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OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 500 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS International also organizes 500 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

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OMICS International is a pioneer and leading science event organizer, which publishes around 500 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS International has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.



Metabolomics study of Ketosis-Associated Increases in FGF21 in Wild-type and PPAR alpha Knockout Mice.

Vladimir Tolstikov, Ph.D.

4th International Conference and Exhibition on Metabolomics & Systems Biology
April 27-29, 2015, Philadelphia, USA

Lilly

Presentation overview

- Analytical Platform and workflow.
- Case study
  - FGF21 (Hepatic dietary adaptation)
- Summary



Lilly Metabolomics Platform

Ultimate combination of targeted and non-targeted approaches.

GC/MS

- Volatiles
- Essential oils
- Esters
- Perfumes
- Terpenes
- Carotenoids
- Flavanoids
- Perfumes

- Alchohols
- Amino acids
- Catecholamines
- Fatty acids
- Phenolics
- Prostanglandins
- Steroids
- Sugar phosphates

- Organic acids
- Organic amines
- Nucleosides
- Nucleotides
- Oligosaccharides
- Peptides
- Co-factors
- Polar Lipids

LC/MS



PEGASUS GC-HRT accurate mass TOF
Gerstel ALEX/CIS MultiPurpose Autosampler



Triple quad 5500

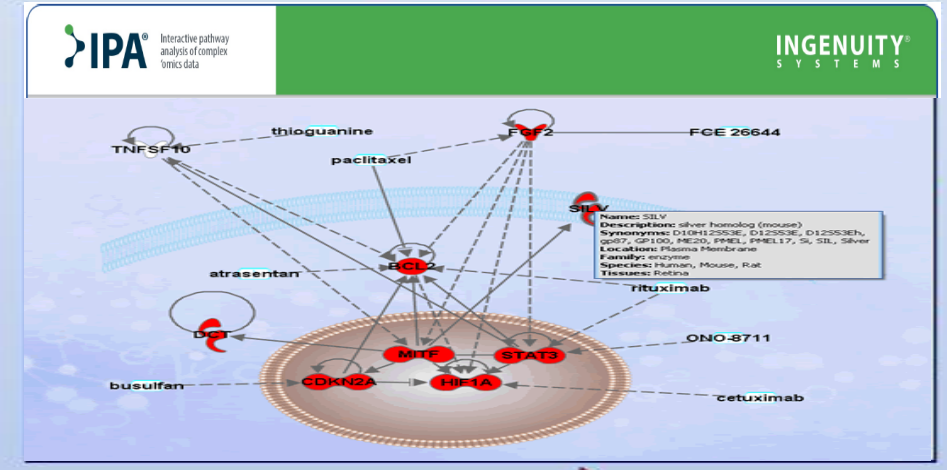


Triple TOF 5600 accurate mass

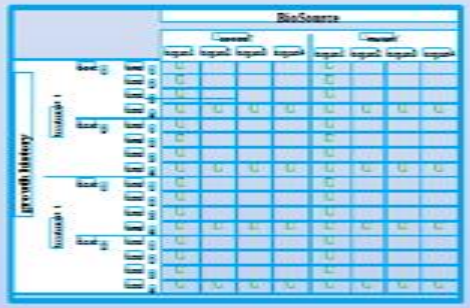
Throughput capabilities 7000 - 15000 samples per year

Lilly

Metabolomics workflow



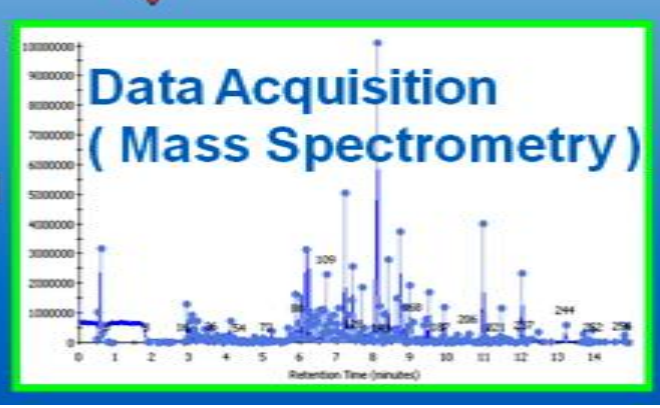
Pathway Analysis

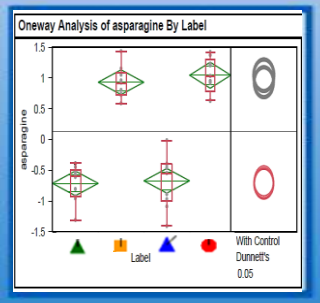


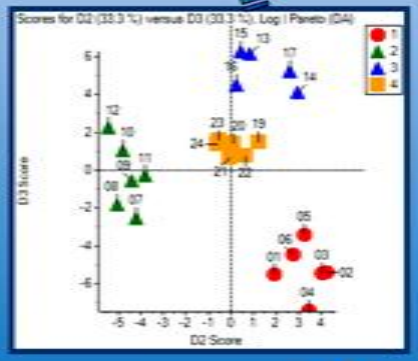
Experimental design



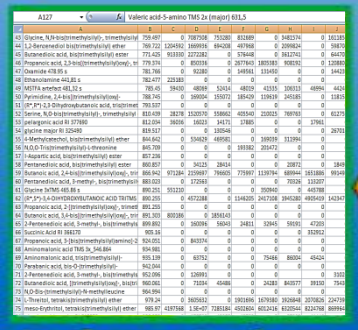
Sample Preparation
(wet chemistry)







Data Analysis
(Univariate and Multivariate Statistics)

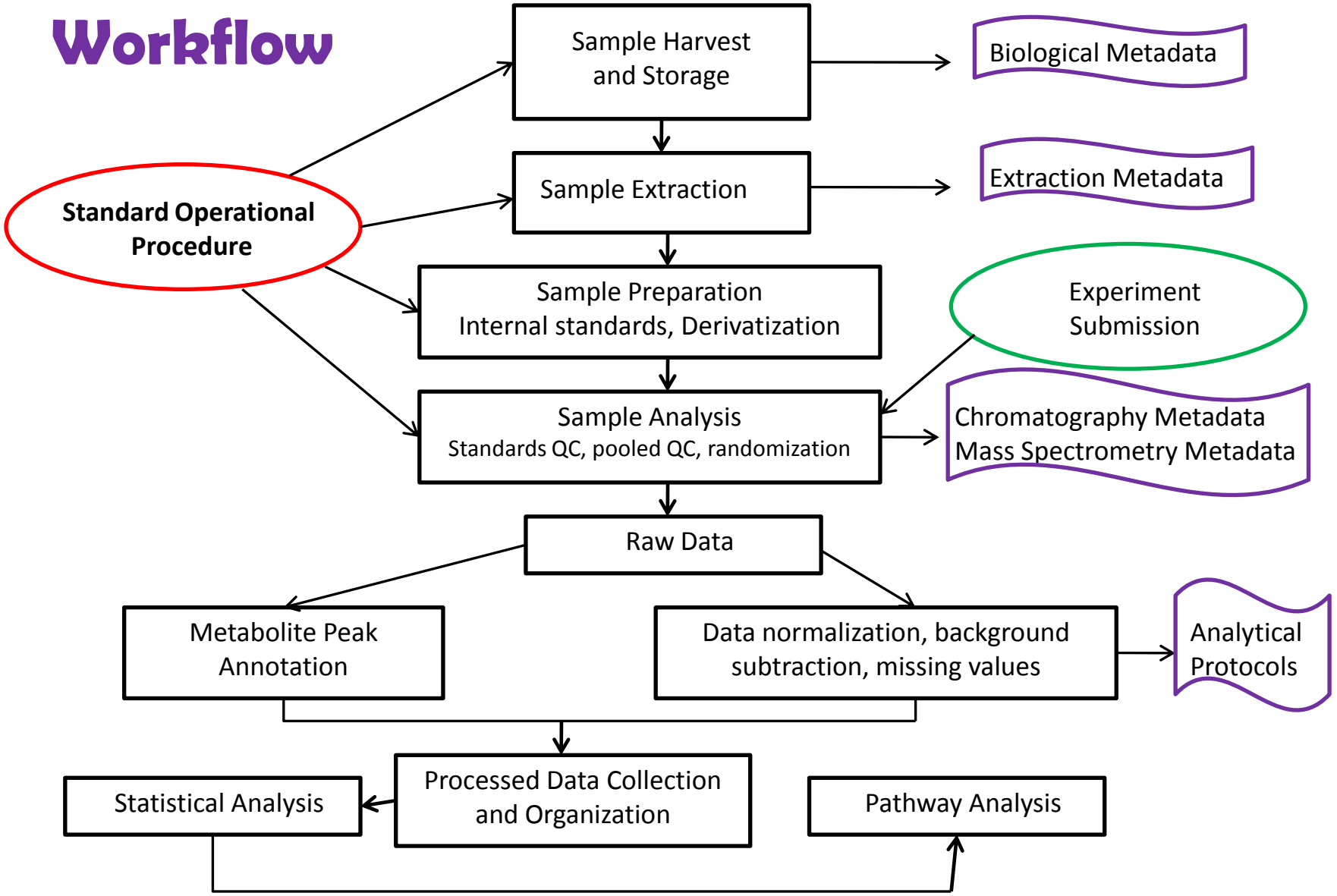


Data Annotation

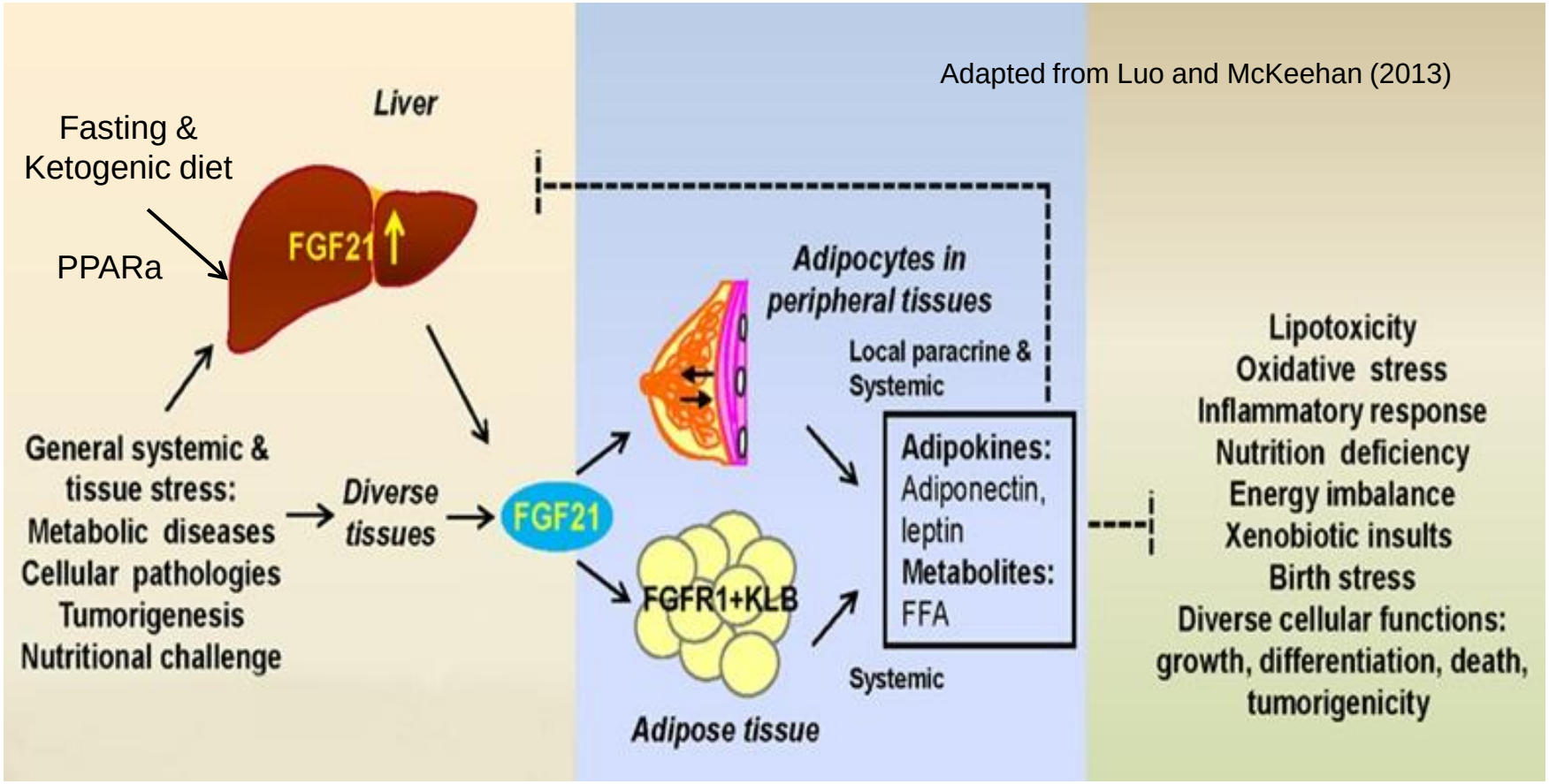
Data pre-processing



Workflow


Sample Harvest and Storage
Biological Metadata
Standard Operational Procedure
Sample Extraction
Extraction Metadata
Sample Preparation
Internal standards, Derivatization
Experiment Submission
Sample Analysis
Standards QC, pooled QC, randomization
Chromatography Metadata
Mass Spectrometry Metadata
Raw Data
Metabolite Peak Annotation
Data normalization, background subtraction, missing values
Analytical Protocols
Statistical Analysis
Processed Data Collection and Organization
Pathway Analysis

Case Study: FGF21 dietary induction



In mice FGF21 is strongly induced in liver by prolonged fasting via PPAR-alpha and in turn induces the transcriptional coactivator PGC-1α and stimulates hepatic gluconeogenesis, fatty acid oxidation, and ketogenesis. *Wiki*

FGF21 regulates mitochondrial activity and enhances oxidative capacity in adipocytes, resulting in increased phosphorylation of AMPK, increased cellular NAD+ levels and activation of SIRT1. *Wiki*

Study design

- 1- wild type fed with chow diet,
- 2- PPARa knockout mice fed with chow diet,
- 3- wild type fed with ketogenic diet,
- 4- PPARa knockout mice fed with ketogenic diet,
- 5-wild type fed fasting,
- 6- PPARa knockout mice fed fasting.

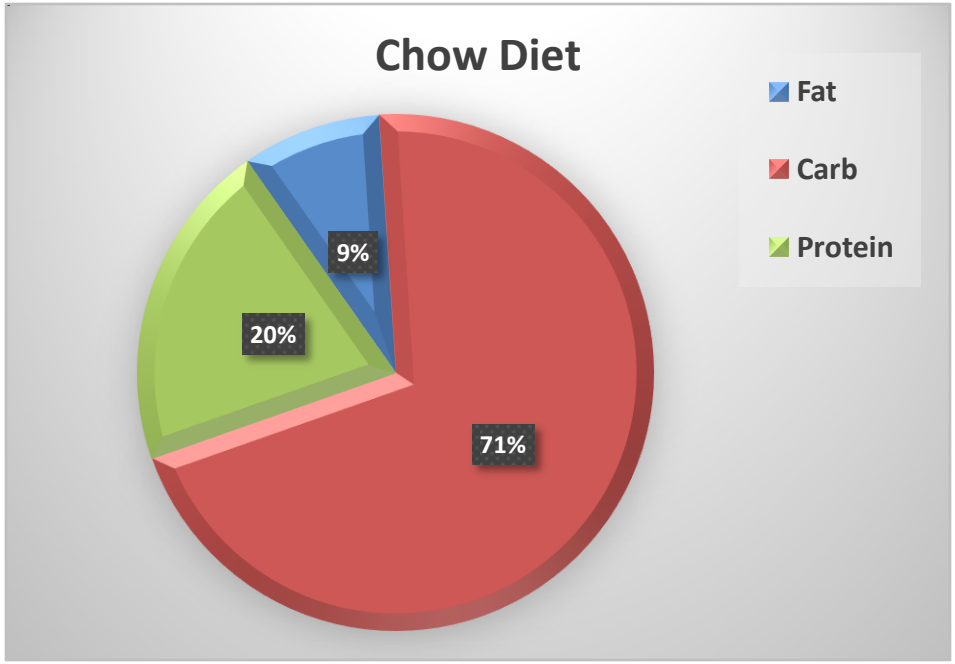
Each group contained 5 animals

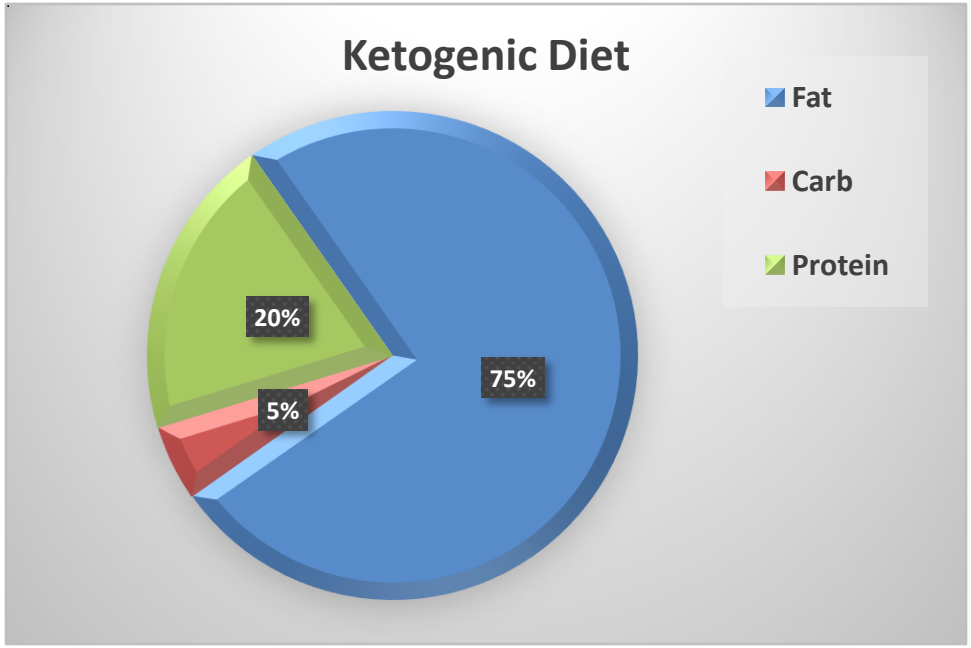
Treatments – 24 hours

Liver samples were harvested for metabolomics analyses

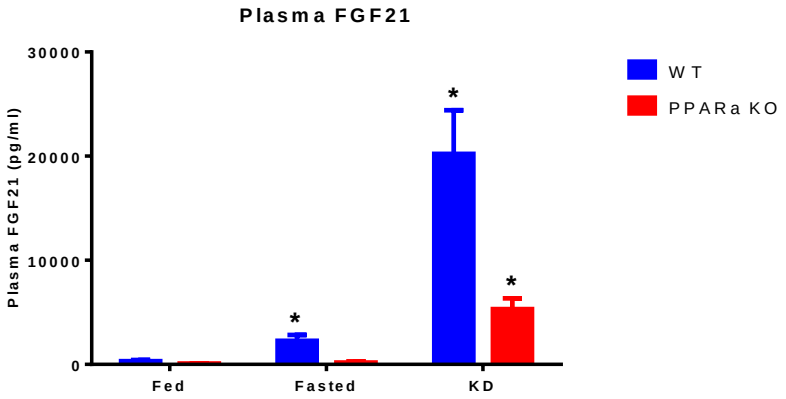


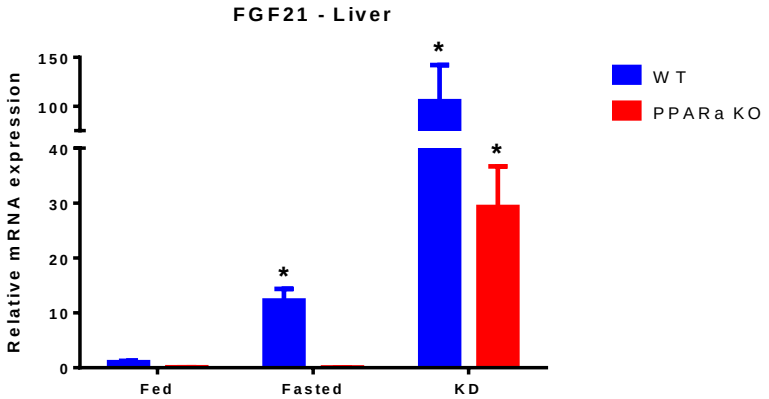
Lilly

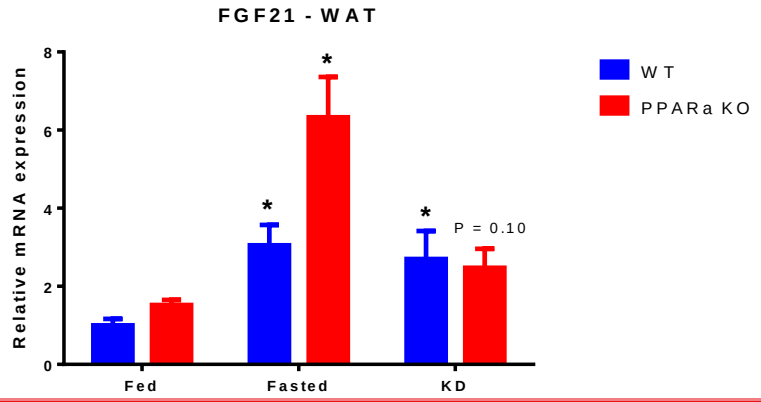

Chow Diet
Fat
Carb
Protein
9%
20%
71%

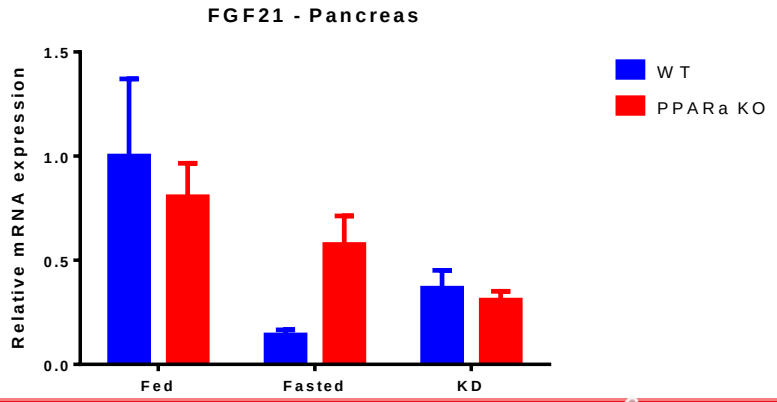

Ketogenic Diet
Fat
Carb
Protein
20%
75%
5%

Fasting and ketogenic diet induce different FGF21 responses in WT and PPAR alpha KO mice

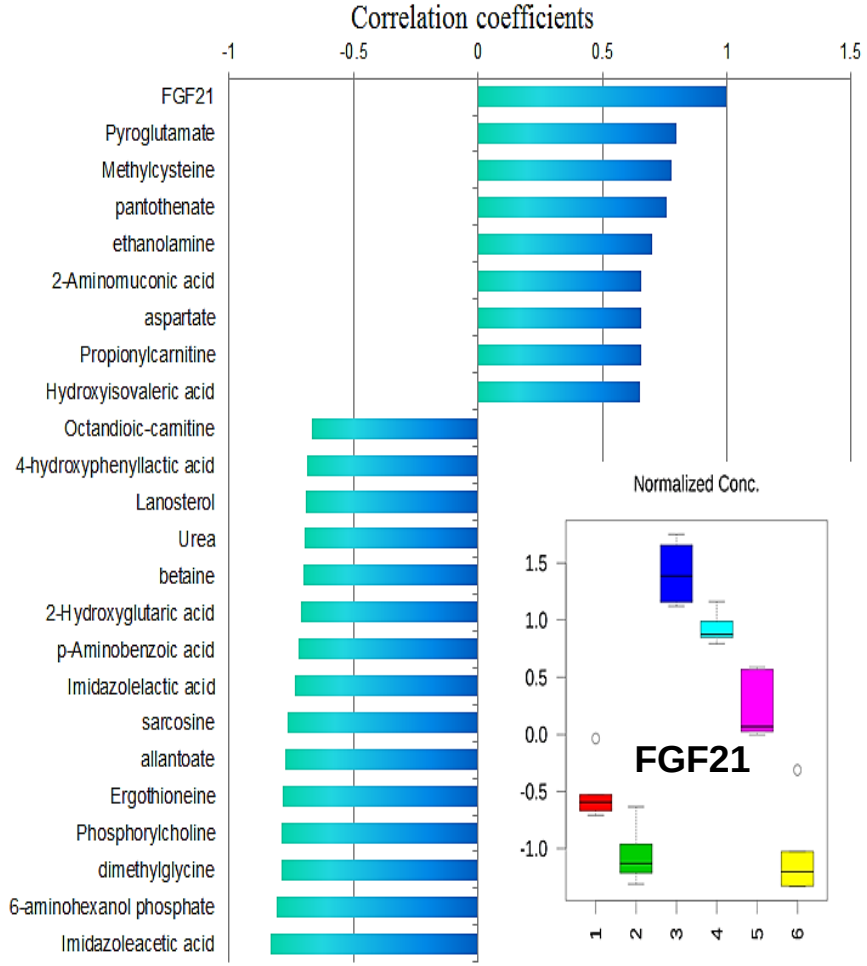


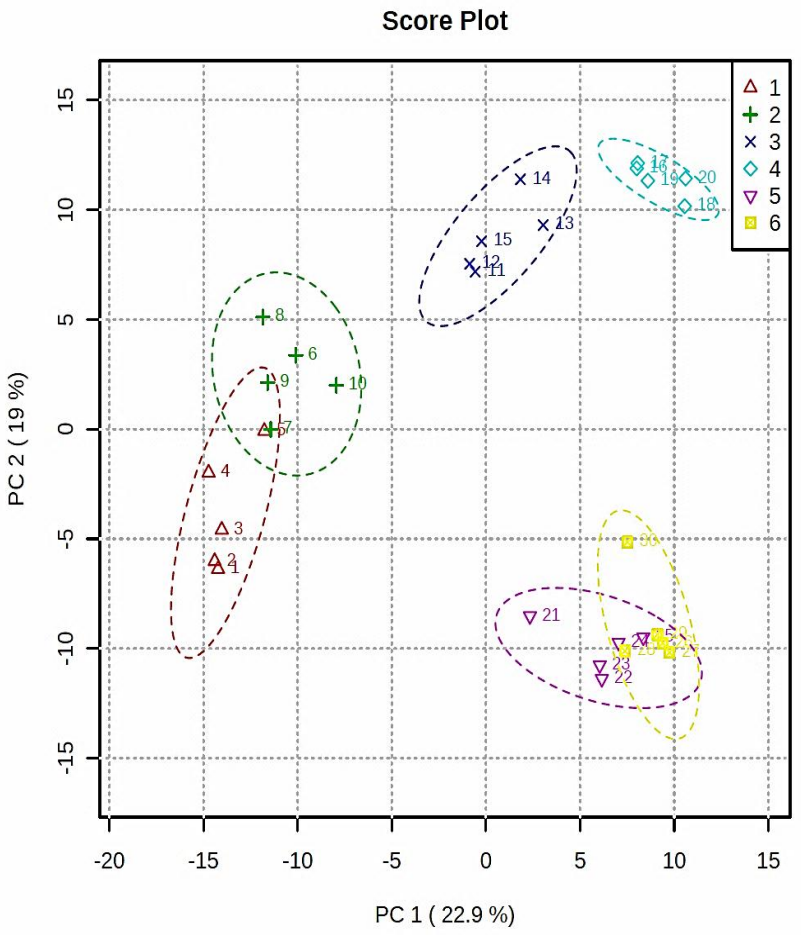






Plasma FGF21 levels correlate with liver expression changes.

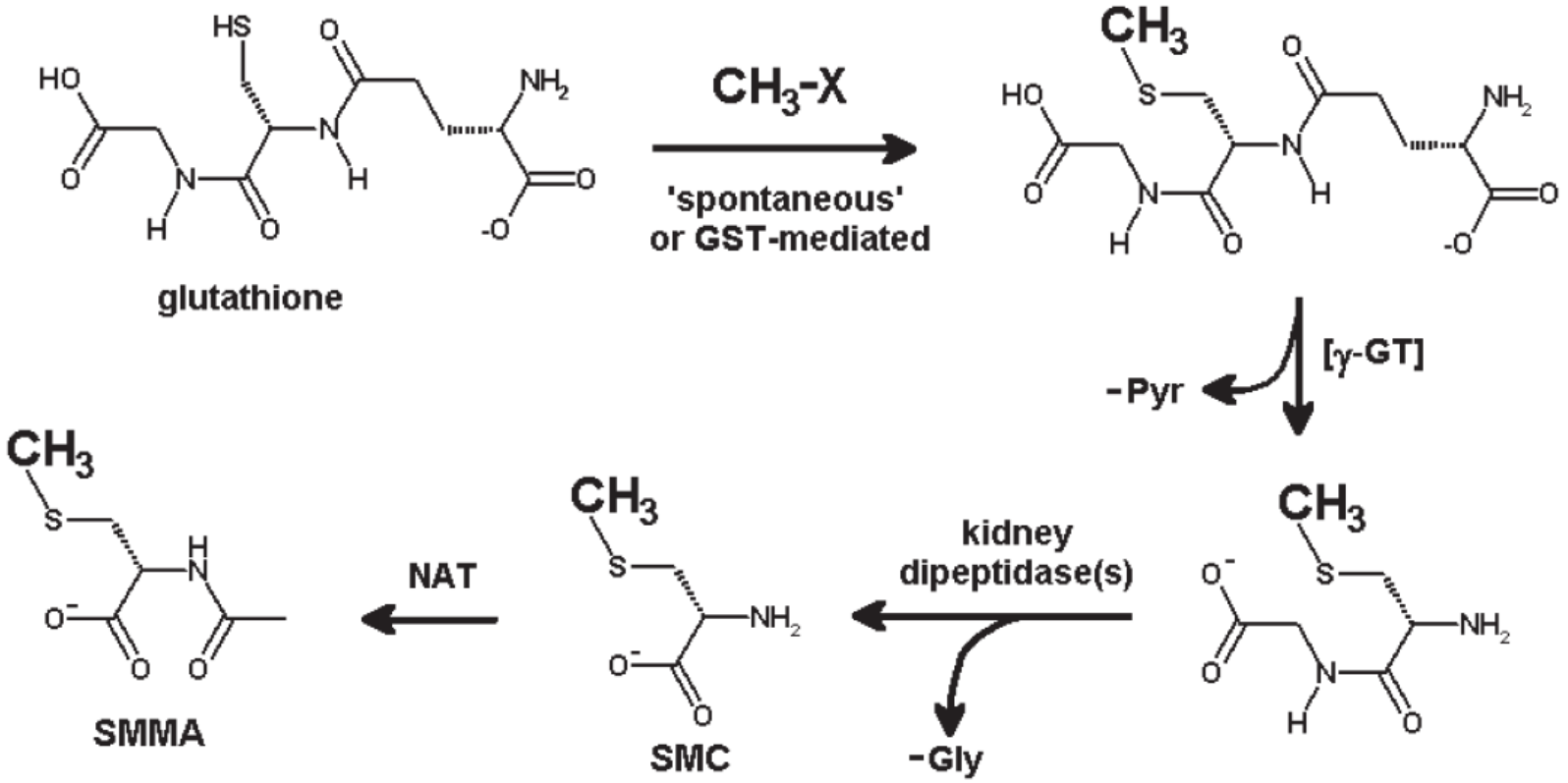




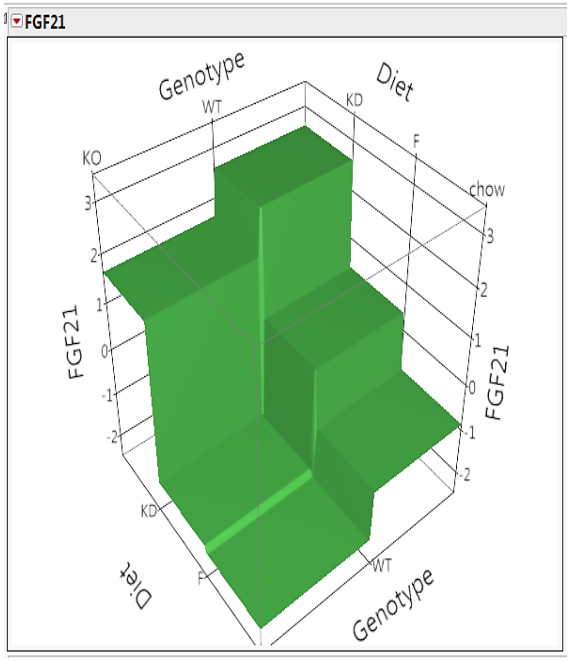
1- wild type fed with chow diet,
2- PPARa knockout mice fed with chow diet,
3- wild type fed with ketogenic diet,
4- PPARa knockout mice fed with ketogenic diet,
5- wild type fed fasting,
6- PPARa knockout mice fed fasting.

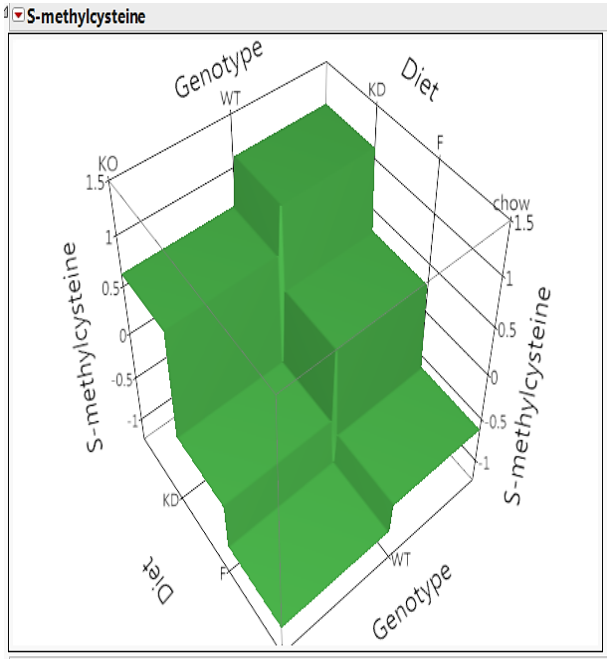
Liver samples were analyzed using global metabolomics - > 300 metabolites

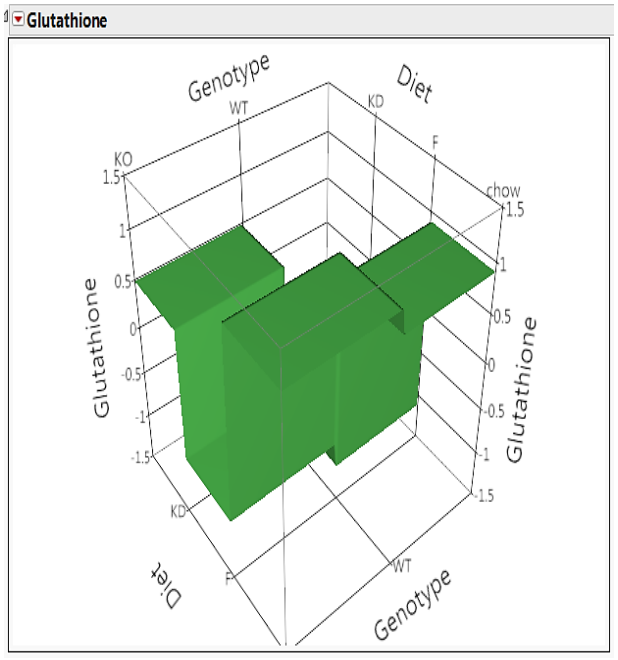
Measurement of S-methylcysteine and S-methyl-mercapturic acid in human urine by alkyl-chloroformate extractive derivatization and isotope-dilution gas chromatography–mass spectrometry, Biomed. Chromatogr. 2011; 25: 330–343

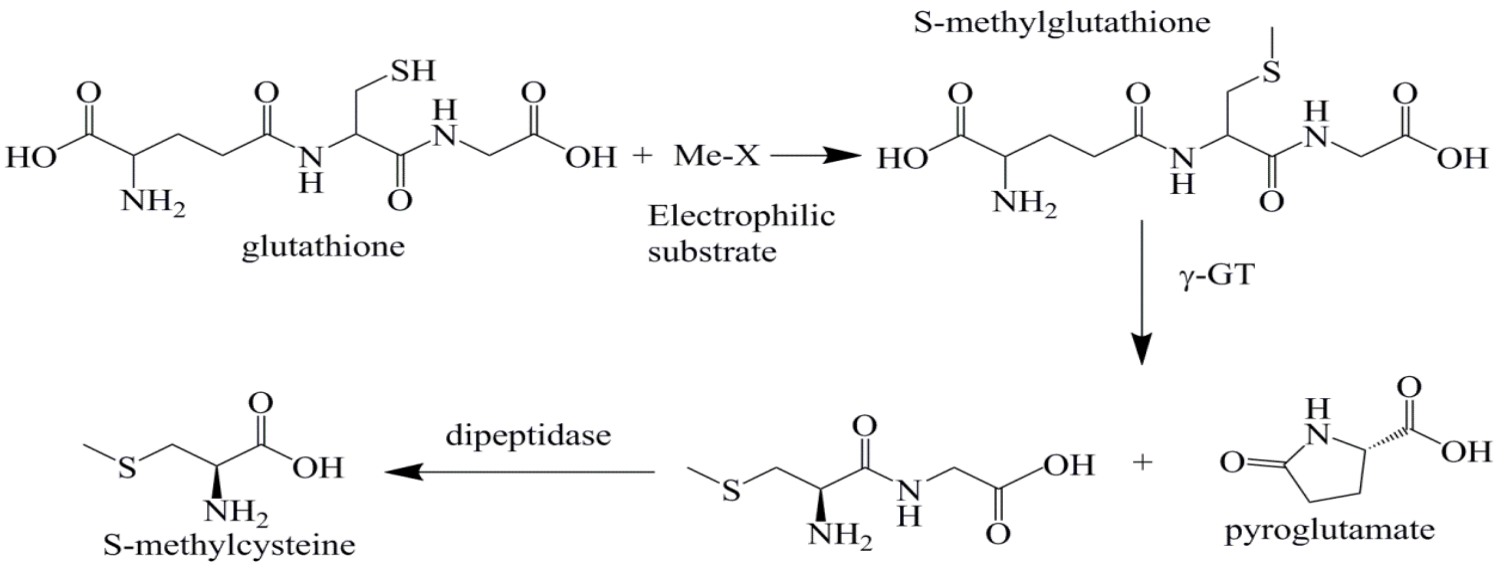


Scheme 3. Biological generation of SMC and SMMA from exogenous methylating agents through the glutathione-mercapturic acid pathway.

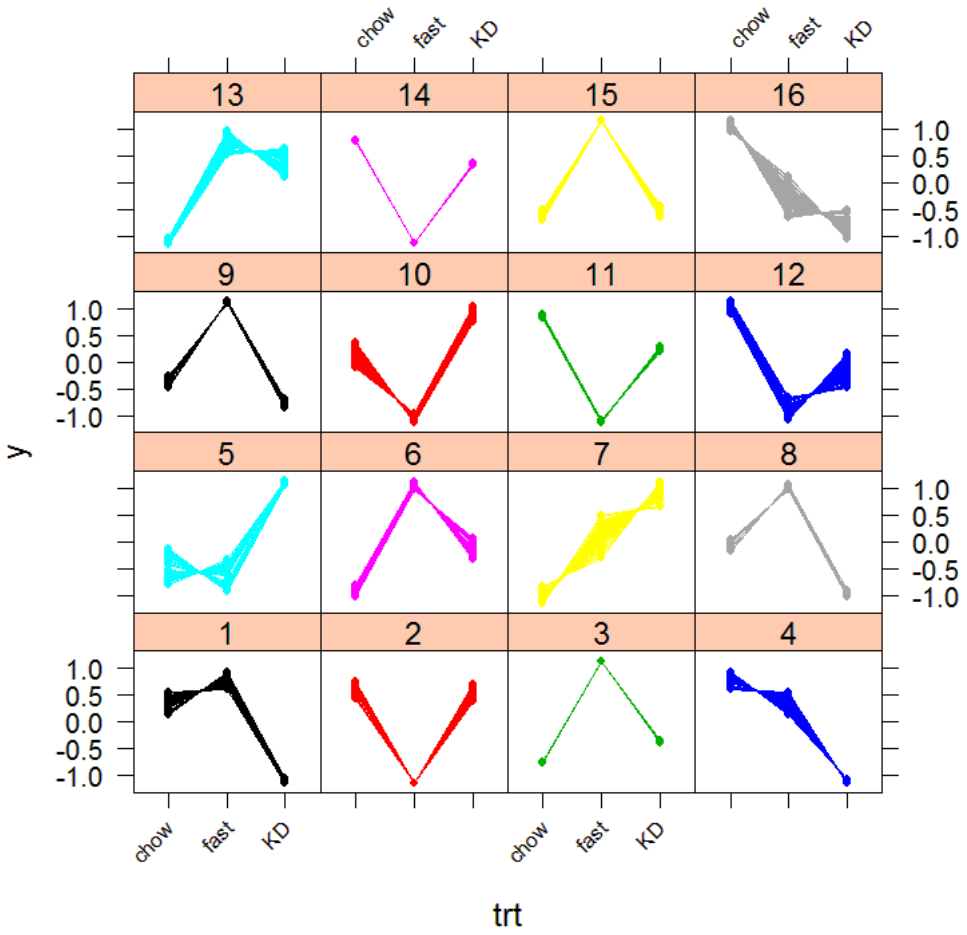


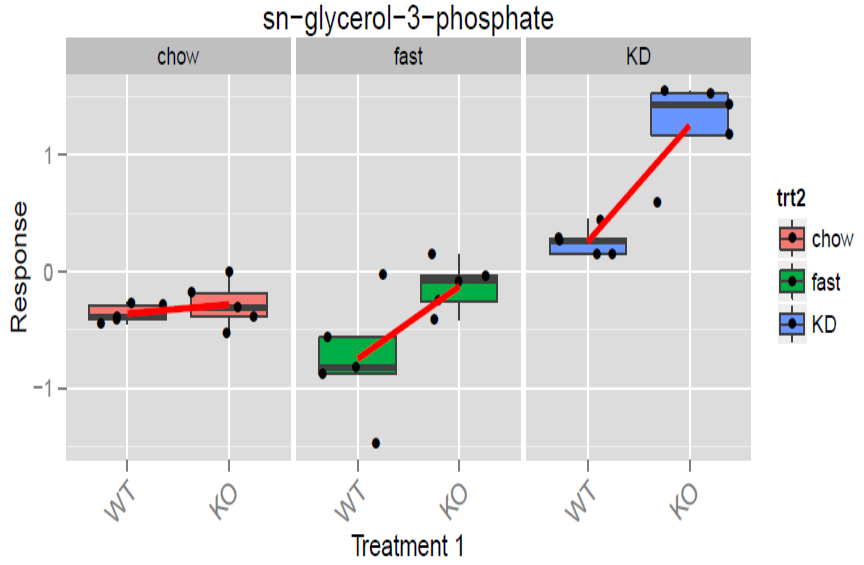


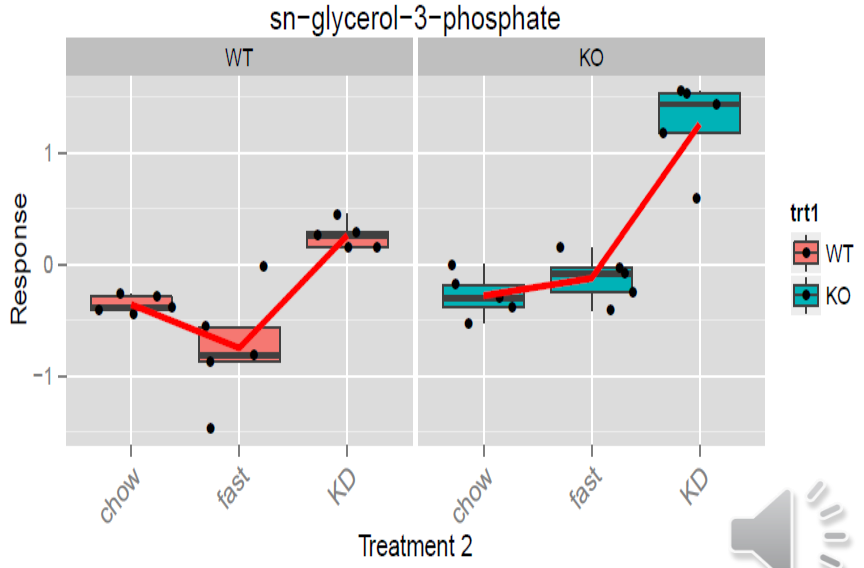




Glutathione-mercapturic pathway. γ-GT - Gamma-glutamyltransferase, dipeptidase – hepatic cysteinylglycine S-conjugate dipeptidase

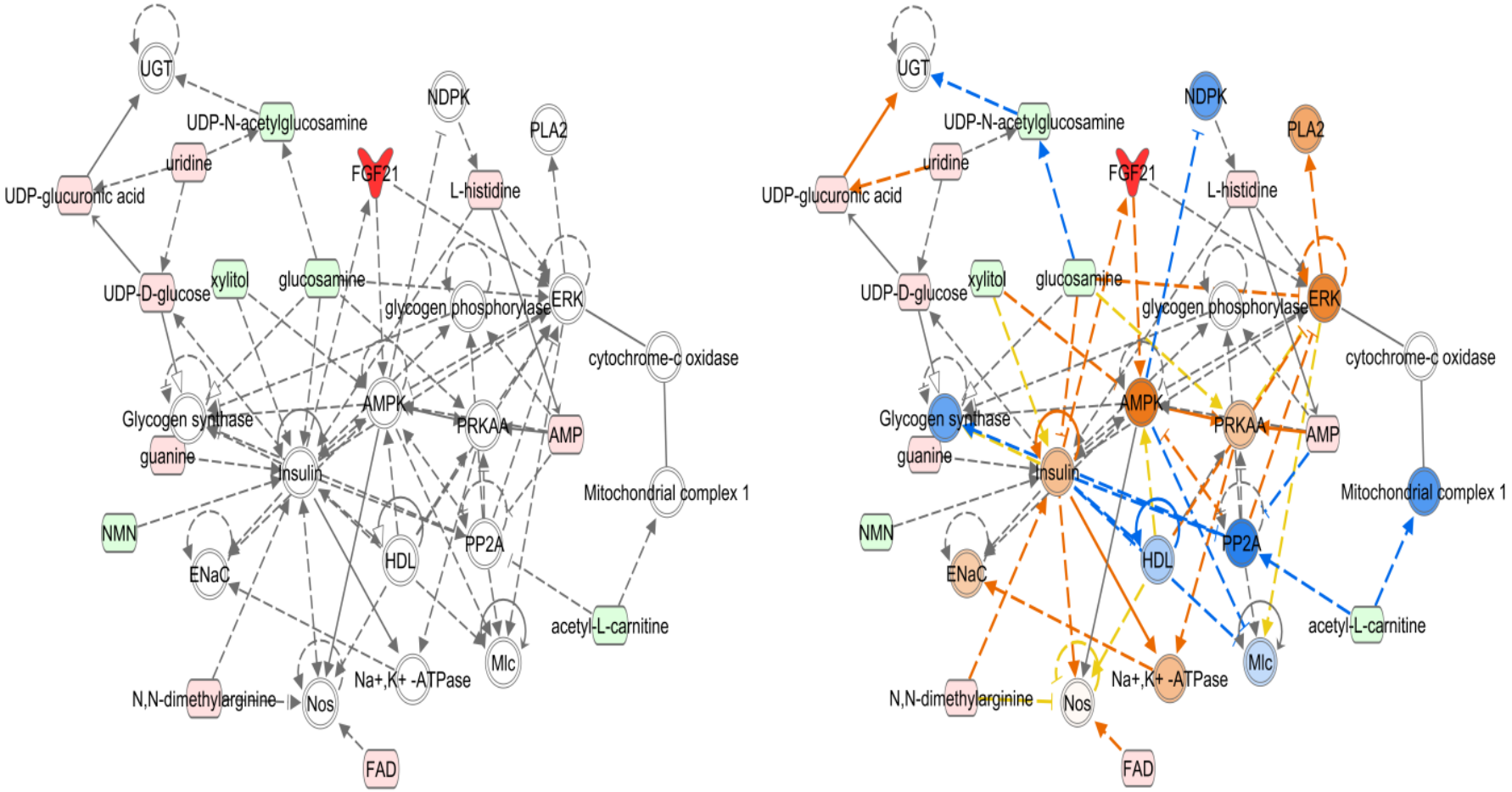






Curve clustering analysis identified 2−dehydro−D−gluconate, deoxyribose−phosphate, and glycerol−3−phosphate as metabolites associated with FGF21 production independent of the regulation of PPARα. This suggests increased TCA cycle activity, elevated lipid accumulation, altered amino acid metabolism and oxidative stress are associated with PPARα-independent increases in FGF21.

Curve clustering analysis revealed potential metabolic biomarkers for PPARa independent regulation of FGF21 production in liver.



Network Analysis in IPA. Experimental data comparing chow and ketogenic diet fed wild type mice (left panel). Molecule Activity Prediction results are shown on the right panel.
Blue = inhibition. Orange = activation.

Summary

- A ketogenic diet dramatically increases and sustains endogenous FGF21 production in wild-type and PPARα KO mice indicating PPARα-independent regulation of FGF21.
- Comprehensive metabolomics platform provided information on metabolic alterations for more than 300 polar metabolites in mouse liver.
- A cluster of metabolites associated with FGF21 production in PPARα KO mice was identified and suggests this increase is associated with increased metabolic stress in the liver.
- Pattern correlation analysis suggests significant changes to the transmethylation pathway.
- Curve clustering analysis isolated group of potential metabolic biomarkers associated with PPARα-independent increases in FGF21 levels.
- Network analysis in IPA provides a mechanistic hypothesis for observed changes.

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Alexander Nikolayev, MS
Consultant Scientist

Dr. Han Wu, PhD
Research Scientist

Anthony Butterfield, MS
Consultant Biologist



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