955000000000000000000000 | upregulated in D1 HF |
| Asparagine (possible bacterial metabolite) | D3 | 0.0127735776278094000000000000 | downregulated in D3 HF |
| Tridecanol (possible bacterial metabolite) | D1 | 0.0000000792403500000000000000 | downregulated in D1 HF |
| Cadaverine (possible bacterial metabolite) | D1 | 0.0009947241000000000000000000 | downregulated in D1 HF |
| Erythrose (possible bacterial metabolite) | D1 | 0.0004821730000000000000000000 | downregulated in D1 HF |
| 1,2,3-trihydroxybenzene | D1 | 0.0051383160000000000000000000 | downregulated in D1 HF |
| 1-monopalmitin | D1 | 0.0100972600000000000000000000 | downregulated in D1 HF |
| 4-methylcatechol | D1 | 0.0162782000000000000000000000 | marginally downregulated in D1 HF |
| Alizarin | D1 | 0.0000000290159500000000000000 | downregulated in D1 HF |
| Behenic acid | D1 | 0.0000000004508335000000000000 | downregulated in D1 HF |
| Caffeic acid | D1 | 0.0064565200000000000000000000 | downregulated in D1 HF |
| Coniferin | D1 | 0.0070677150000000000000000000 | downregulated in D1 HF |
| D-myo-inositol 4-phosphate | D1 | 0.0008985698000000000000000000 | downregulated in D1 HF |
| Pyrrole-2-carboxylic acid | D1 | 0.0000576901100000000000000000 | downregulated in D1 HF |
| Quinic acid | D1 | 0.0000010015490000000000000000 | downregulated in D1 HF |
| Tetracosane | D1 | 0.0006951052000000000000000000 | down regulated in D1 HF, but looks like split response for HF |
| Tetracosanoic acid | D1 | 0.0000000290159500000000000000 | downregulated in D1 HF |
| Trimethylamine-N-oxide | D3 | 0.0029561339818442300000000000 | upregulated in D3 HF |
| Arabinofuranose | D1 | 0.0002146062000000000000000000 | downregulated in D1 HF |
| Hexuronic acid | D1 | 0.0248123900000000000000000000 | downregulated in D1 HF |
| Propyl-l-carnitine | D3 | 0.0417059916448870000000000000 | upregulated in D3 HF |
| Pyrophosphate | D1 | 0.0000000016879950000000000000 | upregulated in D1 HF |
| Myo-inositol phosphate | D1 | 0.0010463640000000000000000000 | downregulated in D1 HF |
| L-cysteine-glycine | D1 | 0.0046854950000000000000000000 | downregulated in D1 HF |
| Dodecanedioic acid | D1 | 0.0228279300000000000000000000 | downregulated in D1 HF |
| Dodecanol | D1 | 0.0001225832000000000000000000 | downregulated in D1 HF |
| Hydroquinone | D1 | 0.0007319480000000000000000000 | upregulated in D1 HF |
| Lanosterol | D1 | 0.0011005240000000000000000000 | downregulated in D1 HF |
| Tranexamic acid | D1 | 0.0004573456000000000000000000 | downregulated in D1 HF |
| 2-amino-7,10-dimethyldibenzo[b,f][1,4]oxazepin-11(10h)-one | D1 | 0.0035415070000000000000000000 | downregulated in D1 HF |
| 3,4-dihydroxyphenylacetic acid (DOPAC) | D1 | 0.0162782000000000000000000000 |  |
| Diethylcarbamic acid | D1 | 0.0000000006829925000000000000 | upregulated in D3 HF |
| Digitoxose | D1 | 0.0051383160000000000000000000 | downregulated in D1 HF |
| N-[(pentafluorophenyl)methylene]-alpha,4-dihydroxy-benzeneethanamine | D1 | 0.0008538479000000000000000000 | downregulated in D1 HF |
| Propane-1,3-diol | D1 | 0.0000069161990000000000000000 | upregulated in D1 HF |
| 2-deoxycytidine | D3 | 0.0002005507359324480000000000 | upregulated in D3 HF |
| Adenosine | D3 | 0.0058833743736860900000000000 | upregulated in D3 HF |
| Cytidine | D3 | 0.0002391390948575170000000000 | upregulated in D3 HF |
| Cytosine | D3 | 0.0002005507359324480000000000 | upregulated in D3 HF |
| Thymidine | D3 | 0.0372617166618133000000000000 | marginally upregulated in D1 and D3 HF; split response |
| Thymidine | D1 | 0.0184955000000000000000000000 | marginally upregulated in D1 and D3 HF; split response |
| Uridine | D3 | 0.0099221603032106000000000000 | upregulated in D3 HF but appears to have split effect in HF |
| Arachidic acid | D1 | 0.0000000002133653000000000000 | downregulated in D1 HF |
| 3-(4-hydroxyphenyl)-1,1,3-trimethylindan-5-ol | D1 | 0.0001225832000000000000000000 | downregulated in D1 HF; marginal effect |
| Thyroxine | D3 | 0.0019170586229779100000000000 | upregulated in D3 HF |
| Citrulline | D3 | 0.0016534168080224000000000000 | upregulated in D3 HF |
| Guanidoacetate | D3 | 0.0332509978829732000000000000 | upregulated in D1 HF |
| N-carbamoyl-aspartate | D1 | 0.0003696032000000000000000000 | marginally downregulated in D1 HF |
| N-carbamoyl-aspartate | D3 | 0.0004717938196814980000000000 | upregulated in D3 HF |
| Putrescine | D1 | 0.0003696032000000000000000000 | downregulated in D1 HF |
| Ureidopropionic-acid | D3 | 0.0208246091663729000000000000 | downregulated in D3 HF |
| Biotin | D3 | 0.0058833743736860900000000000 | upregulated in D3 HF |
| Riboflavin-5-MonoPhosphate | D3 | 0.0000192580090414551000000000 | downregulated in D3 HF |

## Table 3: Top 5 KEGG Pathways

D1 Stool:

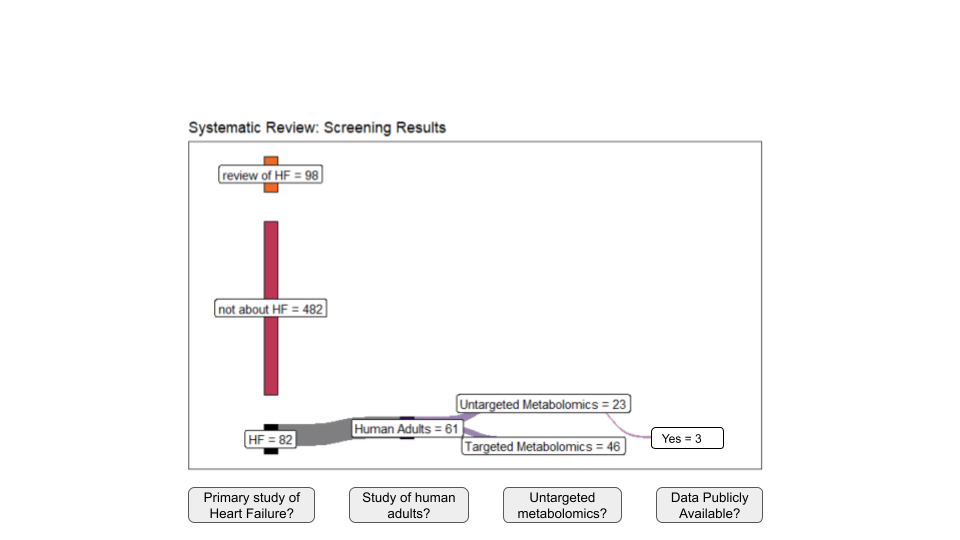
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | KEGG.id | Entry.type | KEGG.name | p.score | studyID |
| 1 | hsa01040 | pathway | Biosynthesis of unsaturated fatty acids | 8.34E-05 | D1 |
| 2 | hsa01232 | pathway | Nucleotide metabolism | 0.002083187464 | D1 |
| 3 | hsa04022 | pathway | cGMP-PKG signaling pathway | 8.34E-05 | D1 |
| 4 | hsa04024 | pathway | cAMP signaling pathway | 0.01608202051 | D1 |
| 5 | hsa04151 | pathway | PI3K-Akt signaling pathway | 0.002083187464 | D1 |

D3 Left Ventricle:

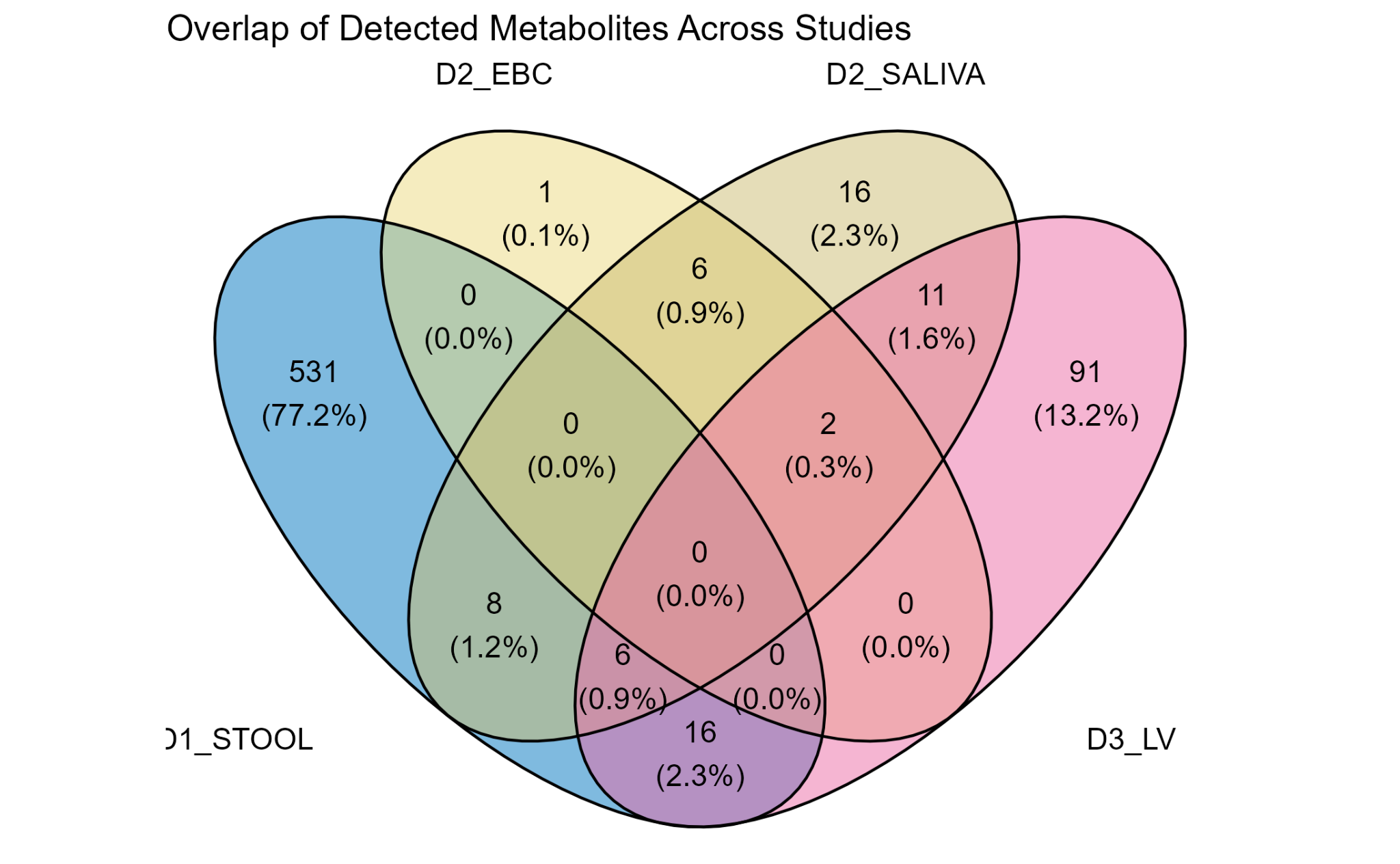
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | KEGG.id | Entry.type | KEGG.name | p.score | studyID |
| 1 | hsa00240 | pathway | Pyrimidine metabolism | 0.002083187464 | D3 |
| 2 | hsa01232 | pathway | Nucleotide metabolism | 8.34E-05 | D3 |
| 3 | hsa02010 | pathway | ABC transporters | 0.004083020755 | D3 |
| 4 | hsa04022 | pathway | cGMP-PKG signaling pathway | 0.002083187464 | D3 |
| 5 | hsa04024 | pathway | cAMP signaling pathway | 0.01208235392 | D3 |

## Supplemental Figure 1: Systematic Screening Results

This is a schematic overview of the papers that we identified, and how many were excluded from each qualification check. We identified a total of 708 citations from PubMed (681), Web of Science (11), MassIVE (10), and Metabolomics Workbench (6). Of these 708 papers, 662 were in English with a PubMedID; 78 were studies of heart failure; 57 of those included human adults; 16 of those papers included an untargeted metabolomics measure; and only 3 of those 16 papers had publicly available data. An additional 3 datasets had a public repository for their raw data that was faulty in some way (repo was not published; no metadata files included; no quality control/internal standards for reference included). One of the studies had two metabolomics measures available (saliva and exhaled breath condensate).



## Supplemental Figure 2: Metabolite Overlap Across Studies



This venn diagram shows the overlapping metabolites between the studies included in this meta-analysis. D1 is a study of GCMS from stool; D2 has exhaled breath condensate (EBC) and saliva for NMR; D3 is a study of left ventricle metabolites from LCMS. This figure does not distinguish between metabolites present in heart failure versus control patients, but rather examines the presence/absence of a metabolite in a cohort as a whole. As all studies used different metabolomic methods and different biospecimen samples, it was not expected to have high overlap between samples.

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