Diabetic Complications Consortium

Application Title: Urine TCA-Cycle Organic Anions in Diabetic Kidney Disease

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1. Project Accomplishments:

We obtained aliquots of 24 hour urine and fasting plasma (where available) from participants in the STOP DKD biorepository. Specimens were tested as indicated in our proposal. We measured 24 hour urine organic anions using targeted MS at the Duke Molecular Physiology Institute (DMPI) in 132 samples. Species measured include lactate, ketones, pyruvate, methylmalonate, ethylmalonate, succinate, methylsuccinate, fumarate, malate, alpha-ketoglutarate, and citrate/isocitrate. For discovery purposes we also measured untargeted metabolites in the urine using GC/MS, identifying 163 metabolites of known identity. In fasting plasma (n=96), we measured keto-acids, amino acids (AA), and acylcarnitines (AC) using targeted MS. We also measured total ketones, lactate, non-esterified fatty acids, triglycerides and pyruvate using clinical chemistries. All measurements were performed at the DMPI on blinded samples.

We have conducted analyses as described in our proposal to support Aims 1 and 2. We noted that follow up was very short limiting our ability to successfully complete Aim 2. As an alternative approach we augmented follow up data using the Duke electronic health records (EHR), a process consistent with STOP DKD consent. We were able to find additional measures of serum creatinine for 122 participants in the EHR. From these we created eGFR trajectories for more successful completion of Aim 2.

Preliminary results are presented below under each Aim.

2. Specific Aims:

Aim 1: Establish urine TCA cycle metabolites as a biomarker of dysregulated metabolism in DKD.

To complete this aim we performed measures of urine TCA metabolites and systemic metabolites as described above. We summarized urine TCA metabolites as the average of z-scored organic anion concentrations and also analyzed them as factors scores from principal component analyses (PCA). 3 PCA derived factors were discovered. These include factor 1: loading directly on fumarate, malate, alpha-ketoglutarate, citrate/isocitrate, lactate and pyruvate; factor 2: loading indirectly on succinate, pyruvate and lactate; and factor 3: loading directly on methylsuccinate, ethylmalonate, and methylmalonate. For exploratory analyses we also factored urine untargeted GC/MS using PCA into 17 factors. We summarized blood metabolites from AA and AC panels using previously published factor loadings from the CathGen cohort.

Results: Higher urine organic anions (z-score average and factor 1 score) were modestly inversely associated with lower levels of non-esterified fatty acids and triglycerides but were not statistically significant (p-value range 0.1 to 0.2). Models were adjusted for age, sex, race, eGFR, and albuminuria. There was no compelling association with branched chain amino acid factor scores, medium chain acylcarnitine factor scores, or long chain acylcarnitine factors scores.

Aim 2: Establish preliminary evidence supporting urine TCA cycle metabolites as risk factors for DKD progression.

To complete this aim we developed extended eGFR data using the EHR where possible, as described above. We used linear mixed models to assess the association between urine TCA-cycle organic anions and eGFR decline.

Results: Urine organic anions defined by the z-score average were not associated with eGFR decline independent of age, sex, race and albuminuria. The z-score average, urine organic anion factors 1 and 3 were each strongly associated with higher eGFR at baseline. Urine organic anion factor 2, which indirectly loads on succinate, pyruvate and lactate, was marginally associated with faster eGFR decline (nominal p=0.05). Because these metabolites indirectly load on the factor, higher levels of the metabolites appear potentially protective. In the untargeted discovery samples, factor 6 which loads indirectly on 3-hydroxyindole, 4-methylcatechol, p-cresol and gentisic acid was marginally associated with slower eGFR decline (nominal p=0.03). Because these metabolites load indirectly on the factor, higher levels of these metabolites appear potentially detrimental.

3. Publications:

Manuscript under development.
Abstract planned for the American Diabetes Association Scientific Session.