Tissue-specific 1H-NMR metabolomic profiling in mice with adenine-induced chronic kidney disease

This project is focused on a metabolomic analyses of the heart, liver, kidney, and skeletal muscles obtained from mice with and without CKD. To accomplish this objective, we extracted tissues from mice with CKD induced by long-term (24 week) adenine-supplemented diet as well as their control-diet fed counterparts with normal kidney function. Metabolites were extracted from tissues and 1H nuclear magnetic resonance (NMR) was performed and coupled with multivariate statistical analysis.

**Abstract**

Chronic kidney disease (CKD) results in impaired filtration of metabolites which may be toxic or harmful to organs/tissues. The objective of this study was to perform unbiased 1H nuclear magnetic resonance (NMR) based metabolomics profiling of tissues from mice with CKD. Five-month-old male C57BL6J mice were placed on either a casein control diet or adenine supplemented diet to induce CKD for 24-weeks. CKD was confirmed by significant increases in blood urea nitrogen (24.1 ± 7.7 vs. 105.3 ± 18.3 mg/dl, *P*<0.0001) in adenine fed mice. Following this chronic adenine diet, the kidney, heart, liver, and quadriceps muscles were rapidly dissected, snap frozen in liquid nitrogen, and metabolites were extracted. Metabolomic profiling coupled with multivariate analyses confirm clear separation in both aqueous and organic phases between control and CKD mice. Severe energetic stress and apparent impaired mitochondrial metabolism were observed in CKD kidneys evidenced by depletion of ATP and NAD+, along with significant alterations in TCA cycle intermediates. Altered amino acid metabolism was observed in all tissues, although significant differences in specific amino acids varied across tissue type. Taken together, this study provides a metabolomics fingerprint of multiple tissues from mice with and without severe CKD induced by chronic adenine feeding.

**Sample Description:**

Five-month old male C57BL/6J (n=17 mice total) were purchased from Jackson Laboratory and chronic kidney disease was induced in about half of the mice via adenine diet regime. Control mice received casein diet for the duration of the study. While under isoflurane anesthesia, tissues were rapidly dissected and snap frozen in liquid nitrogen and stored at -80°C until metabolite extraction. The following tissues were used in this study: kidney, liver, heart (left ventricle), and skeletal muscle (quadriceps). Euthanasia was carried out by thoracotomy followed by cervical dislocation.

To extract metabolites, FOLCH extraction was carried out and two different phases of samples were obtained: aqueous phase and organic phase. Later on, 1H NMR spectra were collected on these resulted samples separately and multivariate analysis was performed.

The data obtained for the NMR metabolomics analysis can be found in the accompanying files:

Procedures: 1. CKD Quad Organic Phase Procedures.docx

Study Design Tables: 2. Study Design Table.xlsx

Metadata: 3. METADATA.xlsx

Processed Data: 4. Weight corrected Data

Raw Data: 5. CKD Quad Organic Phase Raw Data.zip

**Notes:**

Full NMR sample preparation and analysis procedures are available in the accompanying document entitled 1**. CKD Quad Organic Phase Procedures.docx.**

The normalized data that was used in Metaboanalyst 4.0 analysis is available in the accompanying files: **4. Weight corrected Data**

The raw fid as well as 1r file can be found in **5. CKD Quad Organic Phase Raw Data.zip.**