# LIPIDOMIC AND METABOLOMIC ANALYSIS OF HYPERBILIRUBINEMIC PLASMA

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## Background

Gunn rats with reduced UGT1A1 function exhibiting hyperbilirubinemia were examined in IMPC regular pipeline. This data showed major phenotype related to UGT1A1 function: elevated bilirubin and liver enzymes (ALT and AST), reduced iron, RBC and hemoglobin. Besides these expected consequences of hyperbilirubinemia, we found reduced levels of cholesterol, HDL, LDL and triglycerides. Correspondingly, several studies suggested a connection between high levels of bilirubin and reduced adiposity. A recent report showed that hyperbilirubinemia is associated with reduced fat mass and increased hepatic mitochondrial biogenesis, specifically in female animals[1].

These findings made basis for selection of this genotype for lipidomic and metabolomic analysis of plasma.



### Bilirubin

Bilirubin is typically yellow end product of heme breakdown, originating mostly from degradation of red blood cells. Once bilirubin reaches liver, it is conjugated with glucuronic acid by family of UDP-glucuronosyltransferases (UGTs) to create more water-soluble form to limit its toxicity and facilitate its elimination through bile. Gunn rats lack functioning UGT1A1 enzyme and thus exhibit unconjugated hyperbilirubinemia. Defects in gene coding for UGT in humans lead to Gilbert's syndrome or Crigler-Najjar syndrome.



glukuronide

bilirubin

[1] Vidimce J., Pillay J., et al. *Front. Pharmacol.* **2021**, 12, 586715.

## Results

The most prominent differences in plasma metabolite composition between Gunn rats and controls were found in female cohort. 6 main subclasses of metabolites were found: bile acids, flavonoids, fatty acids, amino acids, steroids and, as expected, bilirubin and its metabolites. As opposed to male Gunn rats, lipidomic data analysis in female Gunn rats revealed substantial decrease of all measured lipid classes in comparison with control, with a main difference in compounds containing long unsaturated fatty acids.



Metabolomic screening: 1 PCA for positive and negative mode. 2 Cluster analysis. 3 Metabolic network associated with hyperbilirubinemia.

## Methods

Rat plasma of 14 weeks old animals of both genders and genotypes was examined in metabolomic and lipidomic screening using mass spectrometry.

- Orbitrap ID-X Tribrid (Thermo), Zorbax Eclipse Plus C18 column, water/ACN/methanol extraction for metabolomics
- 6546 LC/Q-TOF (Agilent), Accucore C30 column, MTBE extraction for lipidomics

extraction from plasma → iterative MS/MS → compound libraries → MS scan → extracted peaks



Lipidomic screening: 4 PCA analysis. 5 Volcano plot. 6 Cluster analysis. 7 Fold changes of lipids in Gunn rats characterized by total chain unsaturation and total chain length.

## Conclusions

This is the first study to comprehensively assess metabolomics and lipidomics in hyperbilirubinemic rats. Our findings show that hyperbilirubinemia, specifically in female animals, is associated with changes in cholesterol metabolism and breakdown of fat.