# Metabolomics Workbench and the National Metabolomics Data Repository

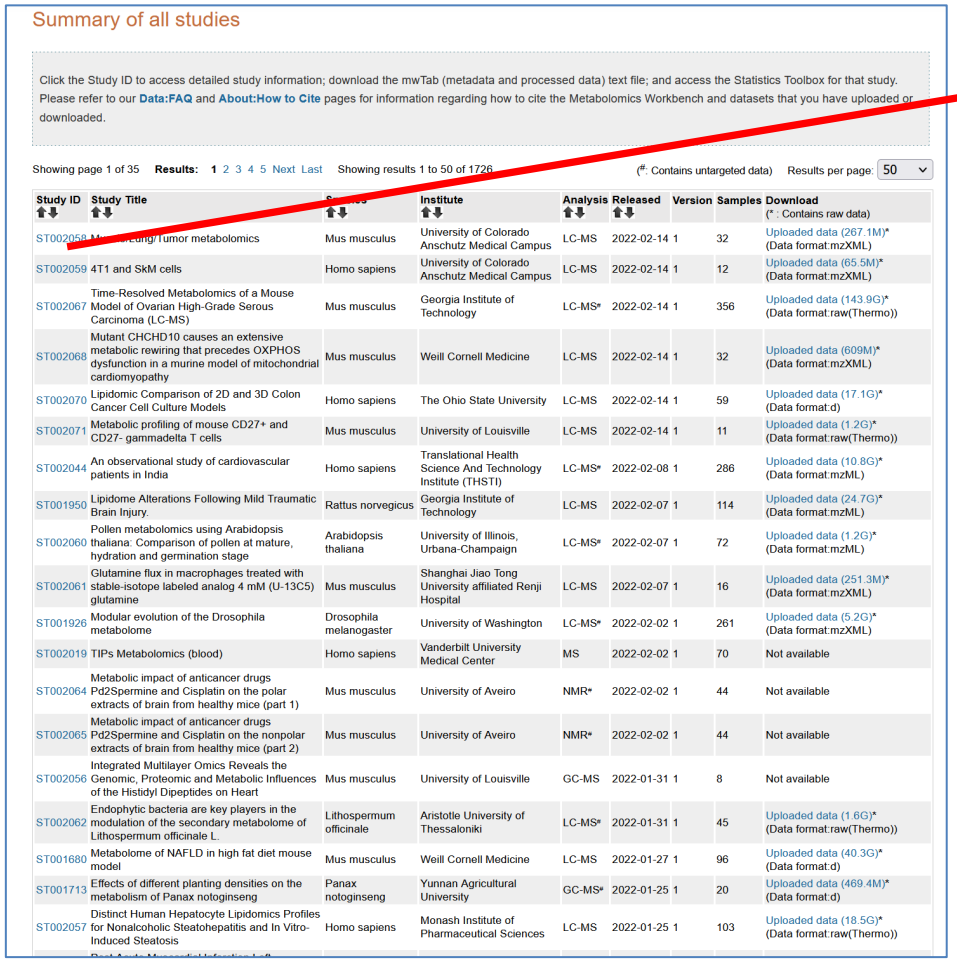
University of California San Diego
and
San Diego Supercomputer Center

## NMDR analysis tools

NIH Common Fund's National Metabolomics Data Repository
(supported by NIH grant, U2C-DK119886)

# NMDR:Study-level analysis options

## Study summary page



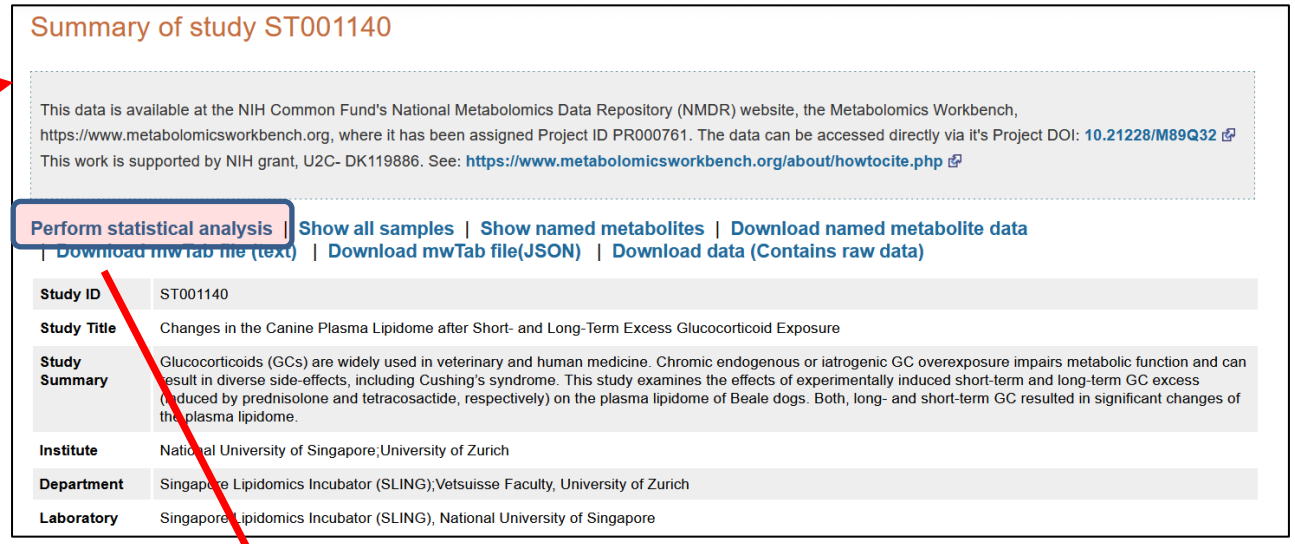
Summary of all studies

Click the Study ID to access detailed study information; download the mwTab (metadata and processed data) text file; and access the Statistics Toolbox for that study. Please refer to our **Data:FAQ** and **About:How to Cite** pages for information regarding how to cite the Metabolomics Workbench and datasets that you have uploaded or downloaded.

Showing page 1 of 35 **Results:** 1 2 3 4 5 Next Last Showing results 1 to 50 of 1726 (#: Contains untargeted data) Results per page: 50

| Study ID | Study Title | Species | Institute | Analysis | Released | Version | Samples | Download (*: Contains raw data) |
|---|---|---|---|---|---|---|---|---|
| ST002058 | M...eding/Tumor metabolomics | Mus musculus | University of Colorado Anschutz Medical Campus | LC-MS | 2022-02-14 | 1 | 32 | Uploaded data (267.1M)* (Data format:mzXML) |
| ST002059 | 4T1 and SkM cells | Homo sapiens | University of Colorado Anschutz Medical Campus | LC-MS | 2022-02-14 | 1 | 12 | Uploaded data (65.5M)* (Data format:mzXML) |
| ST002067 | Time-Resolved Metabolomics of a Mouse Model of Ovarian High-Grade Serous Carcinoma (LC-MS) | Mus musculus | Georgia Institute of Technology | LC-MS* | 2022-02-14 | 1 | 356 | Uploaded data (143.9G)* (Data format:raw(Thermo)) |
| ST002068 | Mutant CHCHD10 causes an extensive metabolic rewiring that precedes OXPHOS dysfunction in a murine model of mitochondrial cardiomyopathy | Mus musculus | Weill Cornell Medicine | LC-MS | 2022-02-14 | 1 | 32 | Uploaded data (609M)* (Data format:mzXML) |
| ST002070 | Lipidomic Comparison of 2D and 3D Colon Cancer Cell Culture Models | Homo sapiens | The Ohio State University | LC-MS | 2022-02-14 | 1 | 59 | Uploaded data (17.1G)* (Data format:d) |
| ST002071 | Metabolic profiling of mouse CD27+ and CD27- gammadelta T cells | Mus musculus | University of Louisville | LC-MS | 2022-02-14 | 1 | 11 | Uploaded data (1.2G)* (Data format:raw(Thermo)) |
| ST002044 | An observational study of cardiovascular patients in India | Homo sapiens | Translational Health Science And Technology Institute (THSTI) | LC-MS* | 2022-02-08 | 1 | 286 | Uploaded data (10.8G)* (Data format:mzML) |
| ST001950 | Lipidome Alterations Following Mild Traumatic Brain Injury. | Rattus norvegicus | Georgia Institute of Technology | LC-MS | 2022-02-07 | 1 | 114 | Uploaded data (24.7G)* (Data format:mzML) |
| ST002060 | Pollen metabolomics using Arabidopsis thaliana: Comparison of pollen at mature, hydration and germination stage | Arabidopsis thaliana | University of Illinois, Urbana-Champaign | LC-MS* | 2022-02-07 | 1 | 72 | Uploaded data (1.2G)* (Data format:mzML) |
| ST002061 | Glutamine flux in macrophages treated with stable-isotope labeled analog 4 mM (U-13C5) glutamine | Mus musculus | Shanghai Jiao Tong University affiliated Renji Hospital | LC-MS | 2022-02-07 | 1 | 16 | Uploaded data (251.3M)* (Data format:mzXML) |
| ST001926 | Modular evolution of the Drosophila metabolome | Drosophila melanogaster | University of Washington | LC-MS* | 2022-02-02 | 1 | 261 | Uploaded data (5.2G)* (Data format:mzXML) |
| ST002019 | TIPs Metabolomics (blood) | Homo sapiens | Vanderbilt University Medical Center | MS | 2022-02-02 | 1 | 70 | Not available |
| ST002064 | Metabolic impact of anticancer drugs Pd2Spermine and Cisplatin on the polar extracts of brain from healthy mice (part 1) | Mus musculus | University of Aveiro | NMR* | 2022-02-02 | 1 | 44 | Not available |
| ST002065 | Metabolic impact of anticancer drugs Pd2Spermine and Cisplatin on the nonpolar extracts of brain from healthy mice (part 2) | Mus musculus | University of Aveiro | NMR* | 2022-02-02 | 1 | 44 | Not available |
| ST002056 | Integrated Multilayer Omics Reveals the Genomic, Proteomic and Metabolic Influences of the Histidyl Dipeptides on Heart | Mus musculus | University of Louisville | GC-MS | 2022-01-31 | 1 | 8 | Not available |
| ST002062 | Endophytic bacteria are key players in the modulation of the secondary metabolome of Lithospermum officinale L. | Lithospermum officinale | Aristotle University of Thessaloniki | LC-MS* | 2022-01-31 | 1 | 45 | Uploaded data (1.6G)* (Data format:raw(Thermo)) |
| ST001680 | Metabolome of NAFLD in high fat diet mouse model | Mus musculus | Weill Cornell Medicine | LC-MS | 2022-01-27 | 1 | 96 | Uploaded data (40.3G)* (Data format:d) |
| ST001713 | Effects of different planting densities on the metabolism of Panax notoginseng | Panax notoginseng | Yunnan Agricultural University | GC-MS* | 2022-01-25 | 1 | 20 | Uploaded data (469.4M)* (Data format:d) |
| ST002057 | Distinct Human Hepatocyte Lipidomics Profiles for Nonalcoholic Steatohepatitis and In Vitro-Induced Steatosis | Homo sapiens | Monash Institute of Pharmaceutical Sciences | LC-MS | 2022-01-25 | 1 | 103 | Uploaded data (18.5G)* (Data format:raw(Thermo)) |

## Study detail page



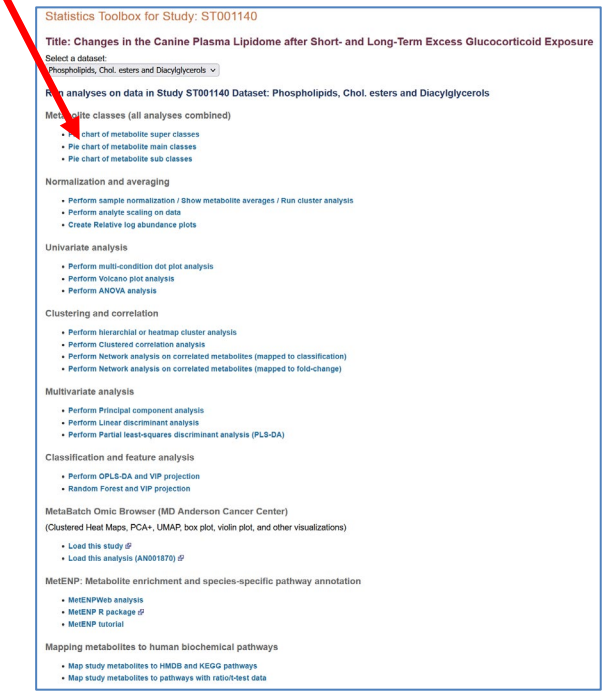
Summary of study ST001140

This data is available at the NIH Common Fund's National Metabolomics Data Repository (NMDR) website, the Metabolomics Workbench, https://www.metabolomicsworkbench.org, where it has been assigned Project ID PR000761. The data can be accessed directly via it's Project DOI: 10.21228/M89Q32
This work is supported by NIH grant, U2C- DK119886. See: https://www.metabolomicsworkbench.org/about/howtocite.php

**Perform statistical analysis** | Show all samples | Show named metabolites | Download named metabolite data | Download mwTab file (text) | Download mwTab file(JSON) | Download data (Contains raw data)

| | |
|---|---|
| **Study ID** | ST001140 |
| **Study Title** | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure |
| **Study Summary** | Glucocorticoids (GCs) are widely used in veterinary and human medicine. Chromic endogenous or iatrogenic GC overexposure impairs metabolic function and can result in diverse side-effects, including Cushing's syndrome. This study examines the effects of experimentally induced short-term and long-term GC excess (induced by prednisolone and tetracosactide, respectively) on the plasma lipidome of Beale dogs. Both, long- and short-term GC resulted in significant changes of the plasma lipidome. |
| **Institute** | National University of Singapore;University of Zurich |
| **Department** | Singapore Lipidomics Incubator (SLING);Vetsuisse Faculty, University of Zurich |
| **Laboratory** | Singapore Lipidomics Incubator (SLING), National University of Singapore |

## Study statistics page



Statistics Toolbox for Study: ST001140

**Title: Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure**

Select a dataset: Phospholipids, Chol. esters and Diacylglycerols

**Run analyses on data in Study ST001140 Dataset: Phospholipids, Chol. esters and Diacylglycerols**

Metabolite classes (all analyses combined)
- Pie chart of metabolite super classes
- Pie chart of metabolite main classes
- Pie chart of metabolite sub classes

Normalization and averaging
- Perform sample normalization / Show metabolite averages / Run cluster analysis
- Perform analyte scaling on data
- Create Relative log abundance plots

Univariate analysis
- Perform multi-condition dot plot analysis
- Perform Volcano plot analysis
- Perform ANOVA analysis

Clustering and correlation
- Perform hierarchial or heatmap cluster analysis
- Perform Clustered correlation analysis
- Perform Network analysis on correlated metabolites (mapped to classification)
- Perform Network analysis on correlated metabolites (mapped to fold-change)

Multivariate analysis
- Perform Principal component analysis
- Perform Linear discriminant analysis
- Perform Partial least-squares discriminant analysis (PLS-DA)

Classification and feature analysis
- Perform OPLS-DA and VIP projection
- Random Forest and VIP projection

MetaBatch Omic Browser (MD Anderson Cancer Center)
(Clustered Heat Maps, PCA+, UMAP, box plot, violin plot, and other visualizations)
- Load this study
- Load this analysis (AN001870)

MetENP: Metabolite enrichment and species-specific pathway annotation
- MetENPWeb analysis
- MetENP R package
- MetENP tutorial

Mapping metabolites to human biochemical pathways
- Map study metabolites to HMDB and KEGG pathways
- Map study metabolites to pathways with ratio/t-test data

# Analysis tools applied to the data for the selected NMDR study
# A study may have more than one analysis (dataset)

**Metabolite classification**
Pie chart of metabolite super classes
Pie chart of metabolite main classes
Pie chart of metabolite sub classes

**Normalization and averaging**
Show Metabolite averages per experimental factor
Perform normalization on data
Create Relative log abundance plots

**Univariate analysis**
Perform multi-condition dot plot analysis
Perform Volcano plot analysis
Perform ANOVA analysis and class enrichment analysis
MetENP analysis

**Clustering and correlation**
Perform hierarchical or heatmap cluster analysis
Perform Clustered correlation analysis
Perform Network analysis on correlated metabolites (mapped to classification)
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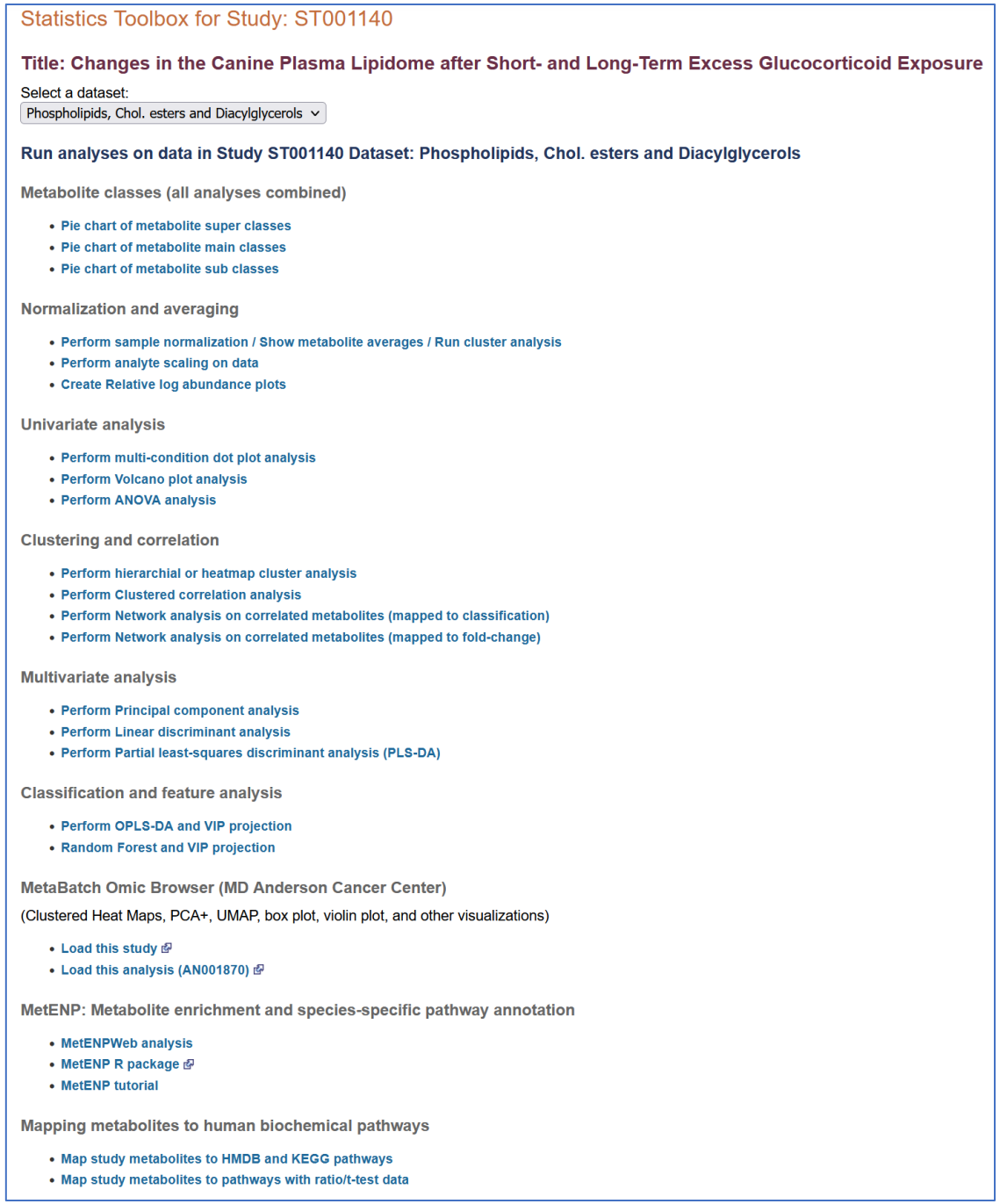
**Mapping metabolites to human biochemical pathways**
Map study metabolites to HMDB and KEGG pathways
Map study metabolites to pathways with ratio/t-test data



# Study-specific analysis toolbox

Statistics Toolbox for Study: ST001140

**Title: Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure**

Select a dataset:

Phospholipids, Chol. esters and Diacylglycerols

**Run analyses on data in Study ST001140 Dataset: Phospholipids, Chol. esters and Diacylglycerols**

### Metabolite classes (all analyses combined)

- Pie chart of metabolite super classes
- Pie chart of metabolite main classes
- Pie chart of metabolite sub classes

### Normalization and averaging

- Perform sample normalization / Show metabolite averages / Run cluster analysis
- Perform analyte scaling on data
- Create Relative log abundance plots

### Univariate analysis

- Perform multi-condition dot plot analysis
- Perform Volcano plot analysis
- Perform ANOVA analysis

### Clustering and correlation

- Perform hierarchial or heatmap cluster analysis
- Perform Clustered correlation analysis
- Perform Network analysis on correlated metabolites (mapped to classification)
- Perform Network analysis on correlated metabolites (mapped to fold-change)

### Multivariate analysis

- Perform Principal component analysis
- Perform Linear discriminant analysis
- Perform Partial least-squares discriminant analysis (PLS-DA)

### Classification and feature analysis

- Perform OPLS-DA and VIP projection
- Random Forest and VIP projection

### MetaBatch Omic Browser (MD Anderson Cancer Center)

(Clustered Heat Maps, PCA+, UMAP, box plot, violin plot, and other visualizations)

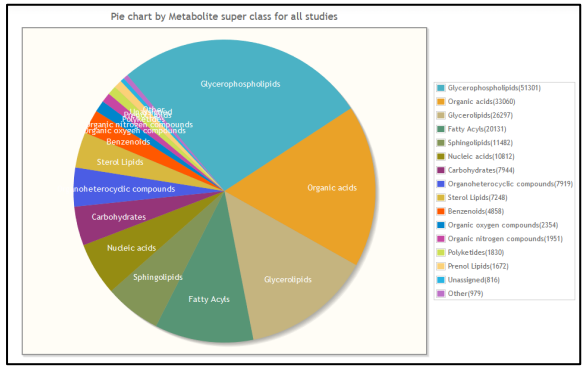
- Load this study
- Load this analysis (AN001870)

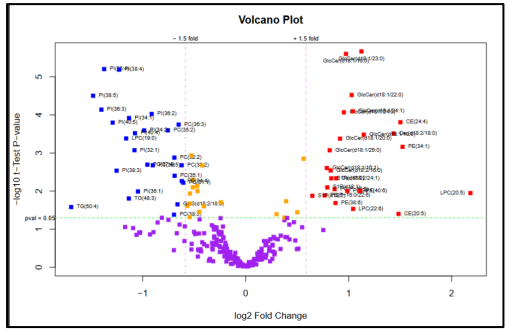
### MetENP: Metabolite enrichment and species-specific pathway annotation

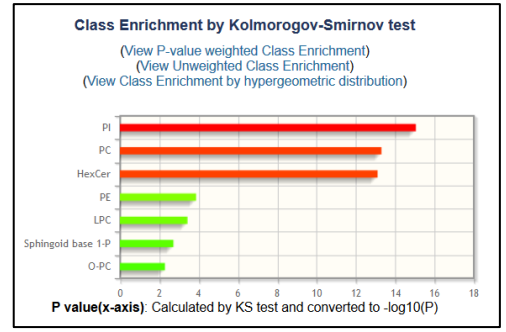
- MetENPWeb analysis
- MetENP R package
- MetENP tutorial

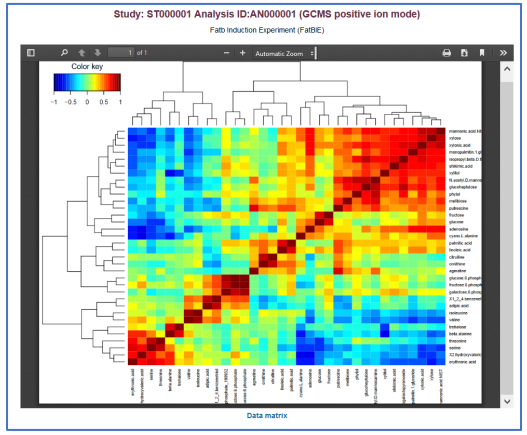
### Mapping metabolites to human biochemical pathways

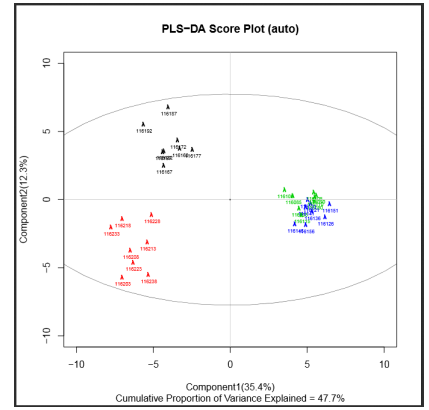
- Map study metabolites to HMDB and KEGG pathways
- Map study metabolites to pathways with ratio/t-test data



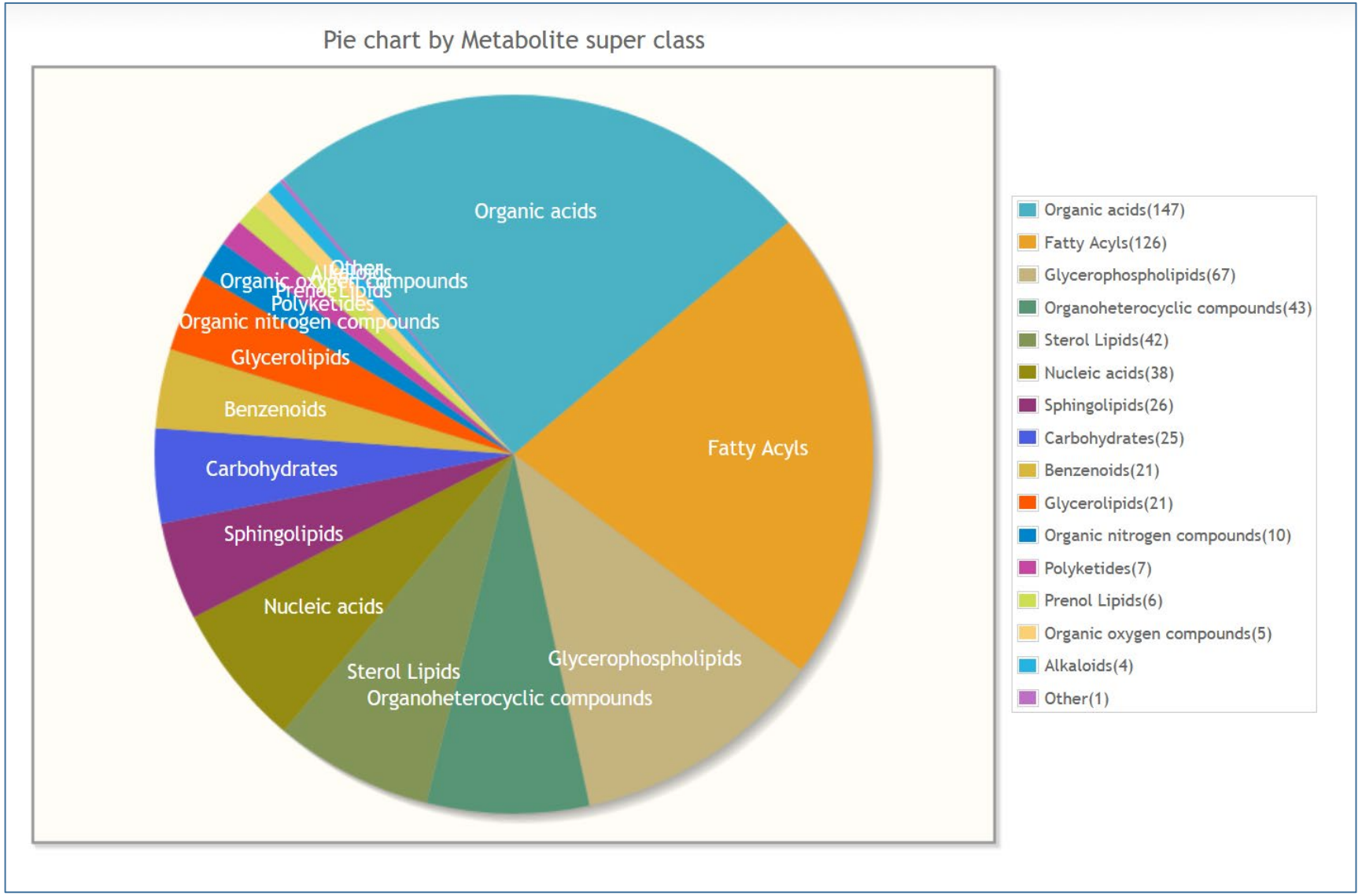



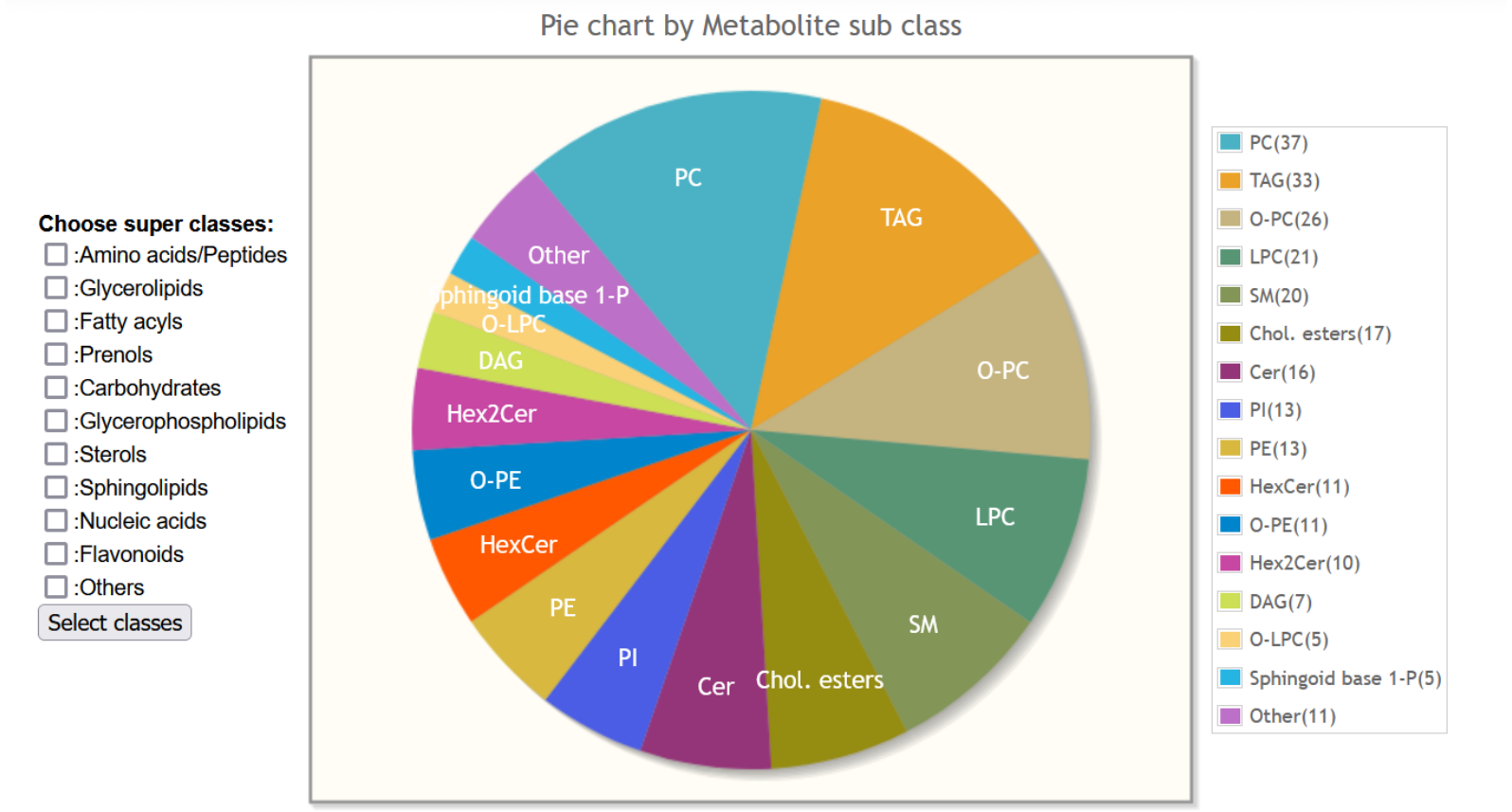



# Pie-chart of metabolite super classes detected in a study

## Metabolite names are mapped to RefMet which is linked to a chemical classification system

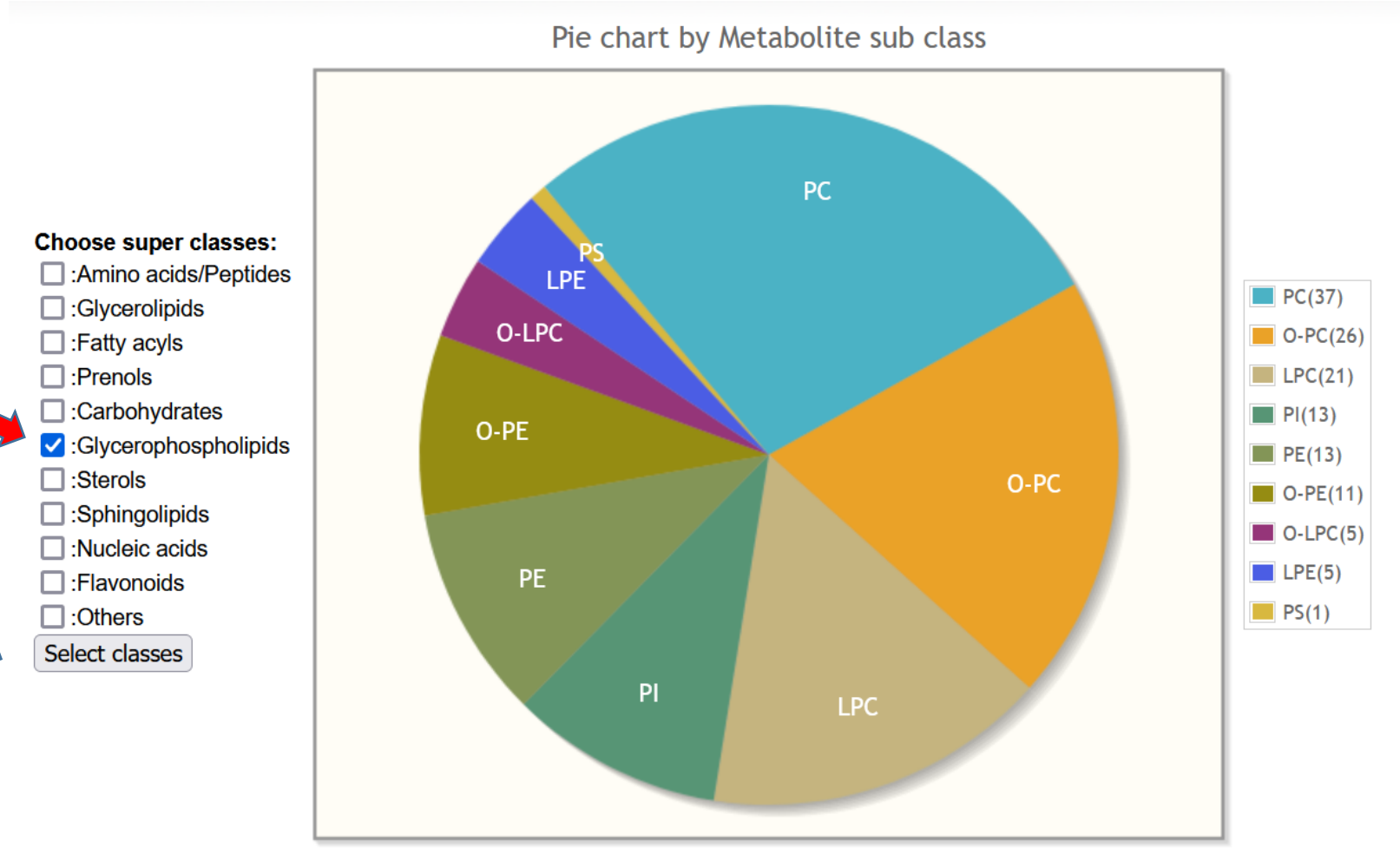


# Pie-chart of metabolite sub classes detected in a study



# Pie-chart of metabolite sub classes detected in a study

## Restrict to a selected super class



# Normalization and averaging: Sample normalization

## Sample normalization

**Normalization and averaging**

- Perform sample normalization / Show metabolite averages / Run cluster analysis
- Perform analyte scaling on
- Create Relative log abundance plo

Values have been normalized by dividing by the sample mean across all metabolites for each experimental condition

Run Hierarchial cluster analysis on this study | Run Heatmap cluster analysis on this study

| Metabolite | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| Cer(d16:1/24:0) | 0.0022 | 0.0022 | 0.0013 | 0.0014 |
| Cer(d18:1/16:0) | 0.0345 | 0.0541 | 0.0344 | 0.0421 |
| Cer(d18:1/18:0) | 0.0354 | 0.0588 | 0.0358 | 0.1090 |
| Cer(d18:1/20:0) | 0.0262 | 0.0248 | 0.0141 | 0.0333 |
| Cer(d18:1/22:0) | 0.1734 | 0.1488 | 0.0848 | 0.1310 |
| Cer(d18:1/23:0) | 0.1474 | 0.1571 | 0.0948 | 0.1476 |
| Cer(d18:1/24:0) | 0.1479 | 0.1616 | 0.0966 | 0.1649 |
| Cer(d18:1/24:1) | 0.0810 | 0.1207 | 0.1002 | 0.1511 |
| Cer(d18:1/25:0) | 0.0144 | 0.0118 | 0.0095 | 0.0130 |
| Cer(d18:1/25:1) | 0.0030 | 0.0039 | 0.0025 | 0.0044 |
| Cer(d18:2/16:0) | 0.0028 | 0.0036 | 0.0042 | 0.0028 |
| Cer(d18:2/18:0) | 0.0016 | 0.0047 | NA | NA |
| Cer(d18:2/22:0) | 0.0134 | 0.0123 | 0.0088 | 0.0083 |
| Cer(d18:2/23:0) | 0.0112 | 0.0174 | 0.0127 | 0.0114 |
| Cer(d18:2/24:0) | 0.0124 | 0.0189 | 0.0120 | 0.0160 |
| Cer(d18:2/24:1) | 0.0051 | 0.0107 | 0.0125 | 0.0091 |
| GlcCer(d18:1/16:0) | 0.0098 | 0.0220 | 0.0141 | 0.0175 |
| GlcCer(d18:1/16:1) | 0.0006 | 0.0012 | 0.0008 | 0.0012 |
| GlcCer(d18:1/18:0) | 0.0009 | 0.0023 | 0.0009 | 0.0028 |

# Normalization and averaging: Analyte scaling

**Normalization and averaging**

- Perform sample normalization / Show metabolite averages / Run cluster analysis
- Perform analyte scaling on data
- Create Relative log abundance plots

## Analyte scaling: choose scaling method and analysis type

| Scale | Method | Study ID | Study Title | MS Analysis Type |
|---|---|---|---|---|
| Run | Median | ST001140 | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Phospholipids, Chol. esters and Diacylglycerols |
| Run | Median | ST001140 | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Sphingolipids |
| Run | Median | ST001140 | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Derivatized Spingosine-1-phosphates |
| Run | Median | ST001140 | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Triacylglycerols |

**View Original data  View scaled data (Median method)**

Scaled datafile (1st 5 columns and 10 rows only)

| Samples | Class | CE.16.0. | CE.16.1. | CE.17.0. |
|---|---|---|---|---|
| Prednisolone-d0-P1 | 1 | -0.0060 | -3.5769 | 1.0000 |
| Prednisolone-d0-P2 | 1 | -10.3174 | -13.8077 | 29.0000 |
| Prednisolone-d0-P3 | 1 | 12.6527 | 10.9615 | -41.0000 |
| Prednisolone-d0-P4 | 1 | 1.1677 | 5.0385 | 15.0000 |
| Prednisolone-d0-P5 | 1 | 0.8323 | 0.7308 | 1.0000 |
| Prednisolone-d0-P6 | 1 | 3.2635 | -3.5769 | -13.0000 |
| Prednisolone-d0-P7 | 1 | 6.0299 | 3.9615 | -27.0000 |
| Prednisolone-d0-P8 | 1 | 6.4491 | 4.5000 | -27.0000 |
| Prednisolone-d4-P1 | 2 | 10.0539 | -4.1154 | -41.0000 |
| Prednisolone-d4-P2 | 2 | -1.2635 | -5.7308 | 15.0000 |

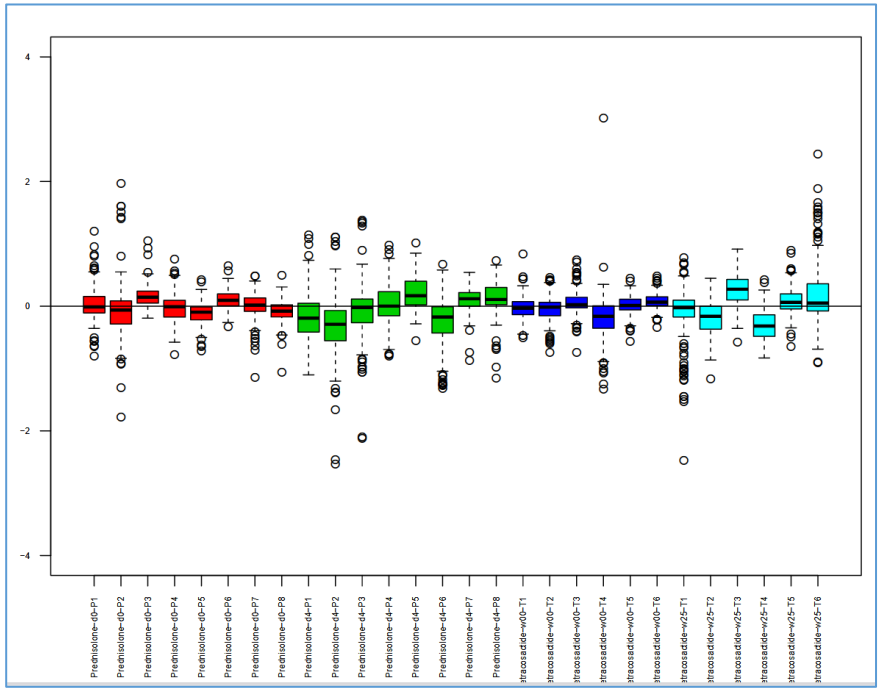
# Normalization and averaging: Abundance plots

**Normalization and averaging**

- Perform sample normalization / Show metabolite averages / Run cluster analysis
- Perform analyte scaling on data
- Create Relative log abundance plots

## Choose mode (within or across sample groups)

| Map | Study ID | Mode | Study Title | MS Analysis Type |
|---|---|---|---|---|
| Run | ST001140 | Within groups | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Phospholipids, Chol. esters and Diacylglycerols |
| Run | ST001140 | Within groups | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Sphingolipids |
| Run | ST001140 | Within groups | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Triacylglycerols |




## Relative log Abundance plots

# Volcano plot analysis tool

**Most tools contain a form where the user selects appropriate experimental groups and analysis parameters prior to running the program**

Univariate analysis

- Perform multi-condition dot plot analysis
- Perform Volcano plot analysis
- Perform ANOVA analysis

## Volcano Plot analysis for Study ST001140

**(Analysis All analyses used)**

Select one or more experimental factors for Groups 1 and 2. The members of each group should be DIFFERENT.

| Group1 | Experimental factor | Group2 |
|---|---|---|
| ☑ | TreatmentGroup:Prednisolone \| TreatmentDuration:0d \| SamplingTimePoint:before (8) | ☐ |
| ☐ | TreatmentGroup:Prednisolone \| TreatmentDuration:4d \| SamplingTimePoint:after (8) | ☑ |
| ☐ | TreatmentGroup:Tetracosactide \| TreatmentDuration:00w \| SamplingTimePoint:before (6) | ☐ |
| ☐ | TreatmentGroup:Tetracosactide \| TreatmentDuration:25w \| SamplingTimePoint:after (6) | ☐ |

**P-value cutoff:** 0.05 **Fold-change cutoff:** 1.5 **Sample normalization:** None

**Group by metabolite classification:** Sub class **Use:** Submitted metabolite names

**Maximum # of (most significant) metabolites per class to use in pvalue group calculation:** All

**Analysis:** Phospholipids, Chol. esters and Diacylglycerols **Combine data for all analyses?:** ☑ Run Volcano Plot

**In this case, samples before and after Prednisolone treatment are compared. This study contains 4 analyses (different metabolite classes) and all data will be combined. A p-value cutoff of 0.5 and fold-change cutoff of 1.5 are selected for the volcano plot analysis step. Metabolites will be classified by sub class (as opposed to main class). No sample normalization will be performed prior to analysis.**

**Analyses for this study:**

**Triacylglycerols**
**Sphingolipids**
**Phospholipids, Cholesterol esters and Diacylglycerols**
**Spingosine-1-phosphates**

# ANOVA analysis tool

**Most tools contain a form where the user selects appropriate experimental groups and analysis parameters prior to running the program**

Univariate analysis

- Perform multi-condition dot plot analysis
- Perform Volcano plot analysis
- Perform ANOVA analysis

## ANOVA analysis for Study ST001140

| ANOVA Setup | |
|---|---|
| Select Factor: | SamplingTimePoint |
| Analysis: | Phospholipids, Chol. esters and Diacylglycerols |
| P-value cutoff: | 0.05 |
| Group by metabolite classification: | Sub class |
| Maximum # of (most significant) metabolites per class to use in group calculation: | 5 |

Run ANOVA

**Choose experimental factor to analyze by ANOVA (in this case it is before and after drug treatment), analysis group, p-value cutoff and classification group.**

**Analyses for this study:**

**Triacylglycerols**
**Sphingolipids**
**Phospholipids, Cholesterol esters and Diacylglycerols**
**Spingosine-1-phosphates**

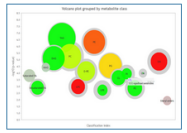
# ANOVA analysis tool: Results

**Most tools contain a form where the user selects appropriate experimental groups and analysis parameters prior to running the program**

Metabolite sub classes (mean values)

Columns are sortable

Individual metabolites (sorted by p-value)



View Bubble plot of results grouped by metabolite classification

Table of results grouped by metabolite class (using 5 most significant analytes per class)

| Metabolite class | F-value | -log10(P-value) | FDR-adjusted P-value(BH) | # of metabolites per class |
|---|---|---|---|---|
| PI | 47.763 | 6.36 | 1.91e-4 | 11(13) |
| PC | 38.360 | 5.56 | 1.26e-3 | 10(37) |
| O-PC | 19.046 | 3.73 | 2.29e-3 | 19(26) |
| O-LPC | 17.402 | 3.53 | 2.93e-3 | 1(5) |
| LPC | 18.045 | 3.34 | 2.38e-2 | 10(21) |
| Chol. esters | 19.437 | 2.92 | 3.18e-2 | 4(17) |
| O-PE | 6.665 | 1.79 | 6.64e-2 | 3(11) |
| PE | 6.261 | 1.72 | 6.68e-2 | 4(13) |

(Analysis: AN001870 )

| Factor:SamplingTimePoint | | | | | |
|---|---|---|---|---|---|
| Metabolite | F value | P-value | FDR adjusted P-value | Main class | Sub class |
| PI(38:4) | 78.118 | 2.597E-9 | 4.077E-7 | Glycerophosphoinositols | PI |
| PC(37:4) | 66.821 | 1.178E-8 | 6.267E-7 | Glycerophosphocholines | PC |
| PI(38:5) | 66.703 | 1.198E-8 | 6.267E-7 | Glycerophosphoinositols | PI |
| PC(38:4) | 44.981 | 4.079E-7 | 1.318E-5 | Glycerophosphocholines | PC |
| PC(39:5) | 44.828 | 4.197E-7 | 1.318E-5 | Glycerophosphocholines | PC |
| PI(36:4) | 42.527 | 6.506E-7 | 1.702E-5 | Glycerophosphoinositols | PI |
| LPC(19:0) | 41.711 | 7.627E-7 | 1.711E-5 | Glycerophosphocholines | LPC |
| CE(24:4) | 52.841 | 4.100E-6 | 8.047E-5 | Sterol esters | Chol. esters |
| PI(40:5) | 26.714 | 2.155E-5 | 3.759E-4 | Glycerophosphoinositols | PI |
| PI(40:4) | 24.754 | 3.587E-5 | 5.631E-4 | Glycerophosphoinositols | PI |
| PC(O-32:2) | 24.240 | 4.113E-5 | 5.668E-4 | Glycerophosphocholines | O-PC |
| LPC(22:0) | 24.047 | 4.332E-5 | 5.668E-4 | Glycerophosphocholines | LPC |
| PC(40:4) | 19.824 | 1.426E-4 | 1.723E-3 | Glycerophosphocholines | PC |
| PC(P-34:2) | 19.543 | 1.550E-4 | 1.739E-3 | Glycerophosphocholines | O-PC |
| PC(P-34:1) | 17.597 | 2.808E-4 | 2.929E-3 | Glycerophosphocholines | O-PC |
| LPC(O-20:0) | 17.402 | 2.985E-4 | 2.929E-3 | Glycerophosphocholines | O-LPC |
| PC(O-34:3) | 17.122 | 3.260E-4 | 3.011E-3 | Glycerophosphocholines | O-PC |
| PC(P-36:2) | 16.727 | 3.696E-4 | 3.224E-3 | Glycerophosphocholines | O-PC |
| PC(O-34:1) | 15.359 | 5.772E-4 | 4.549E-3 | Glycerophosphocholines | O-PC |
| PC(40:5) | 15.347 | 5.795E-4 | 4.549E-3 | Glycerophosphocholines | PC |
| PC(P-34:3) | 14.395 | 7.980E-4 | 5.966E-3 | Glycerophosphocholines | O-PC |


**Bubble plot representation of significant ANOVA metabolite classes**
**Colored circles represent significant metabolites, gray circles represent all metabolites per class**

# Volcano plot analysis results

Clickable icons

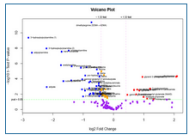
Columns are sortable

Table of results with log2 fold-change,t-test p-values and metabolite classification

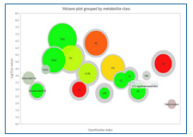
Default sort is by Main class/sub class

Click on “t-test P-value” heading to sort by that item

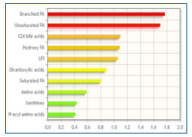
| Experimental factors selected in each group | | | |
|---|---|---|---|
| **Group1:** | TreatmentGroup:Prednisolone \| TreatmentDuration:0d \| SamplingTimePoint:before | **Group2:** | TreatmentGroup:Prednisolone \| TreatmentDuration:4d \| SamplingTimePoint:after |



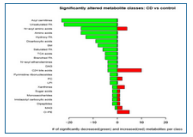
Volcano plot of all significant metabolites



Bubble plot of results grouped by metabolite class



Enrichment by metabolite class



Barplot of significant metabolites by metabolite class

List of significant metabolites (pvalue <=0.05 and fold-change >=1.5)

| Metabolite | log2 Fold Change | t-Test P-value | FDR-adjusted P-value(BH) | Main class | Sub class |
|---|---|---|---|---|---|
| Cer(d18:2/24:1) | 0.878 | 4.60e-3 | 3.08e-2 | Ceramides | Cer |
| Cer(d18:2/18:0) | 1.436 | 3.09e-4 | 4.22e-3 | Ceramides | Cer |
| CE(20:5) | 1.484 | 3.98e-2 | 1.50e-1 | Sterol esters | Chol. esters |
| CE(24:4) | 1.502 | 1.56e-4 | 3.03e-3 | Sterol esters | Chol. esters |
| GlcCer(d18:1/16:1) | 0.788 | 2.48e-3 | 1.91e-2 | Glycosphingolipids | HexCer |
| GlcCer(d18:1/25:0) | 0.816 | 8.50e-4 | 8.71e-3 | Glycosphingolipids | HexCer |
| GlcCer(d18:2/16:0) | 0.826 | 2.88e-3 | 2.11e-2 | Glycosphingolipids | HexCer |
| GlcCer(d18:1/20:0) | 0.916 | 4.21e-4 | 4.93e-3 | Glycosphingolipids | HexCer |
| GlcCer(d18:1/24:0) | 0.953 | 8.57e-5 | 2.33e-3 | Glycosphingolipids | HexCer |
| GlcCer(d18:1/16:0) | 0.974 | 2.51e-6 | 3.09e-4 | Glycosphingolipids | HexCer |
| GlcCer(d18:1/22:0) | 1.029 | 3.01e-5 | 1.29e-3 | Glycosphingolipids | HexCer |
| GlcCer(d18:1/24:1) | 1.040 | 8.03e-5 | 2.33e-3 | Glycosphingolipids | HexCer |
| GlcCer(d18:1/23:0) | 1.124 | 2.16e-6 | 3.09e-4 | Glycosphingolipids | HexCer |
| GlcCer(d18:1/18:0) | 1.147 | 3.31e-4 | 4.28e-3 | Glycosphingolipids | HexCer |
| LPC(19:0) | -1.160 | 4.16e-4 | 4.93e-3 | Glycerophosphocholines | LPC |
| LPC(22:6) | 1.043 | 2.94e-2 | 1.16e-1 | Glycerophosphocholines | LPC |
| LPC(20:5) | 2.182 | 1.13e-2 | 5.65e-2 | Glycerophosphocholines | LPC |
| PC(P-36:5) | 0.864 | 8.89e-3 | 4.97e-2 | Glycerophosphocholines | O-PC |
| PE(P-16:0/22:6) | 0.768 | 1.30e-2 | 6.25e-2 | Glycerophosphoethanolamines | O-PE |
| PC(37:4) | -0.954 | 2.02e-3 | 1.69e-2 | Glycerophosphocholines | PC |
| PC(39:5) | -0.902 | 2.10e-3 | 1.69e-2 | Glycerophosphocholines | PC |
| PC(35:2) | -0.755 | 2.54e-4 | 3.94e-3 | Glycerophosphocholines | PC |
| PC(38:3) | -0.695 | 4.16e-2 | 1.53e-1 | Glycerophosphocholines | PC |
| PC(32:2) | -0.692 | 1.32e-3 | 1.25e-2 | Glycerophosphocholines | PC |

# Volcano plot analysis results (sorted by t-test p-value)

Metabolite sub classes (mean values)

Individual metabolites (sorted by p-value)

Table of results grouped by metabolite class (using most significant analytes per class)

| Metabolite class | log2 Fold Change | -log10(P-value) | FDR-adjusted P-value(BH) | # of metabolites per class |
|---|---|---|---|---|
| LPC | -1.160 | 3.38 | 4.93e-3 | -1(21) |
| PC | -0.719 | 2.65 | 3.22e-2 | -10(37) |
| PI | -1.188 | 3.79 | 8.57e-3 | -12(12) |
| TAG | -1.414 | 1.69 | 8.92e-2 | -2(25) |
| Cer | 1.157 | 2.92 | 1.75e-2 | 2(16) |
| Chol. esters | 1.493 | 2.60 | 7.67e-2 | 2(17) |
| HexCer | 0.961 | 3.90 | 6.47e-3 | 10(10) |
| LPC | 1.613 | 1.74 | 8.65e-2 | 2(21) |
| O-PC | 0.864 | 2.05 | 4.97e-2 | 1(26) |
| O-PE | 0.768 | 1.89 | 6.25e-2 | 1(10) |
| PE | 1.124 | 2.21 | 5.06e-2 | 4(12) |
| Sphingoid base 1-P | 0.812 | 2.22 | 3.83e-2 | 2(5) |

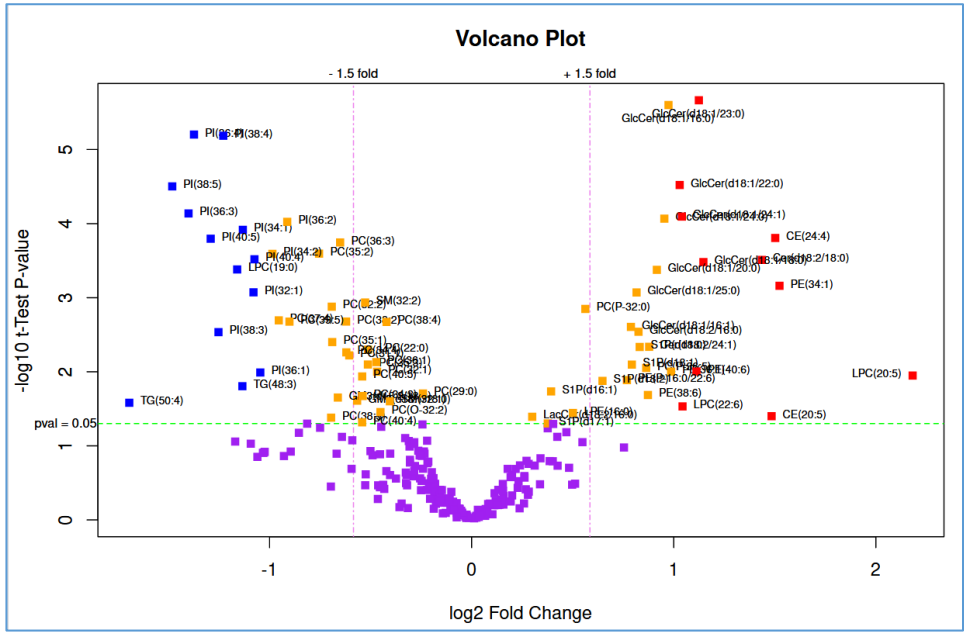
12 out of 12 PI's were significantly downregulated

4 out of 12 PE's were significantly upregulated

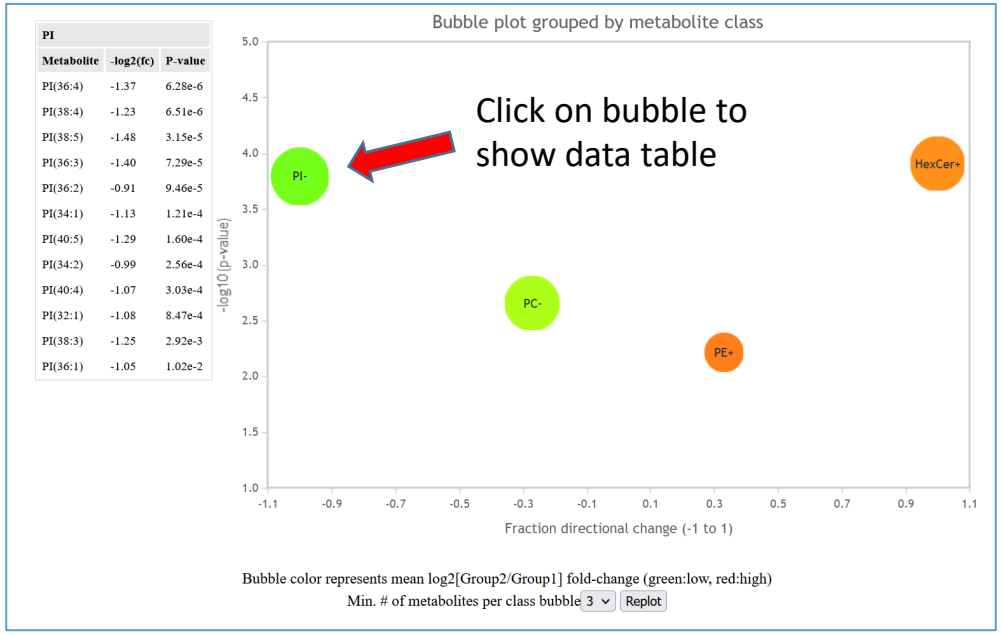
List of significant metabolites (pvalue <=0.05 and fold-change >=1.5)

| Metabolite | log2 Fold Change | t-Test P-value | FDR-adjusted P-value(BH) | Main class | Sub class |
|---|---|---|---|---|---|
| GlcCer(d18:1/23:0) | 1.124 | 2.16e-6 | 3.09e-4 | Glycosphingolipids | HexCer |
| GlcCer(d18:1/16:0) | 0.974 | 2.51e-6 | 3.09e-4 | Glycosphingolipids | HexCer |
| PI(36:4) | -1.374 | 6.28e-6 | 4.01e-4 | Glycerophosphoinositols | PI |
| PI(38:4) | -1.229 | 6.51e-6 | 4.01e-4 | Glycerophosphoinositols | PI |
| GlcCer(d18:1/22:0) | 1.029 | 3.01e-5 | 1.29e-3 | Glycosphingolipids | HexCer |
| PI(38:5) | -1.481 | 3.15e-5 | 1.29e-3 | Glycerophosphoinositols | PI |
| PI(36:3) | -1.401 | 7.29e-5 | 2.33e-3 | Glycerophosphoinositols | PI |
| GlcCer(d18:1/24:1) | 1.040 | 8.03e-5 | 2.33e-3 | Glycosphingolipids | HexCer |
| GlcCer(d18:1/24:0) | 0.953 | 8.57e-5 | 2.33e-3 | Glycosphingolipids | HexCer |
| PI(36:2) | -0.912 | 9.46e-5 | 2.33e-3 | Glycerophosphoinositols | PI |
| PI(34:1) | -1.132 | 1.21e-4 | 2.71e-3 | Glycerophosphoinositols | PI |
| CE(24:4) | 1.502 | 1.56e-4 | 3.03e-3 | Sterol esters | Chol. esters |
| PI(40:5) | -1.291 | 1.60e-4 | 3.03e-3 | Glycerophosphoinositols | PI |
| PC(36:3) | -0.651 | 1.80e-4 | 3.16e-3 | Glycerophosphocholines | PC |
| PC(35:2) | -0.755 | 2.54e-4 | 3.94e-3 | Glycerophosphocholines | PC |
| PI(34:2) | -0.986 | 2.56e-4 | 3.94e-3 | Glycerophosphoinositols | PI |
| PI(40:4) | -1.075 | 3.03e-4 | 4.22e-3 | Glycerophosphoinositols | PI |
| Cer(d18:2/18:0) | 1.436 | 3.09e-4 | 4.22e-3 | Ceramides | Cer |
| GlcCer(d18:1/18:0) | 1.147 | 3.31e-4 | 4.28e-3 | Glycosphingolipids | HexCer |
| LPC(19:0) | -1.160 | 4.16e-4 | 4.93e-3 | Glycerophosphocholines | LPC |
| GlcCer(d18:1/20:0) | 0.916 | 4.21e-4 | 4.93e-3 | Glycosphingolipids | HexCer |
| PE(34:1) | 1.523 | 6.91e-4 | 7.72e-3 | Glycerophosphoethanolamines | PE |
| PI(32:1) | -1.079 | 8.47e-4 | 8.71e-3 | Glycerophosphoinositols | PI |
| GlcCer(d18:1/25:0) | 0.816 | 8.50e-4 | 8.71e-3 | Glycosphingolipids | HexCer |

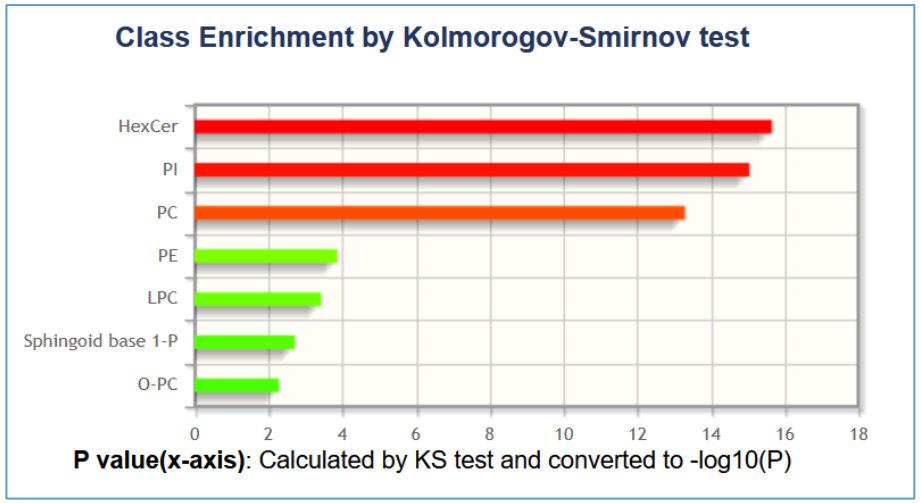
# Volcano plot analysis results (click on icons)



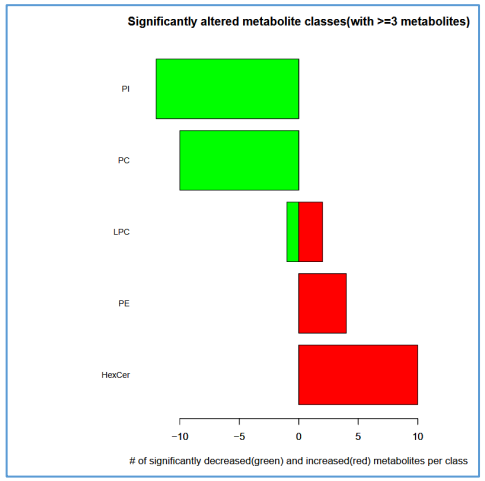

Volcano plot




Bubble plot of –log10 p-value vs fraction directional change




Class enrichment plot




Barplot of significantly altered metabolite classes

# Multi-condition dot-plot analysis

## Useful for plotting time-course data or comparing multiple experimental conditions to controls

Univariate analysis

- Perform multi-condition dot plot analysis
- Perform Volcano plot analysis
- Perform ANOVA analysis

**Example: Metabolite changes associated with methionine stress sensitivity of human breast cancer cells. Use 100uM Methionine group as control and compare 370uM Homocysteine groups at various timepoints.**

**Dot Plot analysis for Study ST000077**

Select one or more experimental factors for control and test groups. The members of each group should be DIFFERENT.

| Control(s) | Experimental factor | Test(s) |
|---|---|---|
| ☑ | Treatment:100uM Met \| Timepoint:0 hours (4) | ☐ |
| ☐ | Treatment:370uM Hcy \| Timepoint:2 hours (4) | ☑ |
| ☐ | Treatment:370uM Hcy \| Timepoint:4 hours (4) | ☑ |
| ☐ | Treatment:370uM Hcy \| Timepoint:8 hours (3) | ☑ |
| ☐ | Treatment:370uM Hcy \| Timepoint:12 hours (4) | ☑ |
| ☐ | Treatment:370uM Hcy \| Timepoint:24 hours (4) | ☑ |
| ☐ | Treatment:370uM Hcy \| Timepoint:48 hours (4) | ☑ |
| ☐ | Treatment:quality check \| Timepoint:quality check (3) | ☐ |

**x-axis labels:** 2hr__4hr__8hr__12hr__24hr__48hr **Show a single class:**

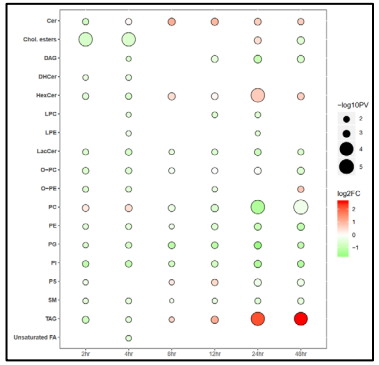
**P-value cutoff:** 0.05 **Fold-change cutoff:** 1.2 **Sample normalization:** Mean

**Group by metabolite classification:** Sub class **# of individual metabolites to display:** 30

**Maximum # of (most significant) metabolites per class to use in group calculation:** 5

**Analysis:** ESI/QTOF positive ion mode **Combine data for all analyses?:** ☑ Run Analysis

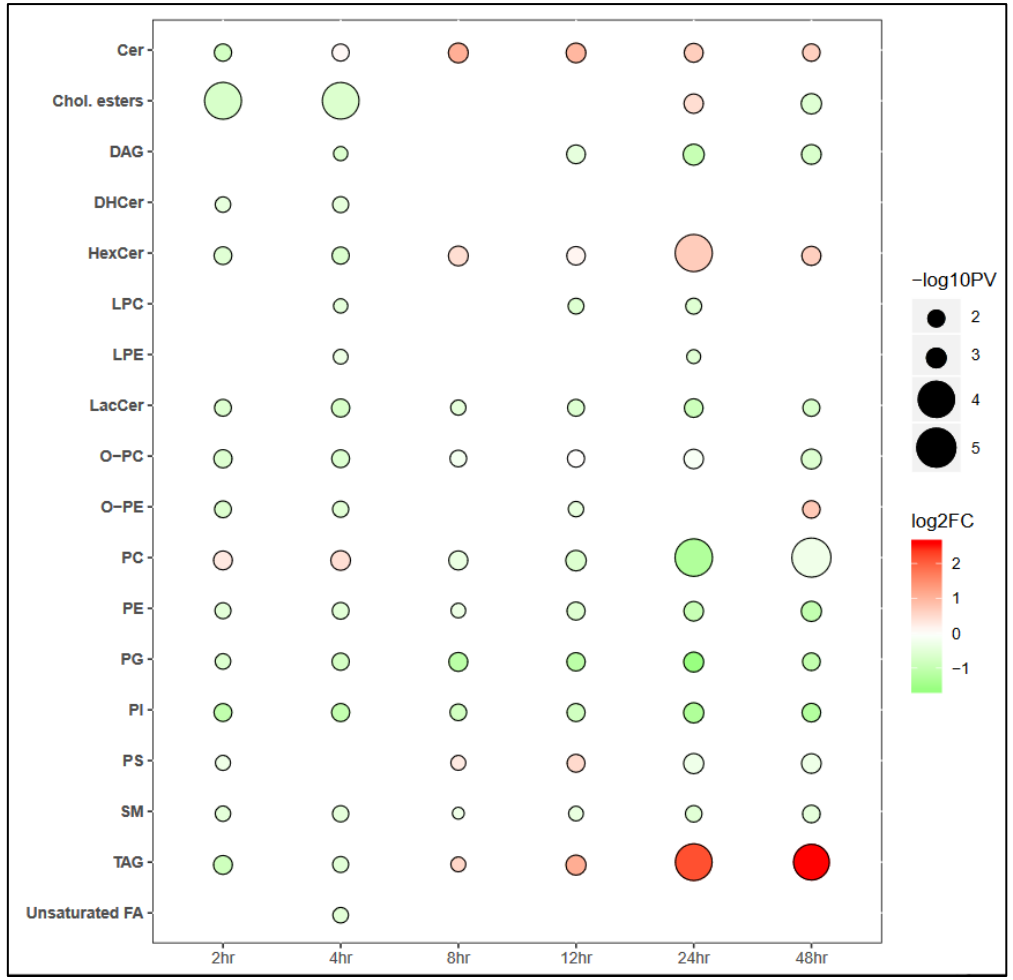
**Query builder GUI**



**Plots**

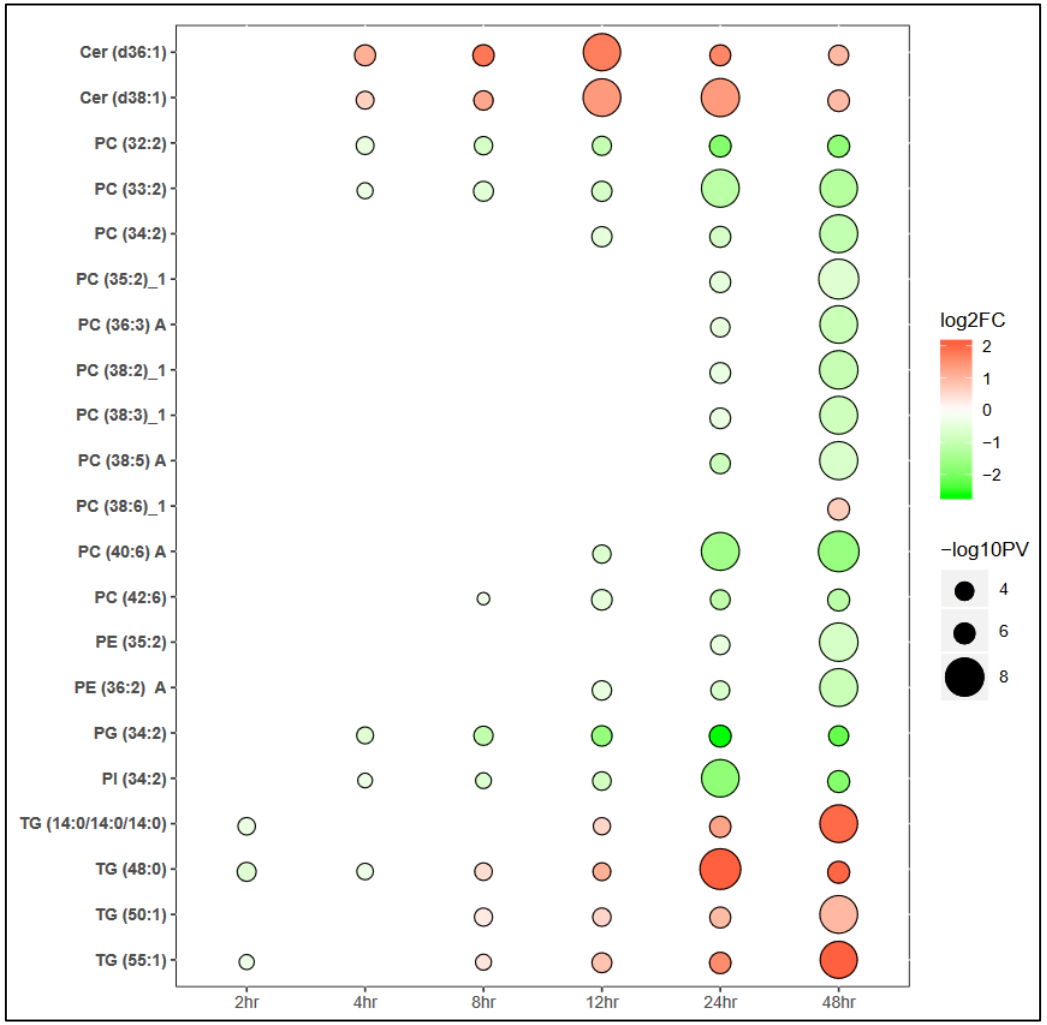
# Multi-condition dot-plot analysis results

**All groups are being compared to the control group (100uM Methionine treatment)**




Increasing time ->

**Metabolite class Plot**




Increasing time ->

**Individual metabolite Plot**

# Multi-condition dot-plot analysis

## Useful for plotting time-course data or comparing multiple experimental conditions to controls

**Dot Plot analysis for Study ST000077**

Select one or more experimental factors for control and test groups. The members of each group should be DIFFERENT.

| Control(s) | Experimental factor | Test(s) |
|---|---|---|
| ☑ | Treatment:100uM Met \| Timepoint:0 hours (4) | ☐ |
| ☐ | Treatment:370uM Hcy \| Timepoint:2 hours (4) | ☑ |
| ☐ | Treatment:370uM Hcy \| Timepoint:4 hours (4) | ☑ |
| ☐ | Treatment:370uM Hcy \| Timepoint:8 hours (3) | ☑ |
| ☐ | Treatment:370uM Hcy \| Timepoint:12 hours (4) | ☑ |
| ☐ | Treatment:370uM Hcy \| Timepoint:24 hours (4) | ☑ |
| ☐ | Treatment:370uM Hcy \| Timepoint:48 hours (4) | ☑ |
| ☐ | Treatment:quality check \| Timepoint:quality check (3) | ☐ |

**x-axis labels ?:** 2hr__4hr__8hr__12hr__24hr__48hr **Show a single class:** TAG

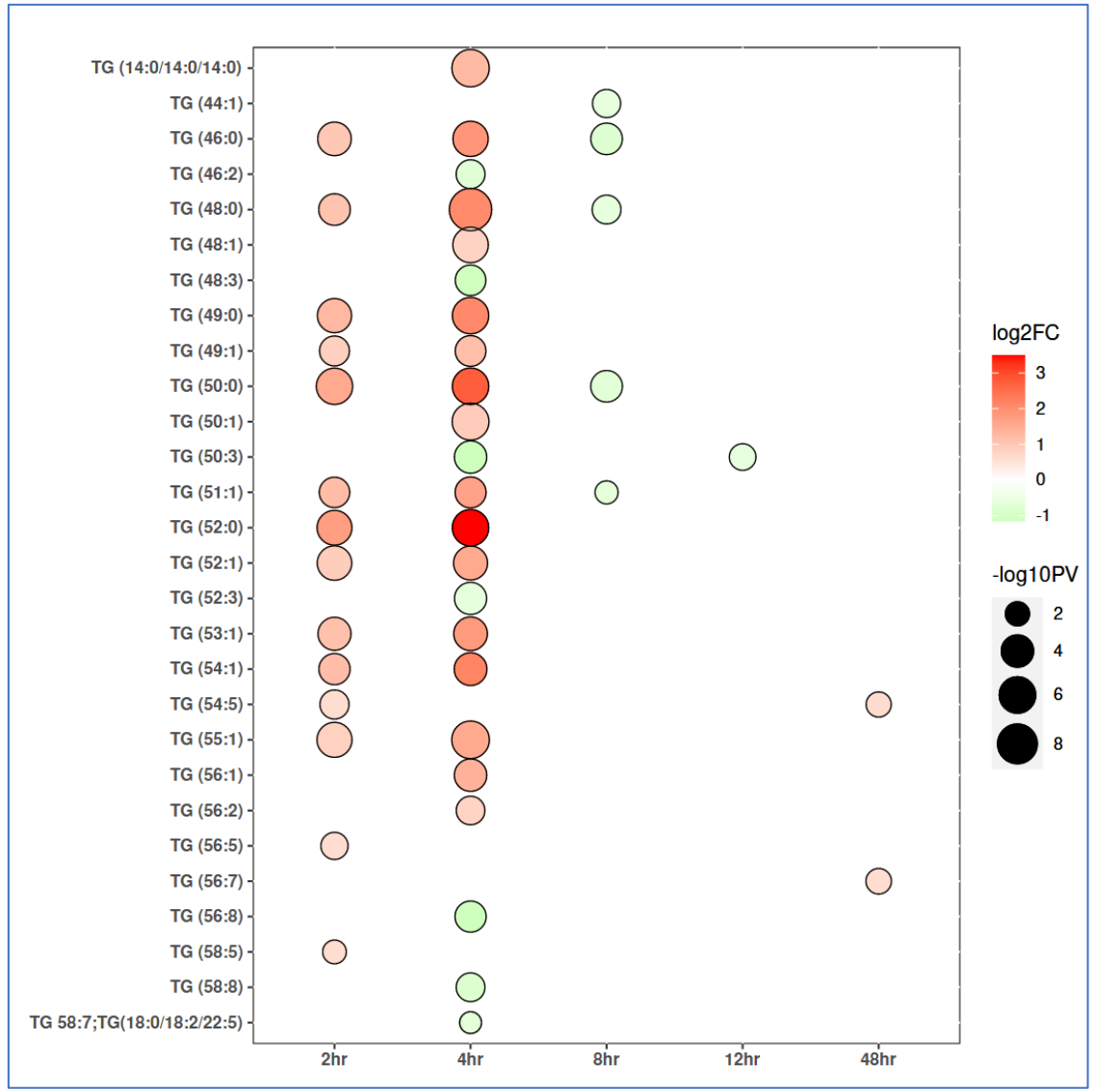
**P-value cutoff:** 0.05 **Fold-change cutoff:** 1.5 **Sample normalization:** None

**Use:** Submitted metabolite names **Maximum # of individual metabolites to display:** 30

**Group by:** Sub class **Maximum # of (most significant) metabolites per class to use in group calculation:** 5

**Analysis:** ESI/QTOF positive ion mode **Combine data for all analyses?:** ☑ Run Analysis

Restrict to a single class (triacylglycerols)




No significant changes compared to control in the TAG class were observed at the 24hr timepoint, so that column is absent in the plot.

# Cluster analysis tools

## Clustering and correlation

- Perform hierarchial or heatmap cluster analysis
- Perform Clustered correlation analysis
- Perform Network analysis on correlated metabolites (mapped to classification)
- Perform Network analysis on correlated metabolites (mapped to fold-change)

**Data for (Study ST001140)**

**(Analysis AN001871)**

Values for each metabolite have been scaled by dividing by the mean across all factors

Run Hierarchial cluster analysis on this study | Run Heatmap cluster analysis on this study

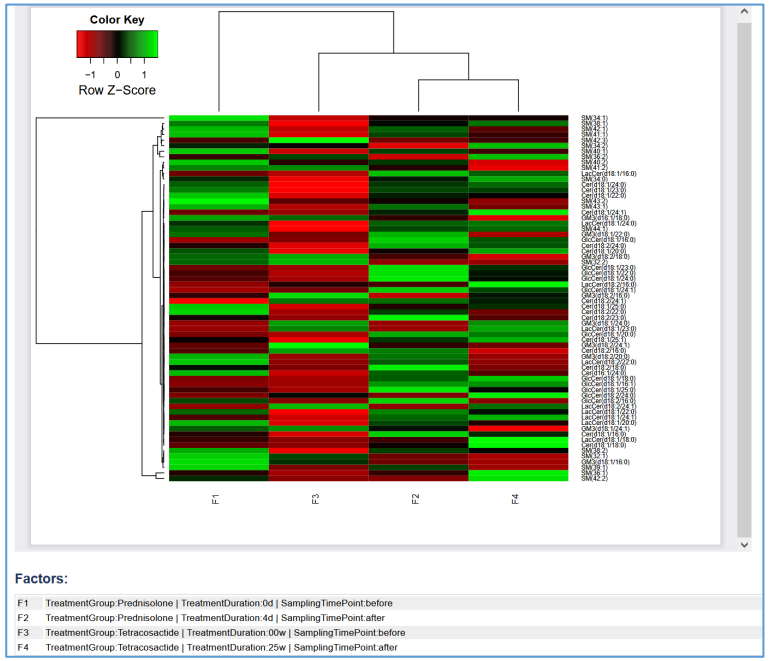
| Metabolite | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| Cer(d16:1/24:0) | 1.3232 | 1.1491 | 0.5756 | 0.7946 |
| Cer(d18:1/16:0) | 0.9360 | 1.2680 | 0.6926 | 1.0354 |
| Cer(d18:1/18:0) | 0.6867 | 0.9859 | 0.5171 | 1.9195 |
| Cer(d18:1/20:0) | 1.1739 | 0.9578 | 0.4702 | 2542 |
| Cer(d18:1/22:0) | 1.3841 | 1.0262 | 0.5037 | 0.949 |
| Cer(d18:1/23:0) | 1.1823 | 1.0882 | 0.5655 | 1.0739 |
| Cer(d18:1/24:0) | 1.1411 | 1.0774 | 0.5541 | 1.1545 |
| Cer(d18:1/24:1) | 0.8238 | 1.0613 | 0.7579 | 1.3952 |
| Cer(d18:1/25:0) | 1.3044 | 0.9207 | 0.6363 | 1.0637 |
| Cer(d18:1/25:1) | 0.9870 | 1.0916 | 0.5991 | 1.2960 |
| Cer(d18:2/16:0) | 0.9557 | 1.0655 | 1.0837 | 0.8881 |
| Cer(d18:2/18:0) | 0.5583 | 1.4417 | NA | NA |
| Cer(d18:2/22:0) | 1.3553 | 1.0771 | 0.6618 | 0.7618 |
| Cer(d18:2/23:0) | 0.9520 | 1.2847 | 0.8033 | 0.8811 |
| Cer(d18:2/24:0) | 0.9376 | 1.2327 | 0.6736 | 1.0994 |
| Cer(d18:2/24:1) | 0.6456 | 1.1770 | 1.1840 | 1.0526 |
| GlcCer(d18:1/16:0) | 0.7048 | 1.3701 | 0.7533 | 1.1468 |
| GlcCer(d18:1/16:1) | 0.7320 | 1.2868 | 0.6996 | 1.2754 |
| GlcCer(d18:1/18:0) | 0.6099 | 1.3018 | 0.4579 | 1.6599 |
| GlcCer(d18:1/20:0) | 0.7235 | 1.3584 | 0.6325 | 1.2584 |
| GlcCer(d18:1/22:0) | 0.7804 | 1.6101 | 0.4553 | 1.0241 |
| GlcCer(d18:1/23:0) | 0.7066 | 1.5278 | 0.5807 | 1.1067 |
| GlcCer(d18:1/24:0) | 0.7881 | 1.5167 | 0.5867 | 1.0069 |
| GlcCer(d18:1/24:1) | 0.6721 | 1.4048 | 0.7622 | 1.1354 |
| GlcCer(d18:1/25:0) | 0.8215 | 1.4817 | 0.6211 | 0.9747 |
| GlcCer(d18:2/16:0) | 0.7297 | 1.2703 | NA | NA |
| GlcCer(d18:2/24:0) | NA | NA | 0.5144 | 1.4856 |
| GM3(d18:1/16:0) | 1.1701 | 0.9053 | 1.0343 | 0.8651 |
| GM3(d18:1/18:0) | 1.3228 | 0.8800 | 1.1933 | 0.5362 |
| GM3(d18:1/22:0) | 1.1302 | 1.2234 | 0.8020 | 0.7264 |
| GM3(d18:1/24:0) | NA | NA | 1.0209 | 0.9791 |

**Clustering data with hclust algorithm for (Study ST001140)**

**(Analysis AN001871)**

| Metabolite | Structure | F1 | F2 | F3 | F4 |
|---|---|---|---|---|---|
| GlcCer(d18:2/24:0) | ME272158 | NA | NA | 0.51 | 1.49 |
| LacCer(d18:2/24:1) | ME272168 | NA | NA | 1.13 | 0.87 |
| GM3(d18:1/24:0) | ME272142 | NA | NA | 1.02 | 0.98 |
| LacCer(d18:1/23:0) | ME272163 | NA | NA | 0.94 | 1.06 |
| GM3(d18:2/20:0) | ME272146 | 1.10 | 0.90 | NA | NA |
| LacCer(d18:2/22:0) | ME272167 | 1.19 | 0.81 | NA | NA |
| Cer(d18:2/18:0) | ME272134 | 0.56 | 1.44 | NA | NA |
| GlcCer(d18:2/16:0) | ME272157 | 0.73 | 1.27 | NA | NA |
| GM3(d18:1/18:0) | ME272140 | 1.32 | 0.88 | 1.19 | 0.54 |
| GM3(d18:2/18:0) | ME272145 | 1.29 | 0.80 | 1.48 | 0.39 |
| Cer(d18:2/24:1) | ME272138 | 0.65 | 1.18 | 1.18 | 1.05 |
| GM3(d18:2/24:1) | ME272147 | 0.82 | 0.93 | 1.55 | 0.79 |
| GM3(d18:2/16:0) | ME272144 | 0.95 | 0.75 | 1.34 | 1.05 |
| SM(42:3) | ME272185 | 0.92 | 0.87 | 1.35 | 0.94 |
| LacCer(d18:2/16:0) | ME272166 | 0.73 | 0.89 | 0.97 | 1.54 |
| Cer(d18:1/24:1) | ME272130 | 0.82 | 1.06 | 0.76 | 1.40 |
| SM(36:1) | ME272174 | 0.95 | 0.91 | 0.66 | 1.53 |
| Cer(d18:1/18:0) | ME272125 | 0.69 | 0.99 | 0.52 | 1.92 |
| GlcCer(d18:1/18:0) | ME272150 | 0.61 | 1.30 | 0.46 | 1.66 |
| GlcCer(d18:1/16:0) | ME272148 | 0.70 | 1.37 | 0.75 | 1.15 |
| GlcCer(d18:1/24:1) | ME272155 | 0.67 | 1.40 | 0.76 | 1.14 |
| GlcCer(d18:1/16:1) | ME272149 | 0.73 | 1.29 | 0.70 | 1.28 |
| GlcCer(d18:1/20:0) | ME272151 | 0.72 | 1.36 | 0.63 | 1.26 |
| GlcCer(d18:1/22:0) | ME272152 | 0.78 | 1.61 | 0.46 | 1.02 |
| GlcCer(d18:1/23:0) | ME272153 | 0.71 | 1.53 | 0.58 | 1.11 |
| GlcCer(d18:1/24:0) | ME272154 | 0.79 | 1.52 | 0.59 | 1.01 |
| GlcCer(d18:1/25:0) | ME272156 | 0.82 | 1.48 | 0.62 | 0.97 |
| SM(32:2) | ME272170 | 1.16 | 0.80 | 1.27 | 0.79 |
| GM3(d18:1/16:0) | ME272139 | 1.17 | 0.91 | 1.03 | 0.87 |
| SM(32:1) | ME272169 | 1.17 | 0.90 | 1.05 | 0.85 |
| GM3(d18:1/24:1) | ME272143 | 1.09 | 1.01 | 1.15 | 0.72 |

## Hierarchial Cluster analysis




**Factors:**

| | |
|---|---|
| F1 | TreatmentGroup:Prednisolone \| TreatmentDuration:0d \| SamplingTimePoint:before |
| F2 | TreatmentGroup:Prednisolone \| TreatmentDuration:4d \| SamplingTimePoint:after |
| F3 | TreatmentGroup:Tetracosactide \| TreatmentDuration:00w \| SamplingTimePoint:before |
| F4 | TreatmentGroup:Tetracosactide \| TreatmentDuration:25w \| SamplingTimePoint:after |

## Heatmap Cluster analysis

# Cluster analysis tools

**Clustering and correlation**

- Perform hierarchial or heatmap cluster analysis
- Perform Clustered correlation analysis
- Perform Network analysis on correlated metabolites (mapped to classification)
- Perform Network analysis on correlated metabolites (mapped to fold-change)

**Clustered correlation heatmap**

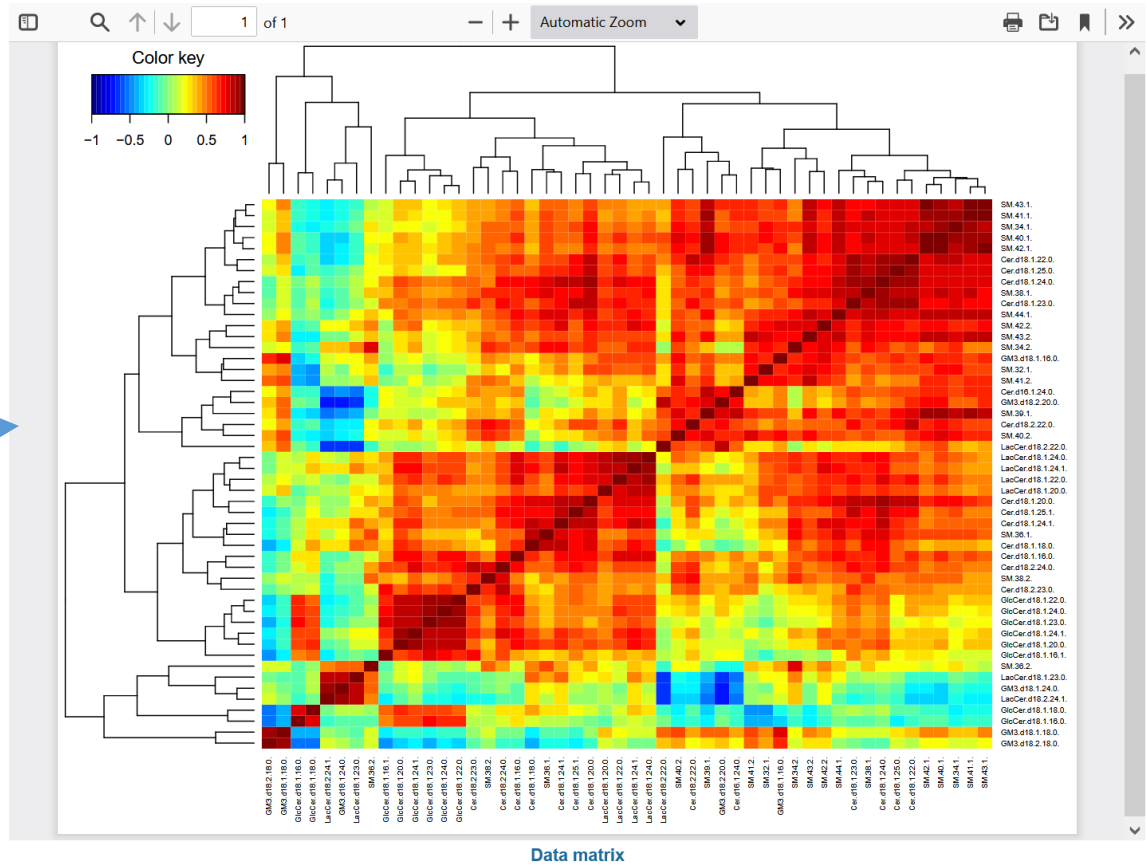
This analysis uses the "cim" function of the "mixOmics" package in the R statistics environment
*Corr_cutoff: Correlation coefficient cutoff. Absolute values less than the cutoff will be discarded (default is 0.75)
Click on links below to perform analysis.

| Map | Study ID | Corr_cutoff | Study Title | MS Analysis Type |
|---|---|---|---|---|
| Run | ST001140 | 0.75 | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Phospholipids, Chol, ... and Triacylglycerols |
| Run | ST001140 | 0.75 | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Sphingolipids |
| Run | ST001140 | 0.75 | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Derivatized Spingosine-1-phosphates |
| Run | ST001140 | 0.75 | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Triacylglycerols |

**Select analysis group and correlation cutoff value (default=0.75)**

**Study: ST001140 Analysis ID:AN001871 (Sphingolipids)**

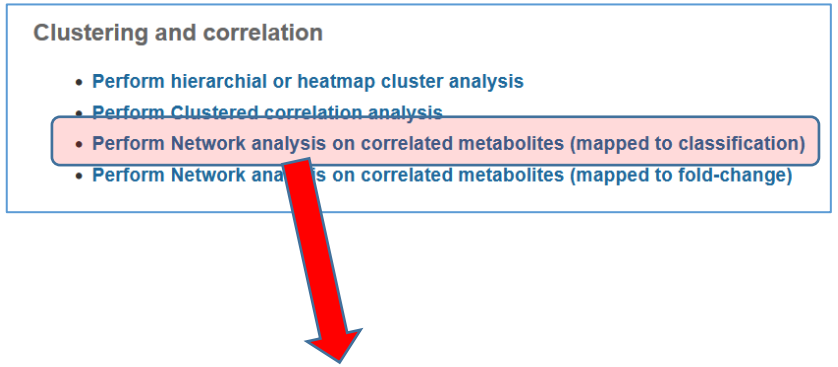
Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure

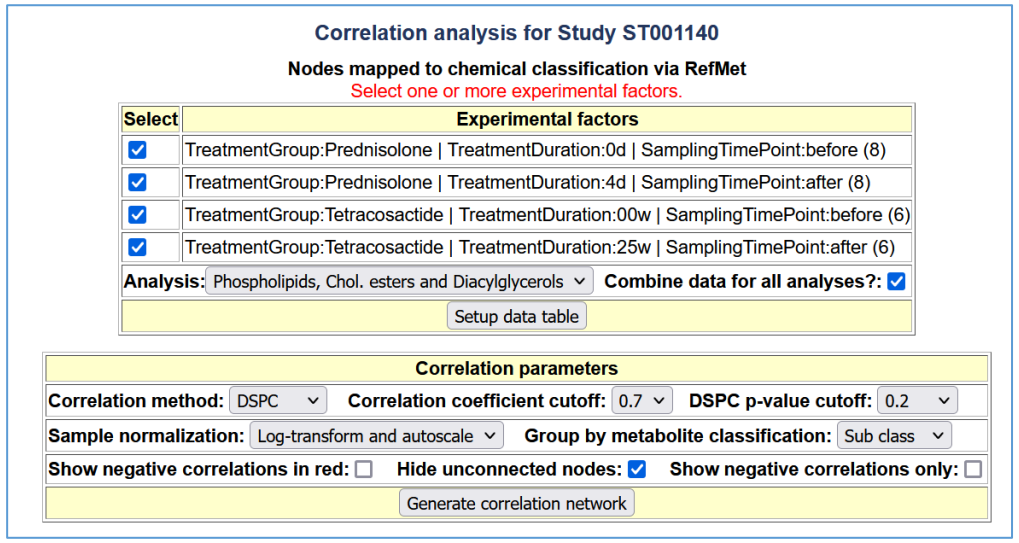



**Clustered correlation analysis**

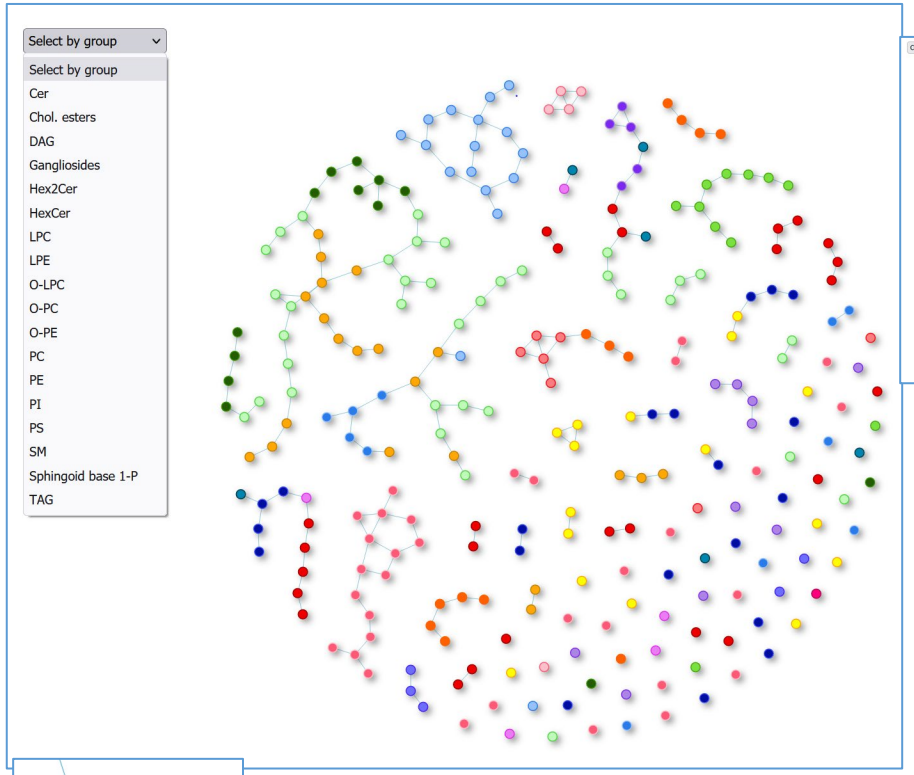
# Network analysis tools (mapped to classification)

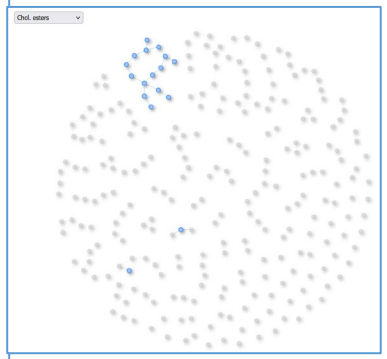
## Pearson correlation or Debiased Sparse Partial Correlation (DSPC)



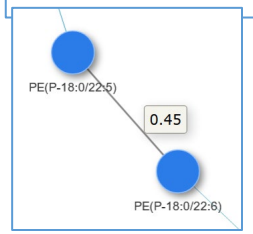




Select groups, correlation method, correlation value cutoff, DSPC p-value cutoff and sample normalization options

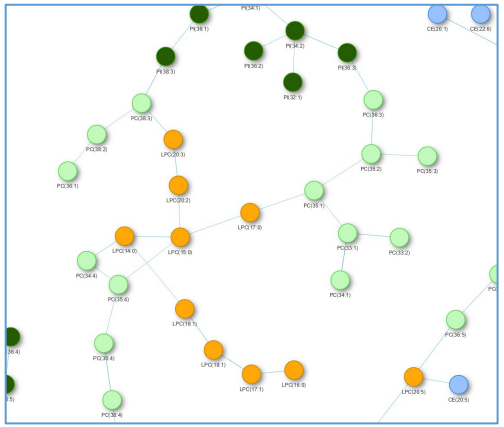


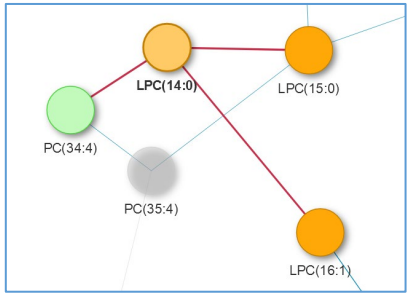



View a single metabolite class using group menu



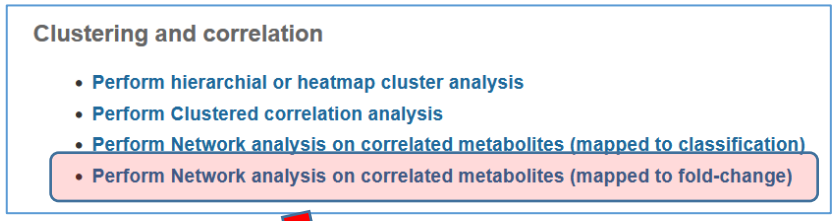

Hover over edge to display correlation coefficient

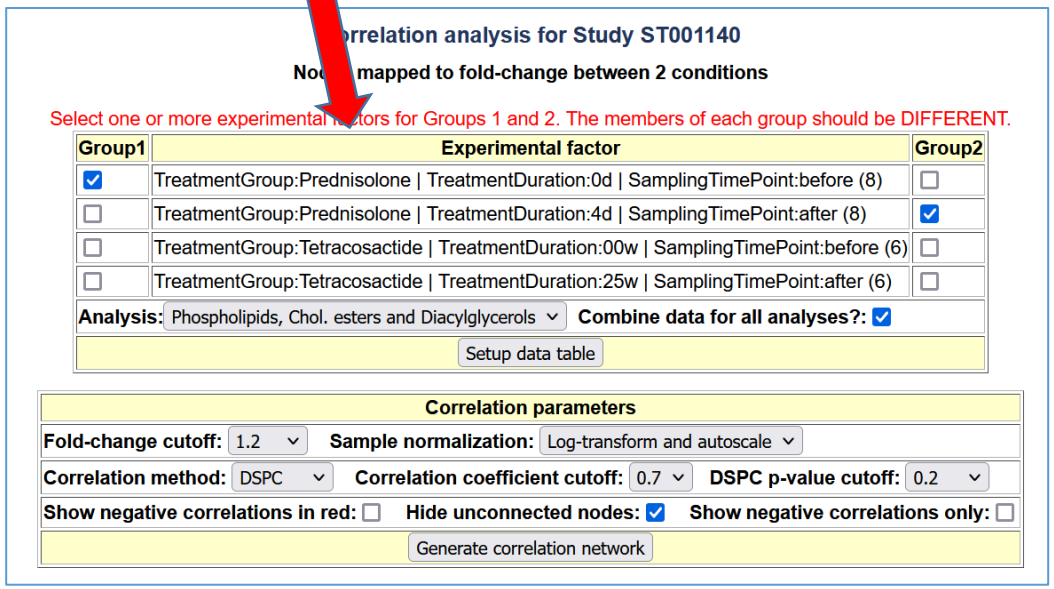




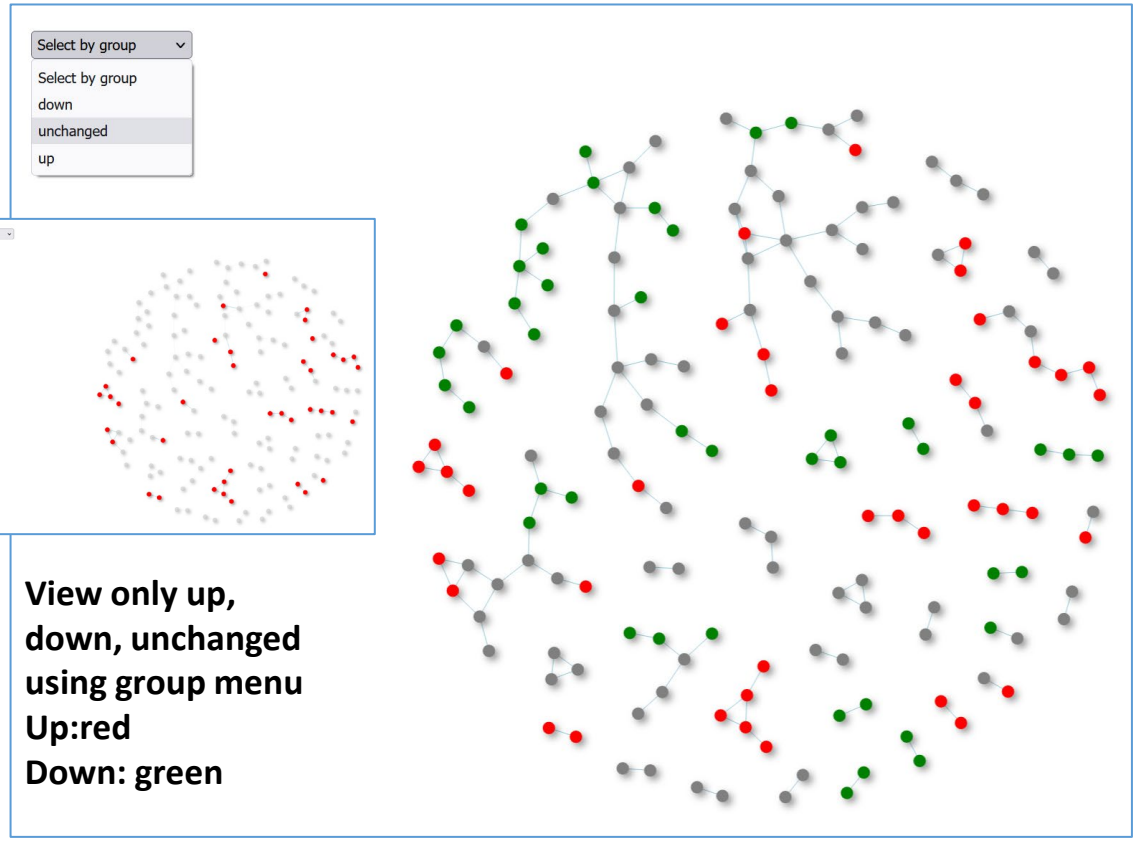

Zoom in to see metabolite labels

# Network analysis tools (mapped to fold-change)

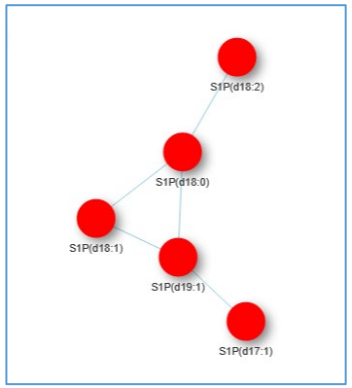



**Select groups to compare by fold-change, correlation method, correlation value cutoff, DSPC p-value cutoff and sample normalization options**




**View only up, down, unchanged using group menu**
**Up:red**
**Down: green**




**Zoom in to see metabolite labels**

# Multivariate analysis tools (LDA example)

**Multivariate analysis**

- Perform Principal component analysis
- Perform Linear discriminant analysis
- Perform Partial least-squares discriminant analysis (PLS-DA)

**Choose conditions to analyze**

**Alternatively, run LDA on all analysis groups in a study combined. This analysis is being run only one group (phospholipids/DAG/ CE)**

**LDA analysis for Study ST001140**

**(Analysis AN001870 )**

Select 3 or more experimental factors for PCA analysis.

| Group | Experimental factor |
|---|---|
| ☑ | TreatmentGroup:Prednisolone \| TreatmentDuration:0d \| SamplingTimePoint:before |
| ☑ | TreatmentGroup:Prednisolone \| TreatmentDuration:4d \| SamplingTimePoint:after |
| ☑ | TreatmentGroup:Tetracosactide \| TreatmentDuration:00w \| SamplingTimePoint:before |
| ☑ | TreatmentGroup:Tetracosactide \| TreatmentDuration:25w \| SamplingTimePoint:after |

Run LDA

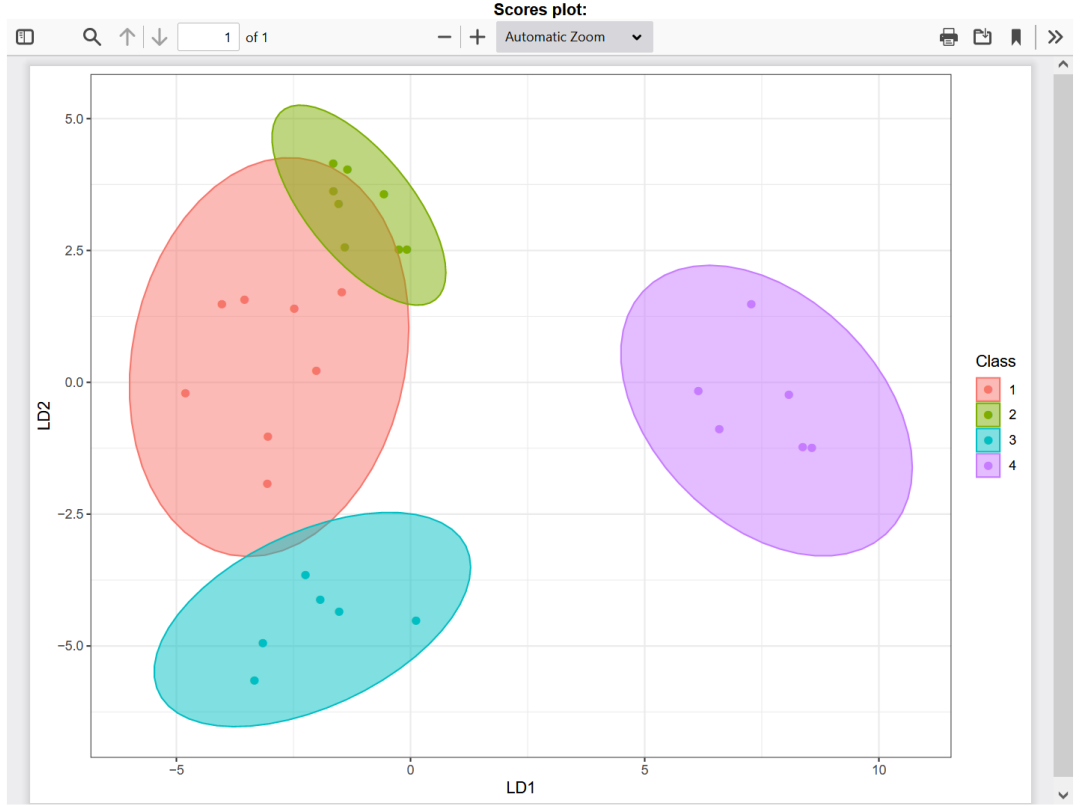
OR

Perform LDA on individual factors (all analyses in study)

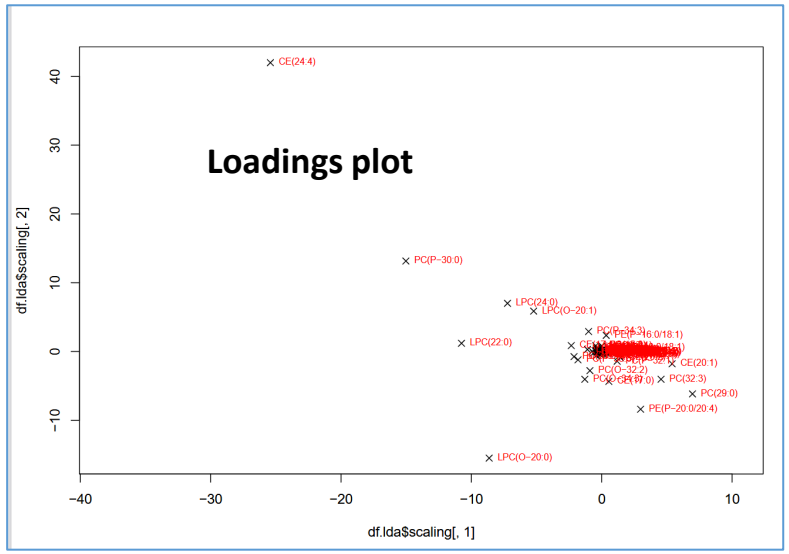
**LDA analysis for (Study ST001140)**

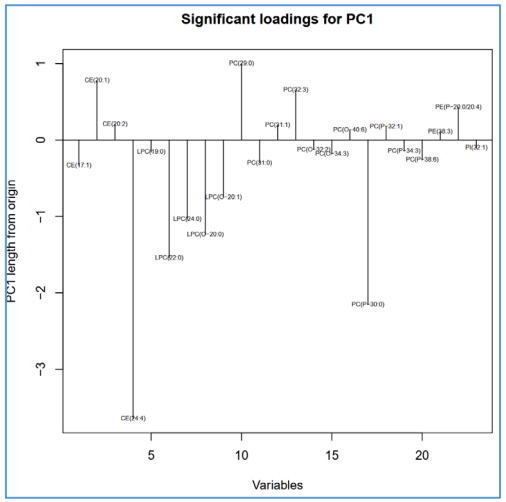
**(Analysis AN001870)**

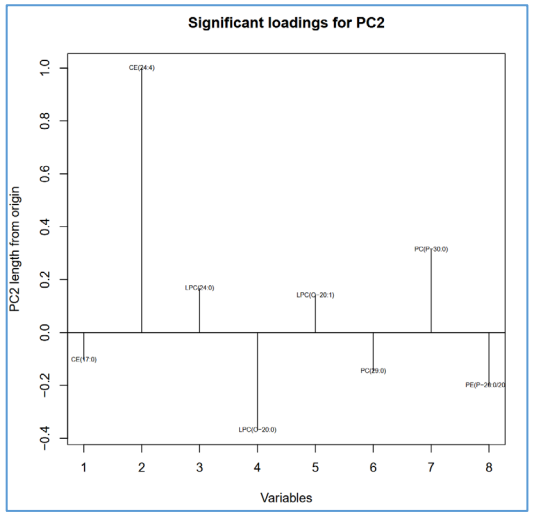
**Scores plot:**




**Scores plot**



# Multivariate analysis tools (PLS-DA example)

**Multivariate analysis**

- Perform Principal component analysis
- Perform Linear discriminant analysis
- Perform Partial least-squares discriminant analysis (PLS-DA)

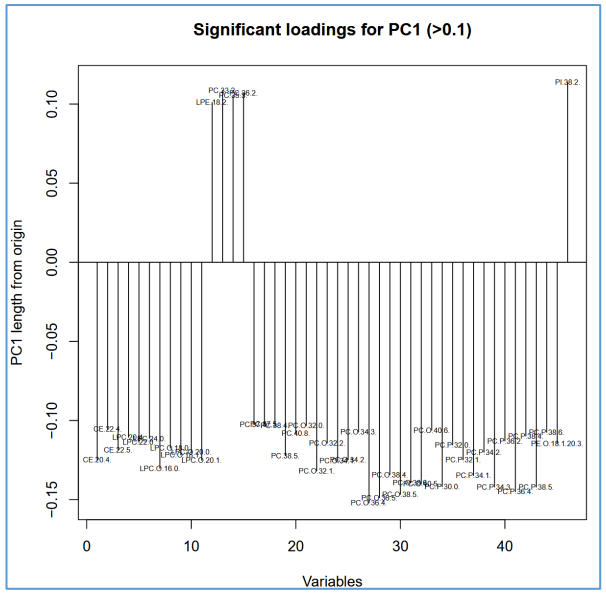
**Choose an analysis group**

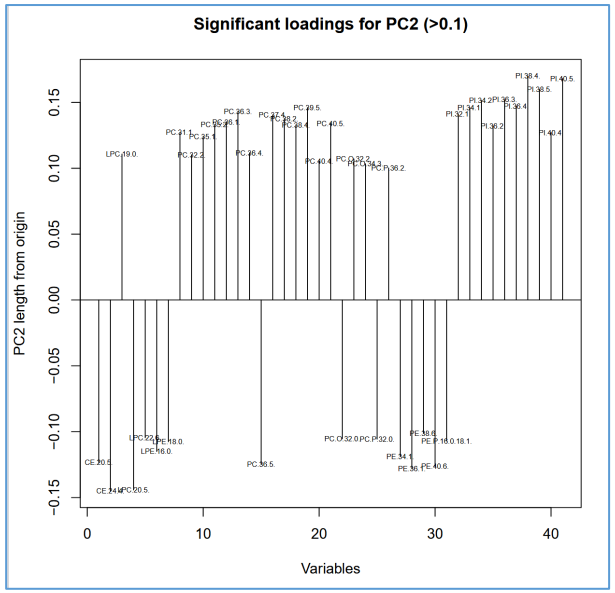
**Partial least squares Discriminant analysis on MS studies**

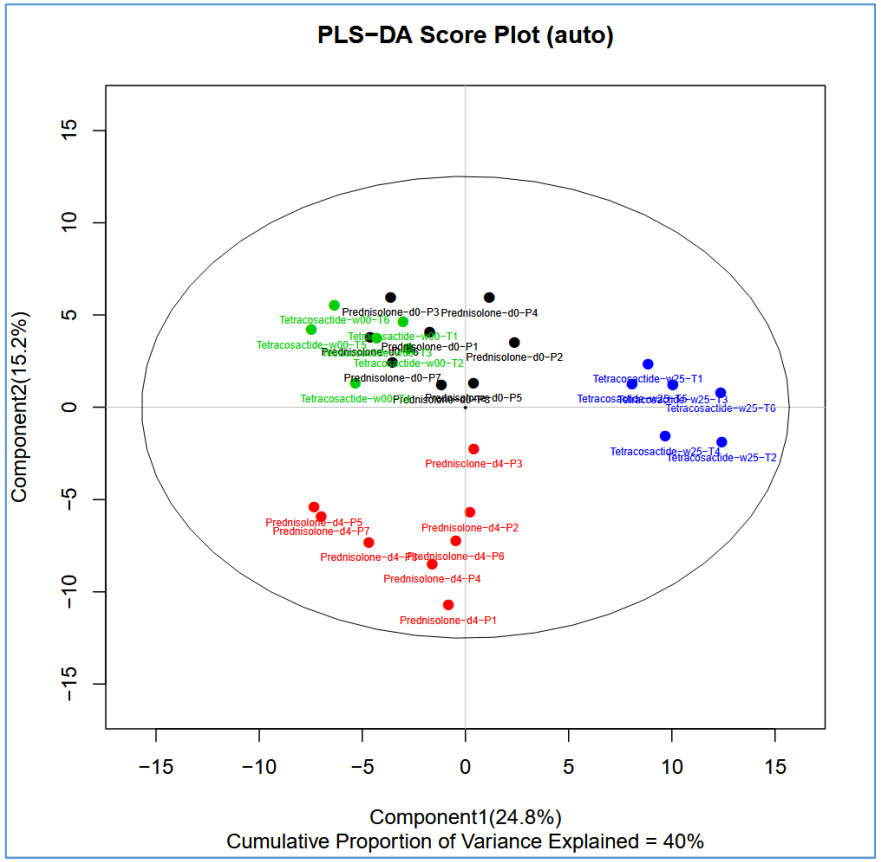
**This analysis uses the "muma" package of the R statistics environment (Reference )**

Click on links below to perform analysis.

| PLSDA | Study ID | Scaling | Study Title | MS Analysis Type |
|---|---|---|---|---|
| Run | ST001140 | Auto | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Phospholipids, Chol. esters and Diacylglycerols |
| Run | ST001140 | Auto | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Sphingolipids |
| Run | ST001140 | Auto | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Derivatized Spingosine-1-phosphates |
| Run | ST001140 | Auto | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Triacylglycerols |




**Scores plot**

# Multivariate analysis tools (PLS-DA example)

**Multivariate analysis**

- Perform Principal component analysis
- Perform Linear discriminant analysis
- Perform Partial least-squares discriminant analysis (PLS-DA)

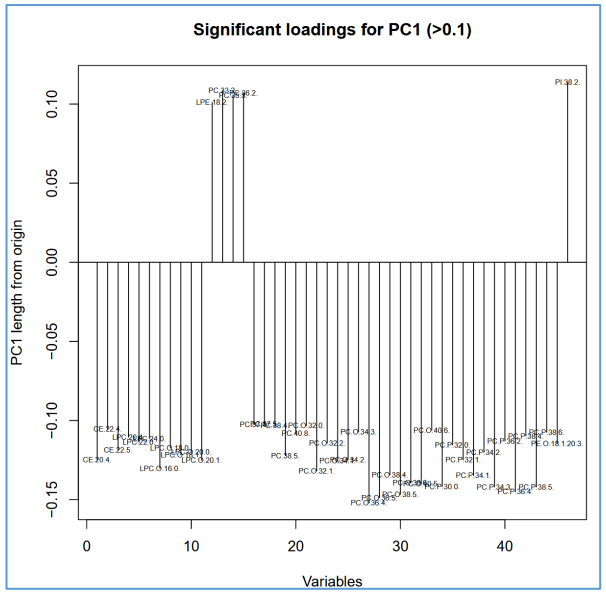
**Choose an analysis group**

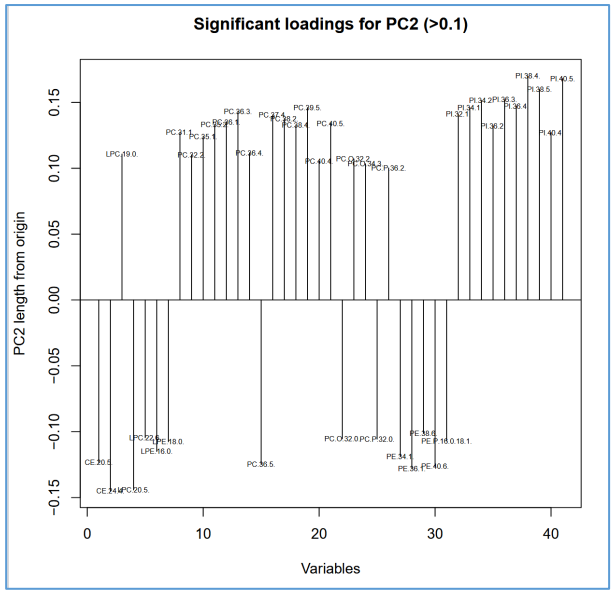
**Partial least squares Discriminant analysis on MS studies**

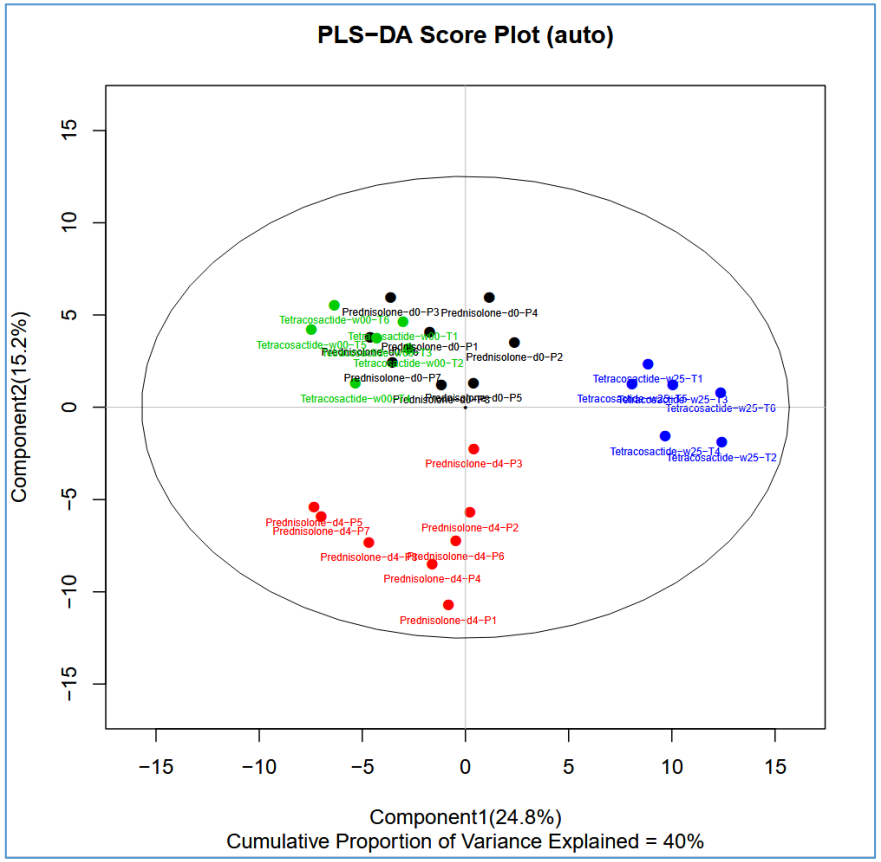
**This analysis uses the "muma" package of the R statistics environment (Reference )**

Click on links below to perform analysis.

| PLSDA | Study ID | Scaling | Study Title | MS Analysis Type |
|---|---|---|---|---|
| Run | ST001140 | Auto | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Phospholipids, Chol. esters and Diacylglycerols |
| Run | ST001140 | Auto | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Sphingolipids |
| Run | ST001140 | Auto | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Derivatized Spingosine-1-phosphates |
| Run | ST001140 | Auto | Changes in the Canine Plasma Lipidome after Short- and Long-Term Excess Glucocorticoid Exposure | Triacylglycerols |




**Scores plot**

# Classification and feature analysis tools (OPLS-DA example)

**Classification and feature analysis**

- Perform OPLS-DA and VIP projection
- Random Forest and VIP projection

**Choose experimental conditions for the 2 groups being compared**

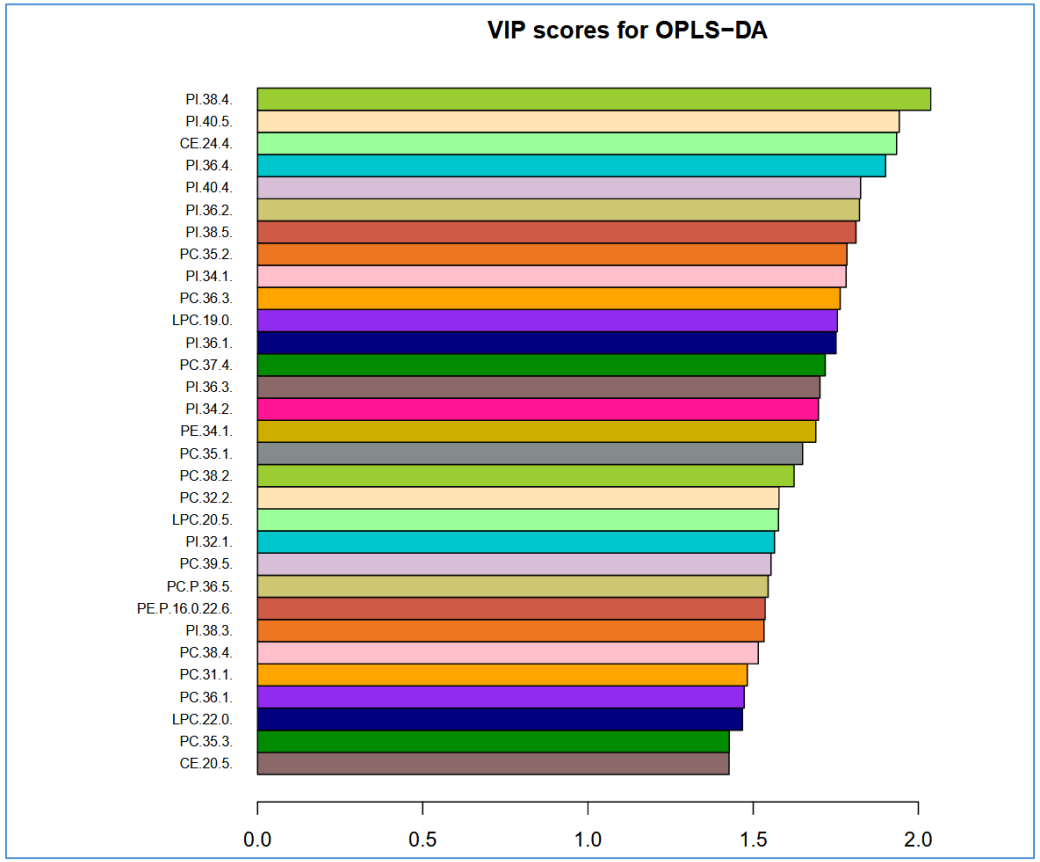
**OPLS-DA analysis for Study ST001140**

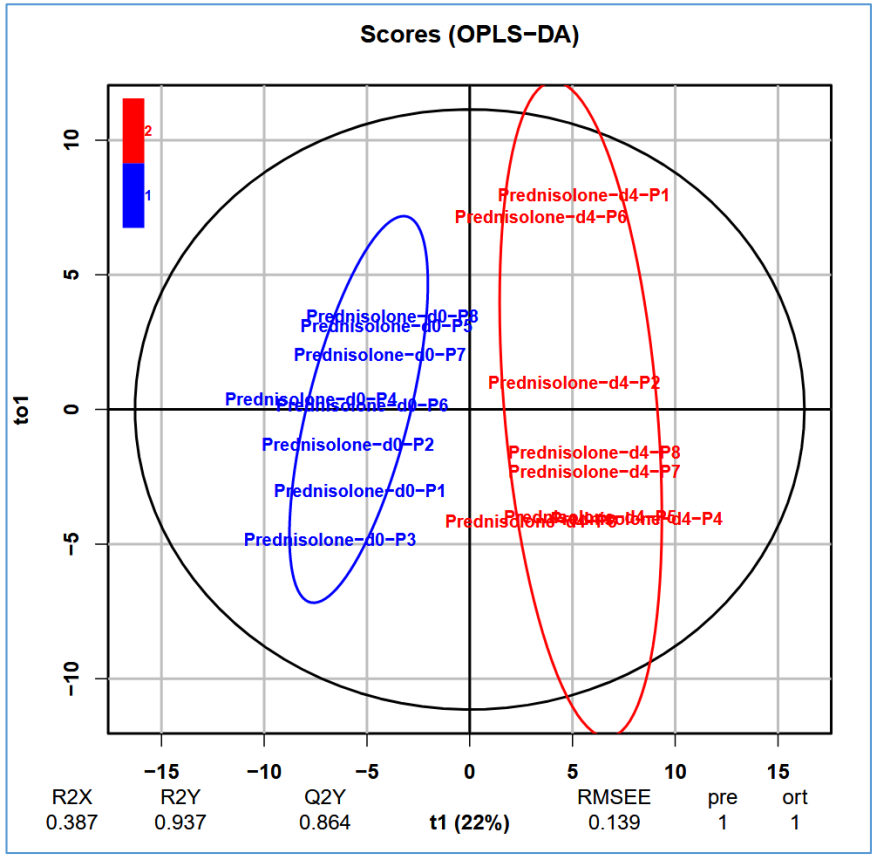
**(Analysis AN001870 )**

Select one or more experimental factors for Groups 1 and 2. The members of each group should be DIFFERENT.

| Group1 | Experimental factor | Group2 |
|---|---|---|
| ☑ | TreatmentGroup:Prednisolone \| TreatmentDuration:0d \| SamplingTimePoint:before | ☐ |
| ☐ | TreatmentGroup:Prednisolone \| TreatmentDuration:4d \| SamplingTimePoint:after | ☑ |
| ☐ | TreatmentGroup:Tetracosactide \| TreatmentDuration:00w \| SamplingTimePoint:before | ☐ |
| ☐ | TreatmentGroup:Tetracosactide \| TreatmentDuration:25w \| SamplingTimePoint:after | ☐ |

Submit Query




**Scores plot**

# Classification and feature analysis tools (Random Forest example)

**Classification and feature analysis**

- Perform OPLS-DA and VIP projection
- Random Forest and VIP projection

**Choose experimental conditions for the 2 groups being compared**

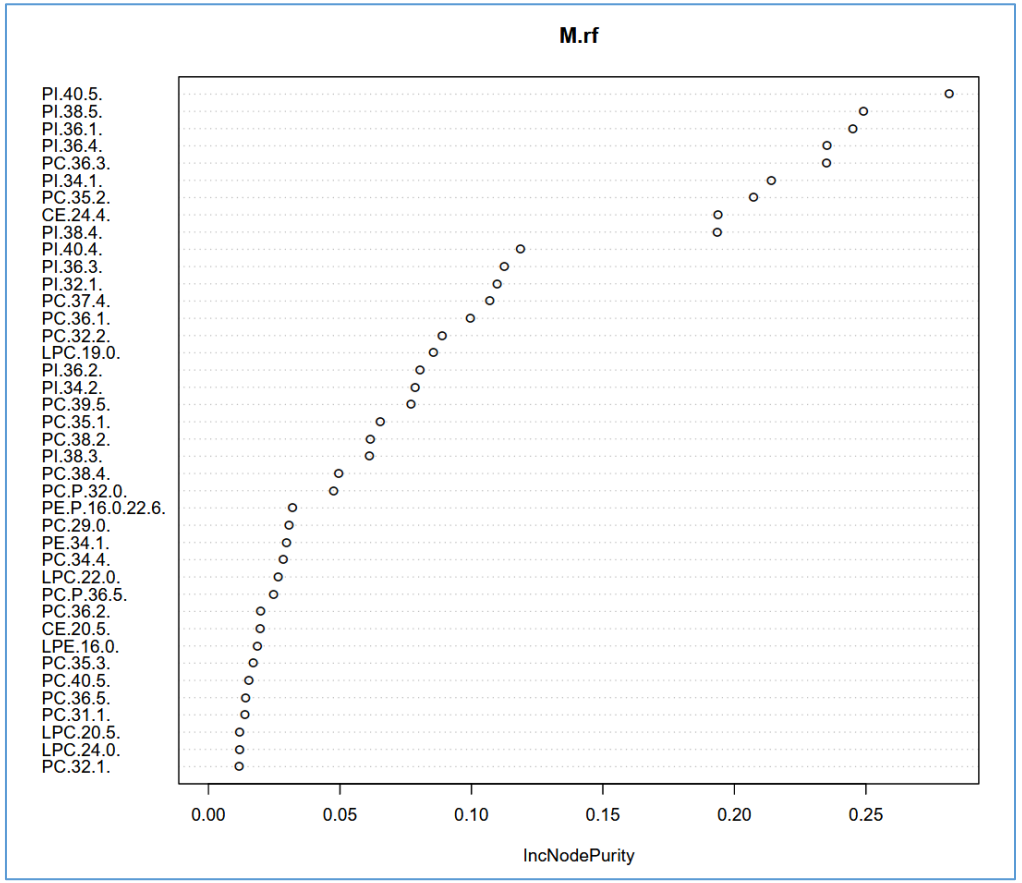
**Random Forest analysis for Study ST001140**

**(Analysis AN001870 )**

Select one or more experimental factors for Groups 1 and 2. The members of each group should be DIFFERENT.

| Group1 | Experimental factor | Group2 |
|---|---|---|
| ☑ | TreatmentGroup:Prednisolone \| TreatmentDuration:0d \| SamplingTimePoint:before | ☐ |
| ☐ | TreatmentGroup:Prednisolone \| TreatmentDuration:4d \| SamplingTimePoint:after | ☑ |
| ☐ | TreatmentGroup:Tetracosactide \| TreatmentDuration:00w \| SamplingTimePoint:before | ☐ |
| ☐ | TreatmentGroup:Tetracosactide \| TreatmentDuration:25w \| SamplingTimePoint:after | ☐ |

Submit Query




**VIP plot**

# Mapping metabolites to human biochemical pathways

**Mapping metabolites to human biochemical pathways**

- Map study metabolites to HMDB and KEGG pathways
- Map study metabolites to pathways with ratio/t-test data

**Choose experimental conditions for the 2 groups being compared**

**Limit by pathway class**

**Pathway mapping for Study ST001140**

**(Analysis All analyses used)**

Select one or more experimental factors for Groups 1 and 2. The members of each group should be DIFFERENT.

| Group1 | Experimental factor | Group2 |
|---|---|---|
| ☑ | TreatmentGroup:Prednisolone \| TreatmentDuration:0d \| SamplingTimePoint:before (8) | ☐ |
| ☐ | TreatmentGroup:Prednisolone \| TreatmentDuration:4d \| SamplingTimePoint:after (8) | ☑ |
| ☐ | TreatmentGroup:Tetracosactide \| TreatmentDuration:00w \| SamplingTimePoint:before (6) | ☐ |
| ☐ | TreatmentGroup:Tetracosactide \| TreatmentDuration:25w \| SamplingTimePoint:after (6) | ☐ |

Limit by pathway class: Metabolic

**Analysis:** Phospholipids, ylglycerols **Combine data for all analyses?:** ☑ Map to pathways

Metabolic
Disease
Drug Action
Drug Metabolism
Physiological
Signaling
All

s Workbench Terms of use | Site m

ego

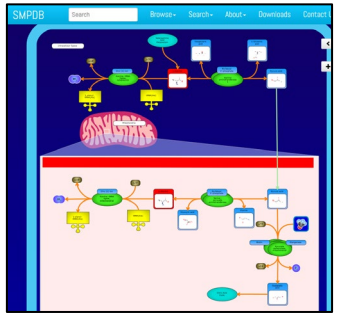
**Results table contains ratios and t-test p-values for each metabolite which is associated with a biochemical pathway**

| | |
|---|---|
| **Numerator:** | TreatmentGroup:Prednisolone \| TreatmentDuration:4d \| SamplingTimePoint:after |
| **Denominator:** | TreatmentGroup:Prednisolone \| TreatmentDuration:0d \| SamplingTimePoint:before |

T-test:If p-value <0.05 metabolites are highlighted*
Red: ratio >1 Green: ratio <1 Gray: insufficient data

| Bile Acid Biosynthesis | Ratio | T-test p-value* | FDR-corrected p-value(BH) |
|---|---|---|---|
| CE(18:0) | 1.26 | 3.413E-1 | 6.066E-1 |
| **Glycerolipid Metabolism** | **Ratio** | **T-test p-value*** | **FDR-corrected p-value(BH)** |
| DG(16:0_20:4) | 0.91 | 8.607E-1 | 9.750E-1 |
| DG(18:0_18:2) | 1.31 | 5.608E-1 | 7.872E-1 |
| DG(18:0_20:4) | 1.04 | 9.192E-1 | 1.000E+0 |
| DG(18:1_18:2) | 1.04 | 0.000E+0 | 0.000E+0 |
| DG(18:1_20:4) | 0.54 | 2.451E-1 | 5.017E-1 |
| DG(18:2_18:2) | 1.27 | 5.280E-1 | 7.643E-1 |
| DG(18:2_20:4) | 0.74 | 5.222E-1 | 7.601E-1 |
| TG(46:1) | 0.57 | 1.312E-1 | 3.511E-1 |
| TG(46:2) | 0.55 | 7.056E-2 | 2.340E-1 |
| TG(48:0) | 1.11 | 8.423E-1 | 9.679E-1 |
| TG(48:1) | 1.06 | 8.760E-1 | 9.767E-1 |
| TG(48:2) | 0.72 | 2.890E-1 | 5.487E-1 |
| TG(48:3) | 0.49 | 4.253E-2 | 1.592E-1 |
| TG(50:0) | 1.41 | 5.078E-1 | 7.484E-1 |
| TG(50:1) | 0.88 | 8.001E-1 | 9.443E-1 |
| TG(50:2) | 0.88 | 0.000E+0 | 0.000E+0 |
| TG(50:3) | 0.88 | 0.000E+0 | 0.000E+0 |
| TG(50:4) | 0.43 | 7.481E-2 | 2.450E-1 |

**Links to human SMPDB pathway**




**A pathway enrichment score table is also generated**

| Pathway Enrichment (Kolmogorov-Smirnov test) | |
|---|---|
| **Pathway (with score >3)** | **Enrichment score (-log10(FDR-corrected p-value))** |
| Sphingolipid Metabolism | 18.000 |
| Phosphatidylinositol Phosphate Metabolism | 15.654 |
| Phospholipid Biosynthesis | 15.051 |
| Glycerolipid Metabolism | 13.416 |
| Steroid Biosynthesis | 3.895 |

# Meta-analysis tools (across different studies)

Home | Data Repository | Databases | Protocols | Tools | Training / Events | About | Search

Overview | Upload / Manage Studies | Browse / Search Studies | Analyze Studies | Data Sharing Policy | Tutorials | FAQ

## Analyze Studies

Analyze studies using **Jupyter Notebooks** or the following online tools.

### MS/NMR studies identifying named metabolites

**Select a study for analysis:**

Select a study

Submit

Analysis tools may also be accessed from within each **study page** using the 'Perform statistical analysis' link

### Comparative analysis across studies

- Perform meta-analysis on selected studies (compare ratios of 2 selected metabolites)
- Compare list of metabolites in 2 selected studies (all analyses)
- Compare list of metabolites in 2 selected studies (individual analyses)

## View metabolite ratios across different studies

Study ID's (comma or space separated):

ST000402 ST000397 ST000395

Start..

Compare 2 individual studies:

ST000001: Fatb Induction Experiment (FatBIE)

ST000002: Intestinal Samples II pre/post transplantation

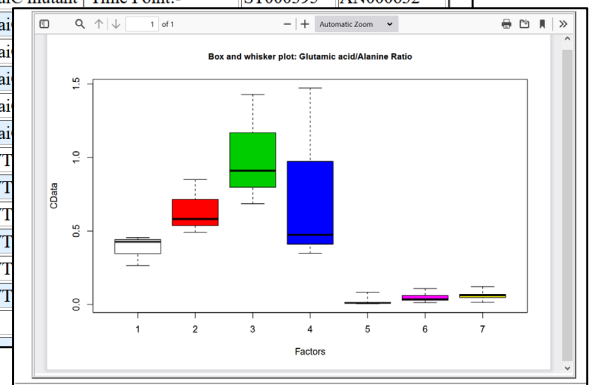
Submit Reset



**Alanine/Glutamic acid Ratio values (Units: AN000641)**

| Boxplot of ratio range | Boxplot of ratios for each factor level | Boxplot of inverse ratios for each factor level |
|---|---|---|
| Box and whisker plot | Boxplot by factor | Boxplot by factor (inverse) |

| Local Sample Id | Alanine | Glutamic Acid | Ratio | Factors | Study Id | Analysis Id |
|---|---|---|---|---|---|---|
| 140619dlvsa01_1 | 24151 | 6385 | 3.782 | Genotype:KaiC mutant \| Time Point:- | ST000395 | AN000632 |
| 140619dlvsa06_2 | 12244 | 5224 | 2.344 | Genotype:Kai | | |
| 140619dlvsa07_2 | 13738 | 6254 | 2.197 | Genotype:Kai | | |
| 140619dlvsa08_3 | 27389 | 23258 | 1.178 | Genotype:Kai | | |
| 140619dlvsa09_2 | 21028 | 12228 | 1.720 | Genotype:Kai | | |
| 140619dlvsa10_2 | 21827 | 10720 | 2.036 | Genotype:Kai | | |
| 140619dlvsa03_1 | 11768 | 10707 | 1.099 | Genotype:WT | | |
| 140619dlvsa05_1 | 10382 | 14825 | 0.700 | Genotype:WT | | |
| 140619dlvsa11_1 | 8548 | 5864 | 1.458 | Genotype:WT | | |
| 140619dlvsa02_1 | 17066 | 5947 | 2.870 | Genotype:WT | | |
| 140619dlvsa04_1 | 10680 | 15722 | 0.679 | Genotype:WT | | |
| 140619dlvsa12_2 | 11958 | 5660 | 2.113 | Genotype:WT | | |
| 140421ajlsa01_1 | 36909 | 1576 | 23.419 | Data:1 | | |




Factor level F1: Genotype:KaiC mutant | Time Point:-
Factor level F2: Genotype:KaiC mutant | Time Point:4
Factor level F3: Genotype:WT | Time Point:-
Factor level F4: Genotype:WT | Time Point:4
Factor level F5: Data:1
Factor level F6: Glucose conc:0 g/L
Factor level F7: Glucose conc:10 g/L

Glutamic acid/Alanine Ratio

| Min. | 1st Qu. | Median | Mean | 3rd Qu. | Max. |
|---|---|---|---|---|---|
| 0.00500 | 0.01295 | 0.03970 | 0.09894 | 0.06530 | 1.47210 |

# Metabolomics Tools:→Load and analyze your own dataset

## Modular, portable suite of statistical tools for metabolomics analysis

- R statistics-based approach
  - **Normalization and scaling**
  - **Bar graphs and Boxplots**
  - **Univariate Analysis**
  - **Multivariate Analysis**
  - **Clustering and Correlation**
  - **Feature Analysis**
- Ability to select and combine groups of experimental conditions (factors)
- Applicable to targeted and untargeted datasets
- Workflow enables classification of metabolite names via RefMet
- Classified datasets are then amenable to class-specific and pathway-specific analysis

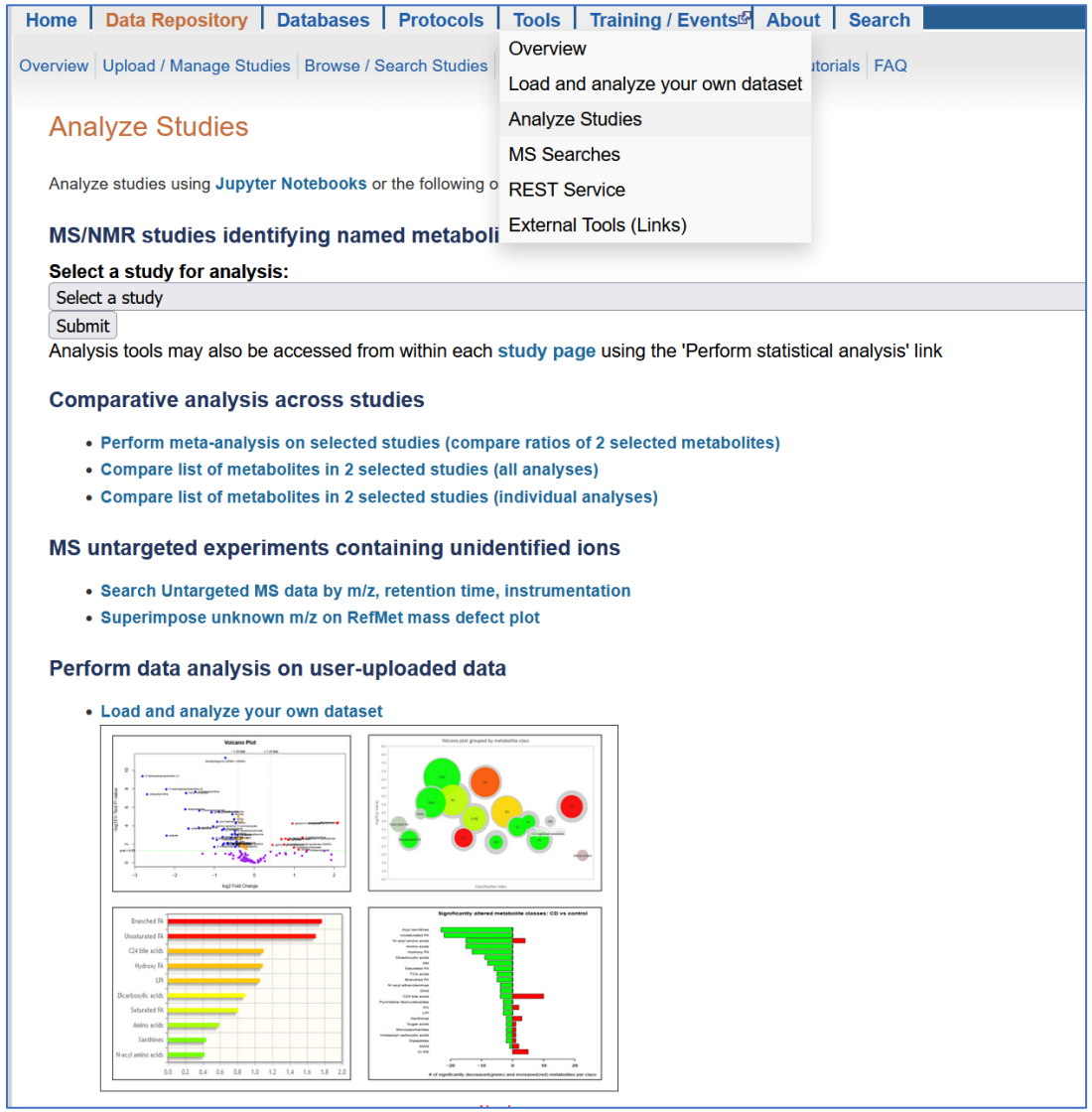
# Metabolomics Tools:→Load and analyze your own dataset

https://www.metabolomicsworkbench.org/data/analyze.php

| Samples | Group | CAR(16:0) | | CAR(18:0) | | CAR(18:1) | | CAR(18:2) | | CE(18:1) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S001_2 | Affected/Male | 32592 | 7400 | 25164 | 16371 | 39797 | 461580 | 342255 | 241473 | 26379 |
| S002_27 | Affected/Male | 37821 | 13552 | 40988 | 26845 | 51799 | 526923 | 409751 | 250720 | 41055 |
| S007_51 | Affected/Male | 9201 | 6037 | 6219 | 10361 | 18848 | 461700 | 168391 | 125282 | 3802 |
| S008_59 | Affected/Male | 132519 | 15845 | 245076 | 159627 | 24173 | 437630 | 326360 | 358552 | 21342 |
| S009_39 | Affected/Male | 24407 | 9146 | 51668 | 32965 | 42774 | 337701 | 362332 | 204264 | 13970 |
| S013_29 | Affected/Male | 30813 | 7299 | 35485 | 25603 | 58491 | 386359 | 385114 | 286028 | 30002 |
| S014_22 | Affected/Male | 33082 | 8830 | 36894 | 21874 | 49050 | 542047 | 420069 | 256991 | 36641 |
| S015_5 | Affected/Male | 29115 | 7472 | 38326 | 23507 | 35022 | 230142 | 298691 | 174860 | 9054 |
| S016_31 | Affected/Male | 34081 | 7571 | 57646 | 48296 | 50157 | 498962 | 426752 | 266928 | 36812 |
| S018_50 | Affected/Male | 58917 | 11048 | 101684 | 70157 | 45607 | 463558 | 429808 | 284710 | 18142 |
| S021_21 | Affected/Male | 22655 | 6631 | 28896 | 22833 | 60510 | 567791 | 461084 | 409043 | 40955 |
| S022_14 | Affected/Male | 23852 | 7132 | 33083 | 20959 | 56129 | 465535 | 475318 | 425279 | 17626 |
| S023_41 | Affected/Male | 26156 | 6751 | 44201 | 26734 | 57518 | 482054 | 447923 | 338220 | 46731 |
| S024_43 | Affected/Male | 24502 | 7108 | 36540 | 25172 | 37975 | 488013 | 379549 | 371133 | 21571 |
| S025_33 | Affected/Male | 10231 | 5945 | 9475 | 14291 | 22012 | 391757 | 281674 | 189573 | 2667 |
| S026_23 | Affected/Male | 31683 | 9410 | 39957 | 30026 | 40384 | 477841 | 385080 | 341657 | 19780 |
| S027_18 | Affected/Male | 24153 | 5860 | 36417 | 28030 | 41637 | 476109 | 382987 | 348275 | 20344 |
| S028_35 | Affected/Male | 32603 | 6541 | 64274 | 44075 | 62381 | 480321 | 527889 | 425970 | 32329 |
| S029_34 | Affected/Male | 29696 | 7858 | 39767 | 36869 | 51518 | 483914 | 494792 | 379614 | 31195 |
| S031_9 | Affected/Male | 30138 | 6312 | 26999 | 22104 | 40489 | 476062 | 401627 | 325777 | 30745 |
| S032_64 | Affected/Female | 32551 | 9934 | 45279 | 30568 | 50255 | 329084 | 417248 | 412746 | 12094 |
| S034_66 | Affected/Female | 40129 | 7901 | 54879 | 52292 | 51006 | 515113 | 355455 | 367787 | 28220 |
| S037_46 | Affected/Female | 55349 | 7426 | 103693 | 35440 | 22463 | 173160 | 191218 | 172002 | 6157 |
| S038_8 | Affected/Female | 16663 | 9111 | 9982 | 11166 | 49852 | 365320 | 332450 | 208024 | 3605 |
| S040_26 | Affected/Female | 30737 | 11822 | 30133 | 19357 | 28450 | 464703 | 349594 | 352426 | 25960 |
| S041_69 | Affected/Female | 20351 | 9616 | 33138 | 15191 | 60271 | 308696 | 365188 | 384615 | 18781 |
| S042_61 | Affected/Female | 44531 | 10508 | 87680 | 70868 | 34093 | 507718 | 452632 | 536826 | 22587 |
| S044_3 | Affected/Female | 26159 | 7195 | 34041 | 31696 | 33092 | 482180 | 363697 | 362897 | 29765 |
| S045_58 | Affected/Female | 53023 | 9926 | 96073 | 71568 | 34687 | 403564 | 295048 | 467228 | 32331 |
| S046_24 | Affected/Female | 21720 | 5712 | 23667 | 10882 | 41203 | 279409 | 267775 | 243040 | 17383 |
| S047_16 | Affected/Female | 17094 | 5225 | 24567 | 17196 | 42917 | 349825 | 298969 | 278524 | 36062 |
| S049_48 | Affected/Female | 55655 | 10899 | 63535 | 62495 | 42110 | 401089 | 387996 | 400073 | 17062 |
| S051_44 | Affected/Female | 22293 | 5128 | 36012 | 26083 | 38486 | 475328 | 410951 | 371971 | 18243 |
| S053_11 | Affected/Female | 12268 | 4303 | 24253 | 21592 | 52598 | 385859 | 259803 | 369865 | 16433 |
| S057_1 | Affected/Female | 26327 | 7078 | 29278 | 21698 | 61240 | 504927 | 420130 | 390436 | 28532 |
| S059_28 | Affected/Female | 3859 | 2676 | 1881 | 2439 | 31575 | 296196 | 186595 | 106324 | 2445 |

File format required:
Column 1: sample names
Column 2: group identifier (letters, numbers or text)
Columns 3 to n: Variables

This is an option to analyze your own dataset (as opposed to a submitted NMDR study)



# Analysis tools available on user-uploaded data

**(these are NOT submitted studies –a data table is uploaded via a form )**

**Normalization and scaling**

- Sample normalization
- Analyte scaling
- Relative log abundance plot

**Bargraphs and Boxplots**

- Bargraph
- Bargraph ratio
- Boxplot

**Univariate Analysis**

- Volcano Plot
- ANOVA analysis
- Debiased sparse partial correlation analysis

**Clustering and Correlation**

- Hierarchical Cluster Analysis
- Pearson Correlation:
- Clustered correlation analysis

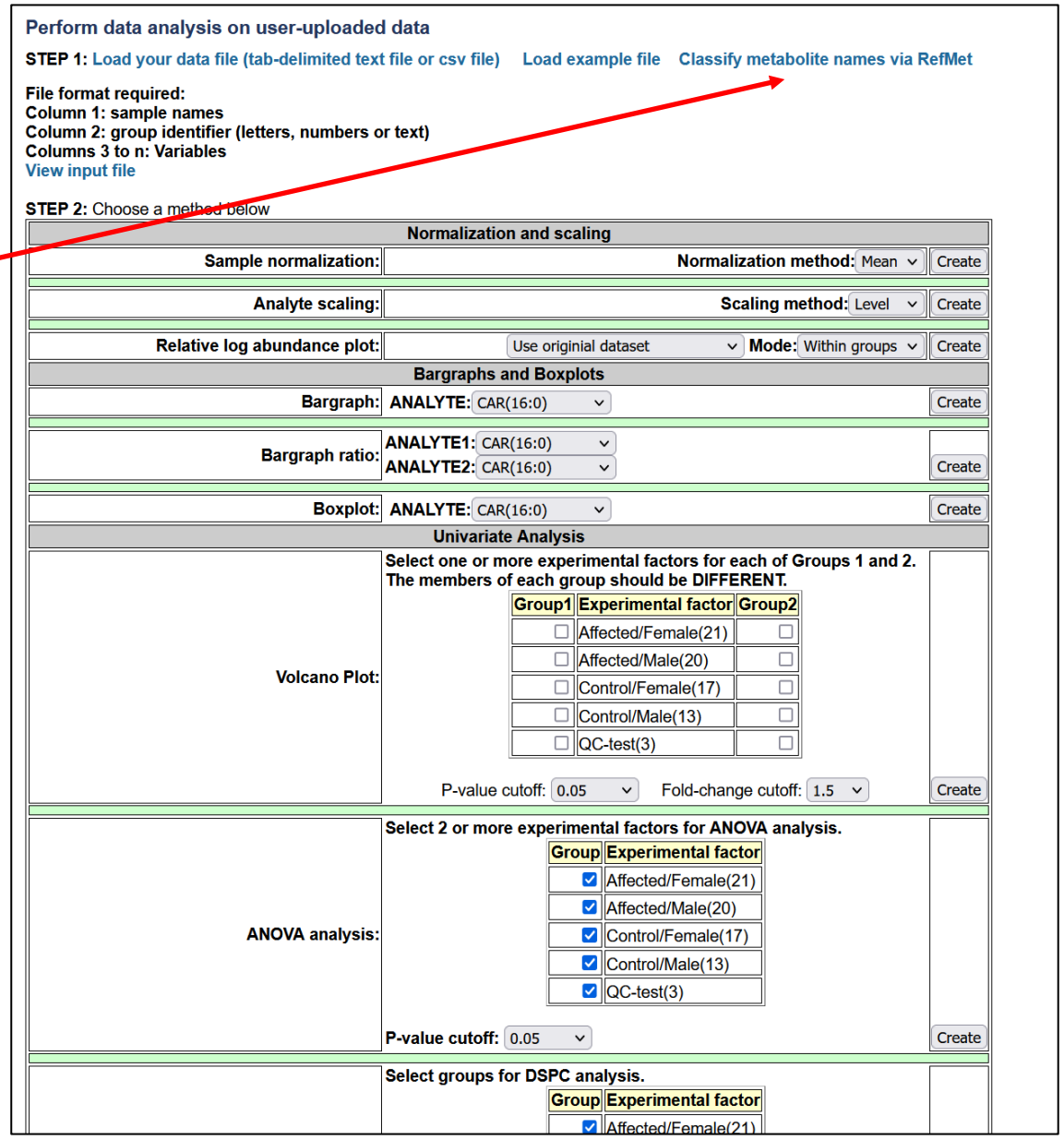
**Multivariate Analysis**

- Principal Component Analysis
- Linear Discriminant Analysis

**Classification and Feature Analysis**

- OPLS-DA/VIP analysis
- Random Forest/VIP analysis

Mapping of input metabolite names to RefMet facilitates enables deployment of classification tools



# Metabolomics Tools:→Load and analyze your own dataset

## Examples of output from online tools