Quantitative metabolomic profiling of blood serum during the autophagy modulation by NMR spectroscopy

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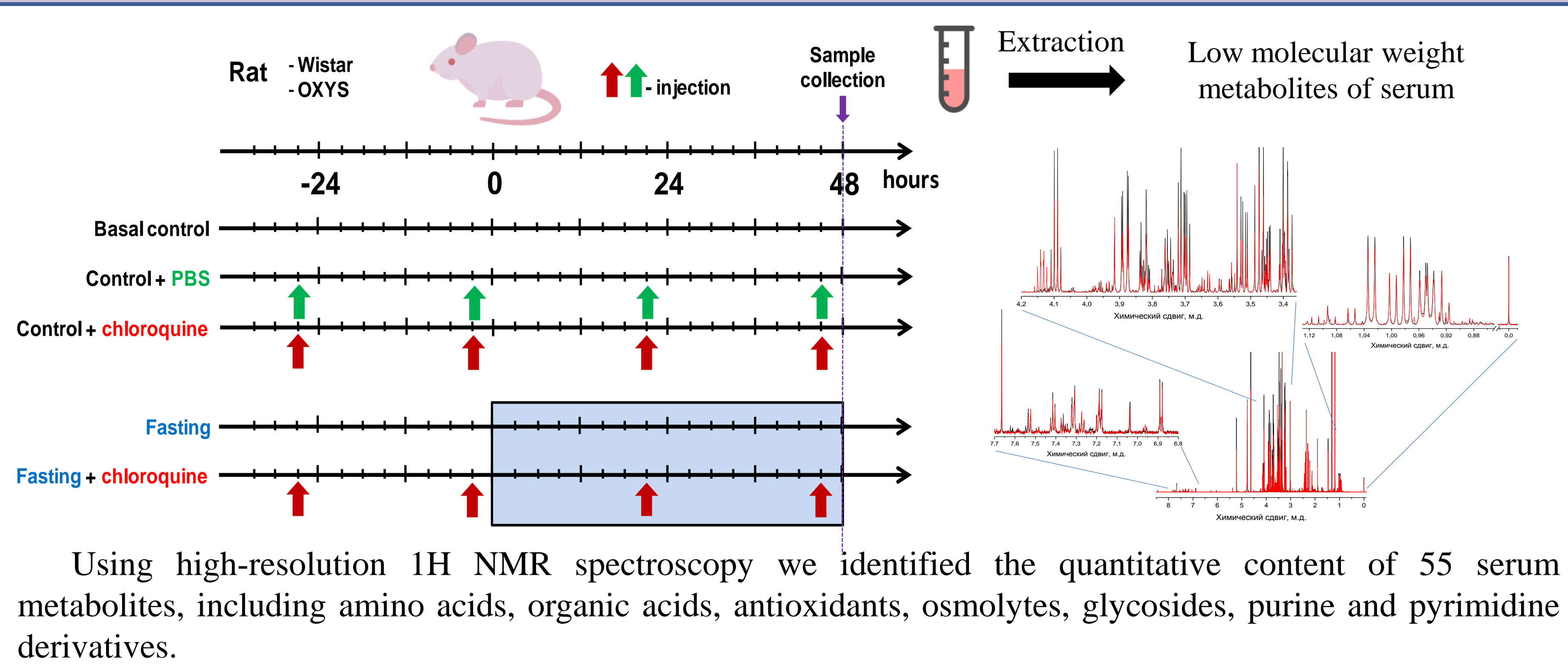
Background

Autophagy is a process of intracellular self-destruction that balances synthesis and degradation. It is a process of delivering the cytoplasmic material into lysosomes and then getting rid of unnecessary or damaged cellular components in order to maintain cellular function. Autophagy allows cells to adapt to stress, mobilize their energy reserves, and decompose potentially harmful components. Autophagy is involved in various processes: from fighting bacterial and viral infections to cell renewal in a developing embryo, it is also one of the main mechanisms for maintaining cellular and organismal homeostasis under conditions of starvation, diabetes, cardiovascular and infectious diseases, neurodegenerative diseases and many age-related diseases.

Understanding the molecular mechanisms of activation and inhibition of autophagy, as well as the mechanisms of its regulation, can serve as the basis for the development of new drugs and increase the effectiveness of cancer treatment methods.

The main goal of the present work was to study the quantitative changes in the concentrations of metabolites under conditions of induction and inhibition of autophagy in blood serum of senescent-accelerated OXYS and Wistar at the age of 4 months. We explored the effects of (1) autophagy activation by 48-h fasting, (2) inhibition by chloroquine (CQ) treatment and (3) combined effects of fasting and CQ.

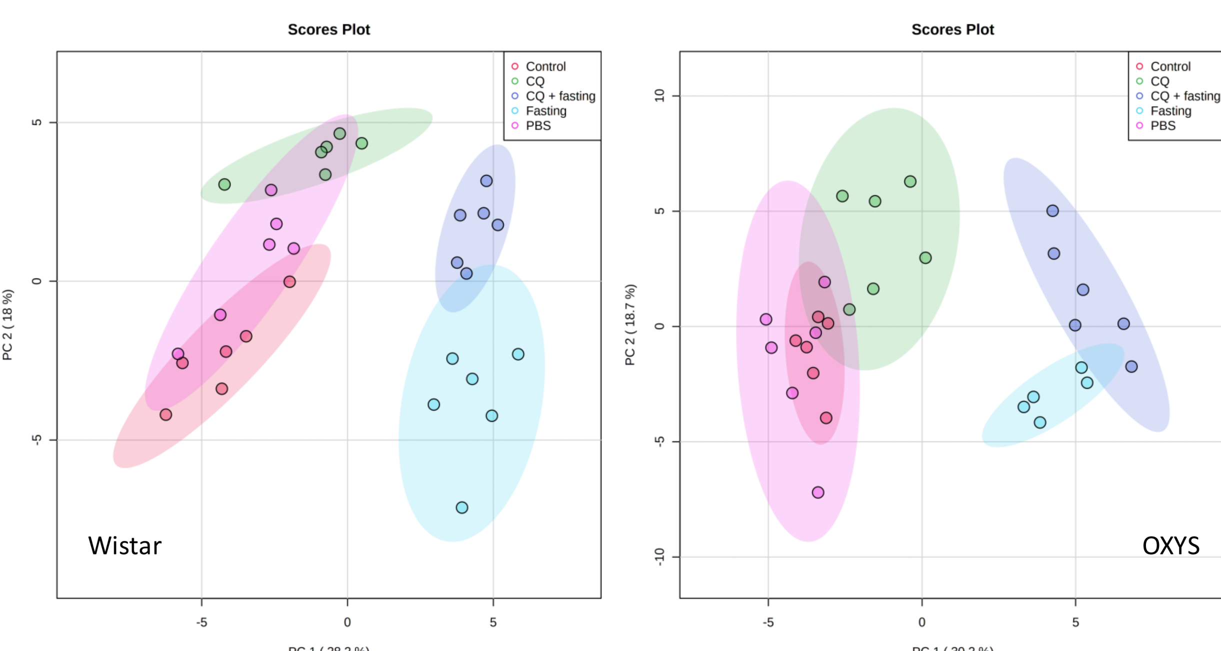
Materials and methods



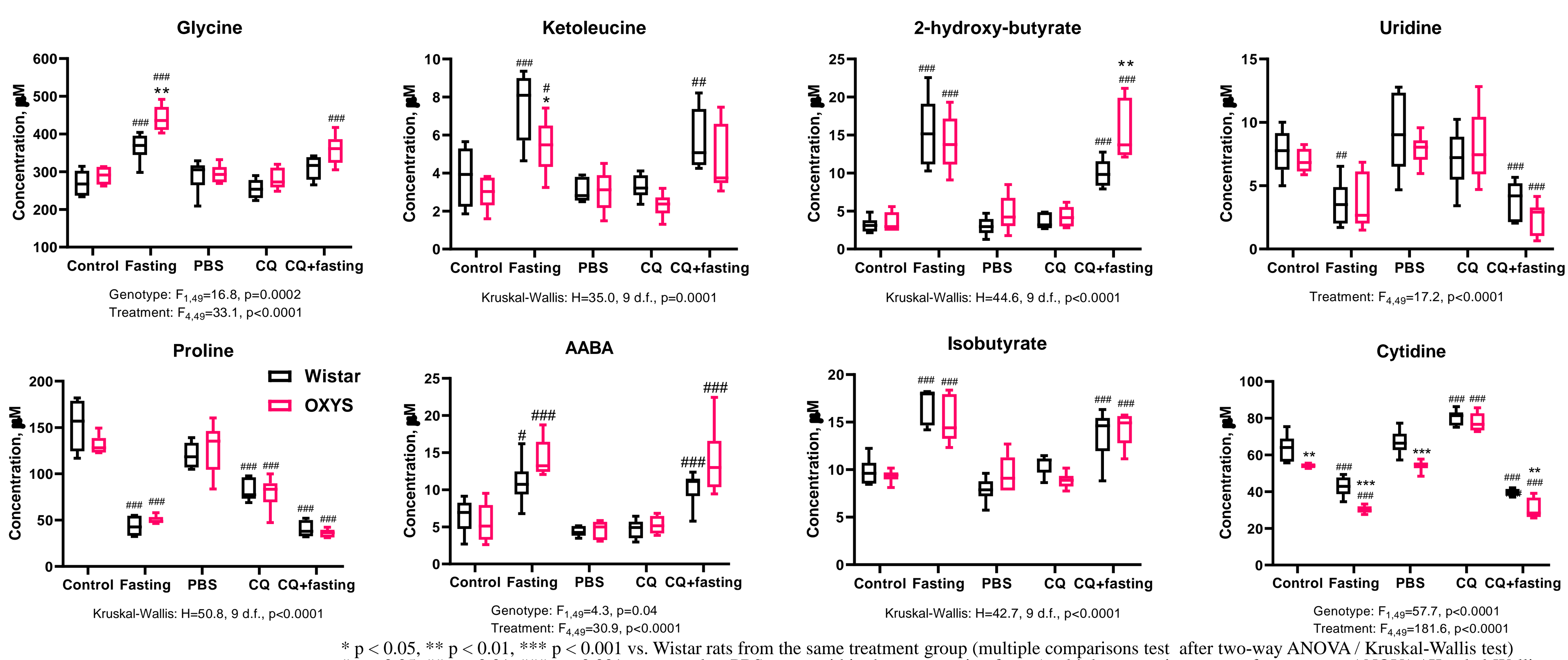
Using high-resolution 1H NMR spectroscopy we identified the quantitative content of 55 serum metabolites, including amino acids, organic acids, antioxidants, osmolytes, glycosides, purine and pyrimidine derivatives.

Results

Quantitative metabolomic profile of blood serum

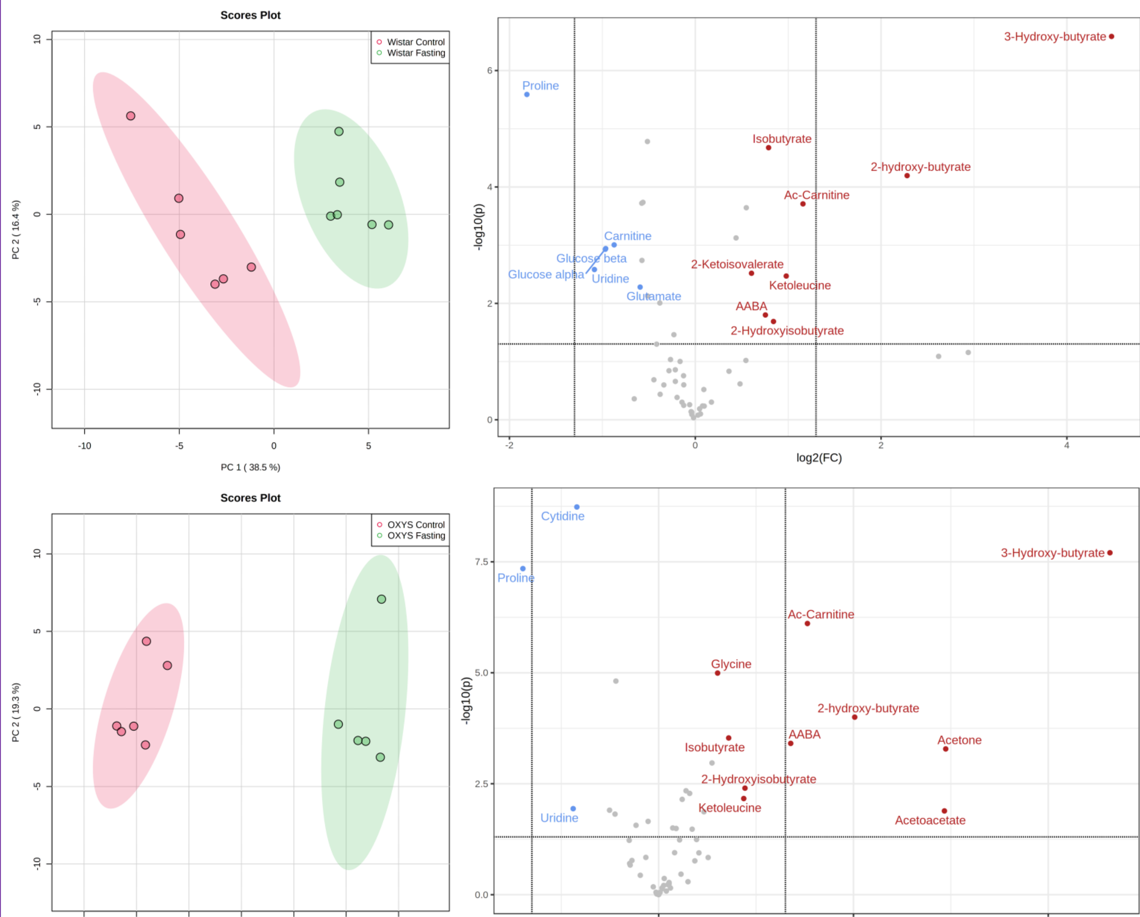


To evaluate the differences in the metabolomic profiles of studied groups we performed principal component analysis (PCA). Fasting induces significant metabolomic changes along the first principal component (PC1), while changes related to CQ administration mostly correspond to PC2. CQ influence is noticeable for both fasting animals and for the group of animals without food restriction.



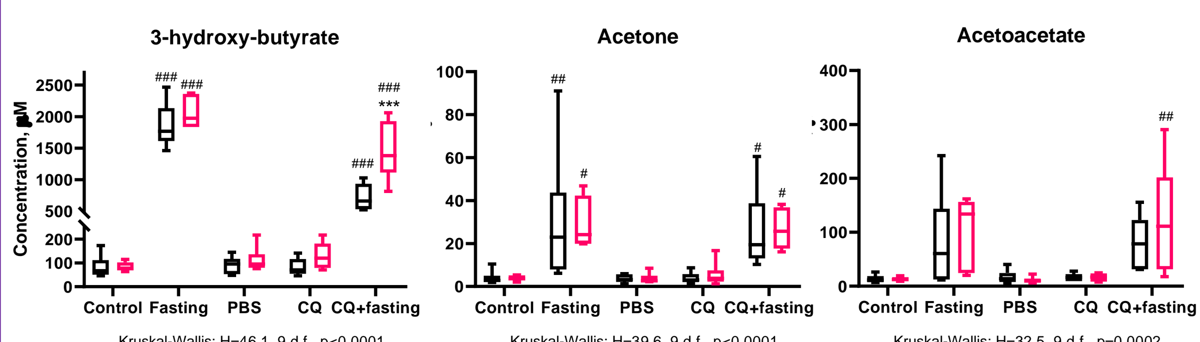
* p < 0.05, ** p < 0.01, *** p < 0.001 vs. Wistar rats from the same treatment group (multiple comparisons test after two-way ANOVA / Kruskal-Wallis test)
# p < 0.05, ## p < 0.01, ### p < 0.001 vs. control or PBS group within the same strain of rats (multiple comparisons test after two-way ANOVA / Kruskal-Wallis test)

Changes of metabolomic profile during fasting



The metabolites with the highest (fold change >1.5) and statistically significant (p < 0.05) differences between fasting and control groups

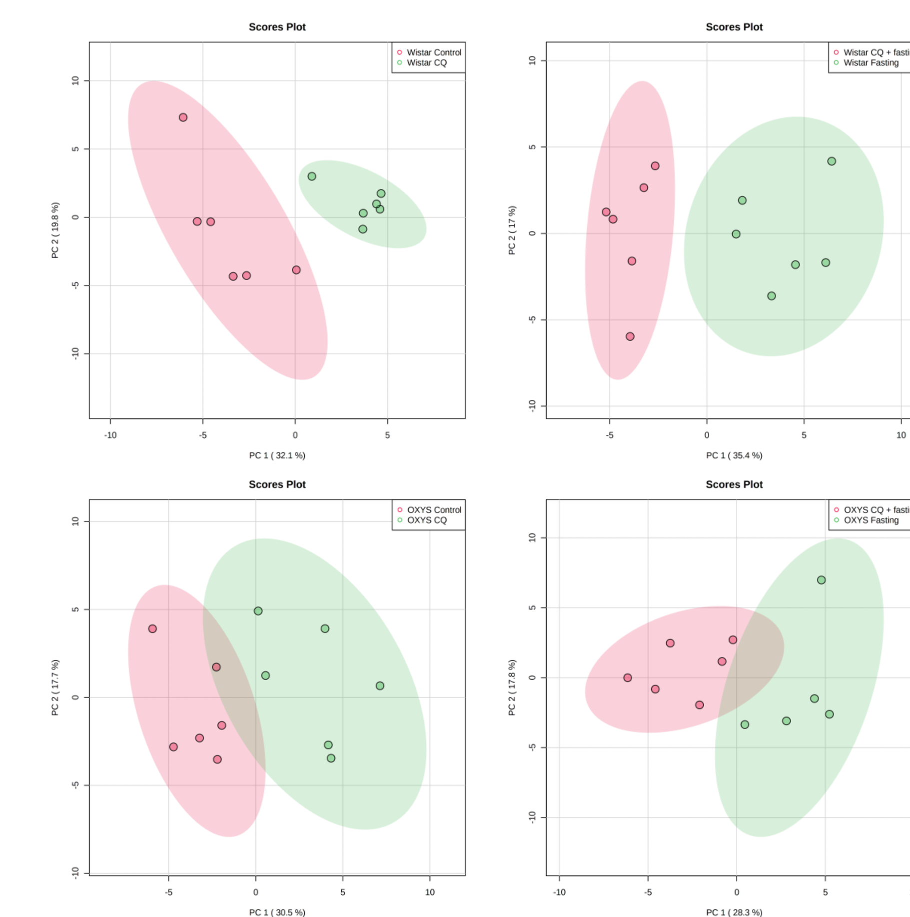
The most drastic changes correspond to increase of concentrations of ketone bodies and organic acids: acetone, acetoacetate, ketoleucine, AABA, 2-hydroxybutyrate, 3-hydroxybutyrate, isobutyrate, and so on. The elevation of 3-hydroxybutyrate level was more than 20-fold from 80 μM in control animals to approximately 2 mM in rats with the dietary restrictions. This may indicate the state of ketose in rats after 48 hours of fasting. The set of differential metabolites for Wistar and OXYS rats are similar, but two-way ANOVA indicates that for several metabolites the effect of fasting depends on genotype.



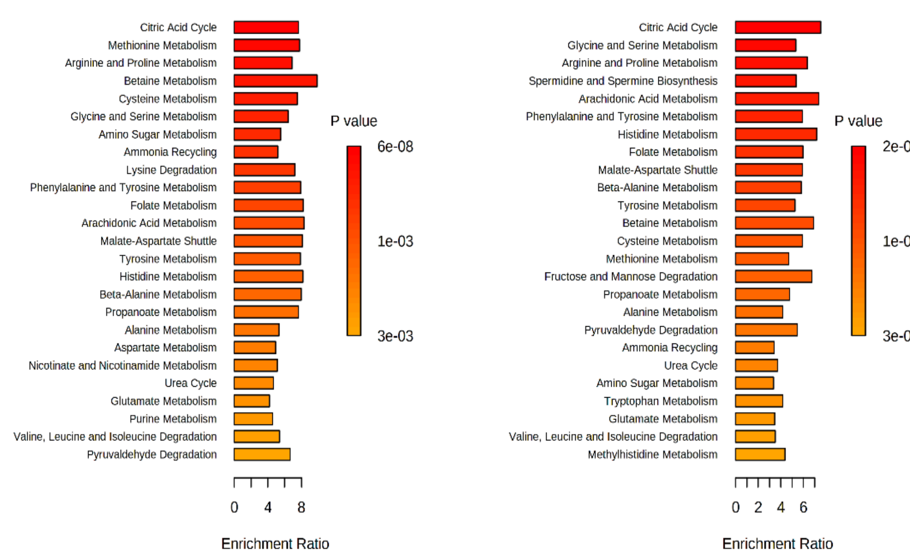
Metabolite Set Enrichment Analysis (MSEA)

For both rat strains the most affected metabolic pathways are fatty acid metabolism, beta oxidation of very long chain fatty acids, and metabolism of glycine, serine, glutamate, alanine, arginine, proline, and glutathione.

Changes of metabolomic profile with CQ treatment

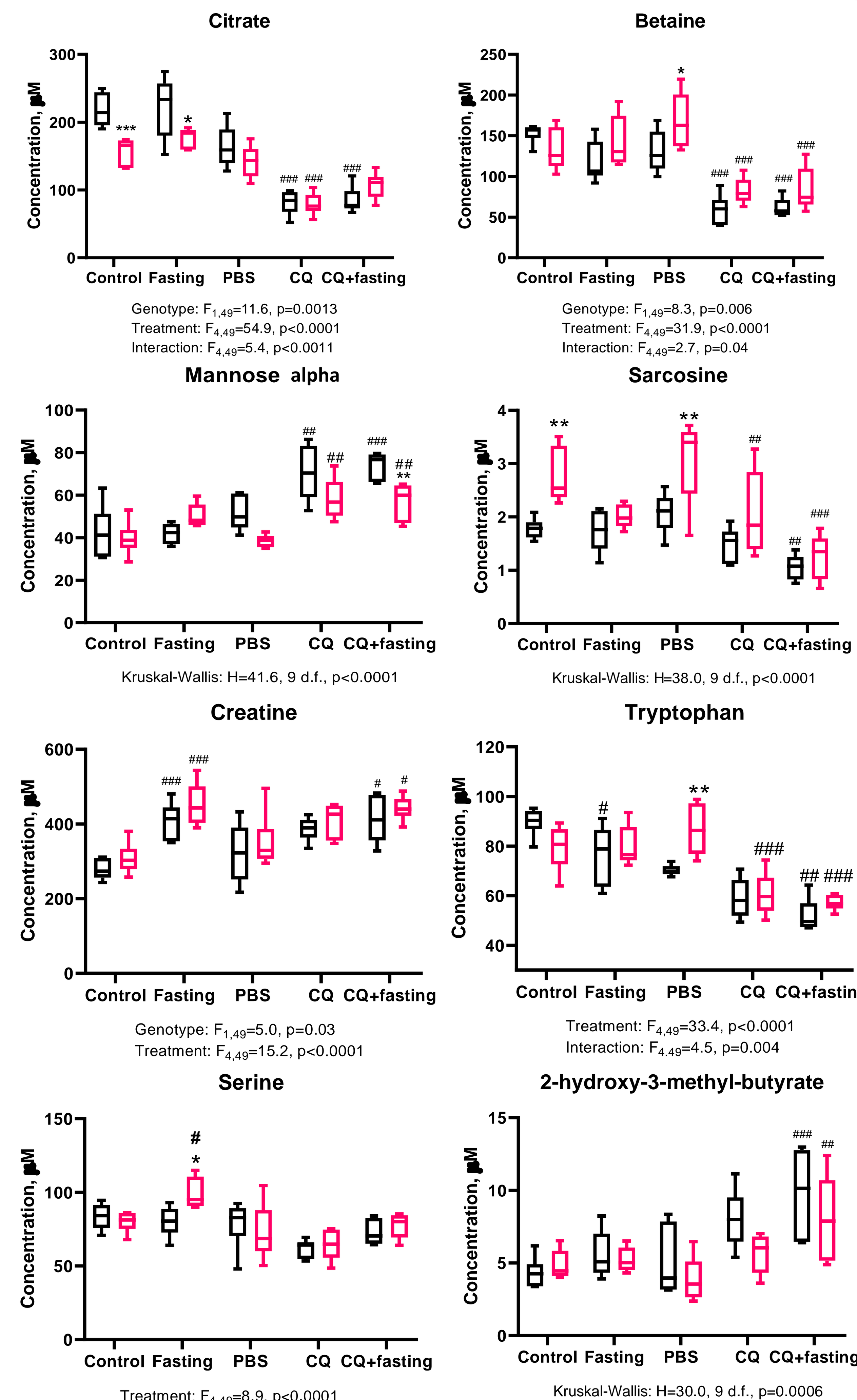


According to the univariate analysis, CQ increases the concentrations of cytidine, mannose, 2-hydroxy-3-methylbutyrate, phenylalanine, creatine. Exposure to chloroquine reduces the concentration of 13 metabolites, for four compounds the decrease was more than twofold (citrate, betaine, proline, ascorbate). Two-way analysis of variance shows that for many metabolites, the effects of chloroquine depends on the genotype of animals (cytidine, β-mannose, tryptophan, sarcosine, methionine, glucose, ascorbate, betaine, asparagine).



The most affected metabolic pathways are citric acid cycle and metabolism of arginine, proline, glycine, serine, methionine, betaine, cisteine, folate, and tyrosine.

The major interstrain differences correspond to different contributions of these pathways into the total picture of metabolomic changes.



- **48-hour fasting leads to significant changes in the serum metabolomic profile, primarily affecting metabolic pathways related to fatty acid metabolism and to metabolism of several amino acids.**
- **Under CQ treatment, the most affected metabolites were citrate, betaine, cytidine, proline, tryptophane, glutamate and mannose.**
- **For many metabolites the effects of autophagy modulation depended on the animal genotype indicating a dysregulation of metabolome reactivity in OXYS rats. Thus, the metabolic responses to modulation of autophagy in OXYS and Wistar rats are different .**
- **Altered metabolites in OXYS rats serum may serve as potential biomarkers of the manifestation of the signs of accelerated aging.**
- **Revealed metabolic signatures characteristic to fasting and CQ treatment might provide an enhanced understanding for the mechanism connecting metabolism and autophagy.**

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