Protocol #: **15-0541**
Project Title: **Effect of Urinary Alkalinization on Urine Uric Acid Precipitation and Crystallization in Adults with Type 1 Diabetes**

Principal Investigator: Petter Bjornstad, M.D.
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I. Hypotheses and Specific Aims: A brief statement of the purpose of the research project. This section should include the hypotheses and specific aims being tested in the research. *Note: If you are applying for QA/QI/PE determination, you should not use terms like "hypothesis" or "research;" Instead, use the terms "aims" or "goals" and "evaluation."*(Approx. 1 paragraph)

Specific Aim 1: To evaluate the ability of 2 doses of oral sodium bicarbonate (1950mg each) to alkalize urine over 24 hours in adults with type 1 diabetes and without chronic kidney disease.

Hypothesis 1: Sodium bicarbonate over 24 hours can increase urine pH by 0.5-1.0 in adults with type 1 diabetes and without chronic kidney disease.

Specific Aim 2: To evaluate the effect of urinary alkalinization on urine uric acid concentration, precipitation and crystallization in adults with type 1 diabetes and without chronic kidney disease.

Hypothesis 2: Increasing urine pH with oral sodium bicarbonate in adults with type 1 diabetes and without chronic kidney disease will solubilize urine uric acid thereby increasing urine uric acid, decreasing urine acid precipitation and crystallization

Specific Aim 3: To evaluate the relationship between urine uric acid concentration, precipitation and crystallization with glomerular function and markers of tubular injury in adults with type 1 diabetes and without chronic kidney disease.

Hypothesis 3.1: Urine uric acid concentration, precipitation and crystallization will be associated with markers of tubular injury (e.g. NGAL, MMP9) in adults with type 1 diabetes and without chronic kidney disease.

Hypothesis 3.2: Urine uric acid concentration, precipitation and crystallization will be associated with estimated glomerular filtration rate (CKD-EPI creatinine and cystatin C) in adults with type 1 diabetes and without chronic kidney disease.

In summary, this will be a pilot study with the aims to: 1) determine whether 2 doses of oral sodium bicarbonate (1950 mg per dose) over 24 hours can alkalinize urine in adults with type 1 diabetes and without chronic kidney disease; 2) determine the effect of urinary alkalinization on urine uric acid; and 3) examine the relationships between urine uric acid and glomerular function and markers of tubular injury.

The importance of this proposal relates to the major research and clinical need to understand the relationship between urine uric acid and diabetic tubular nephropathy in type 1 diabetes.

II. **Background and Significance**: Explain the background of this project so that we will understand why it is important to perform this research project. (Approx. 1 page)

Diabetic nephropathy is characterized not only by glomerular disease but also tubulointerstitial injury. The tubular changes associated with diabetic nephropathy, include basement membrane thickening, tubular hypertrophy, epithelial-mesenchymal transition, glycogen accumulation and interstitial inflammation (1). Although glomerular changes has received significantly more attention from researchers and clinicians than tubulointerstitial changes in diabetes, tubular injury is known to associate better with renal function than glomerular injury (2). In fact, tubular proteinuria may precede microalbuminuria with type 1 diabetes (3), suggesting that tubular damage may be induced earlier than glomerular injury in the course of diabetic nephropathy.

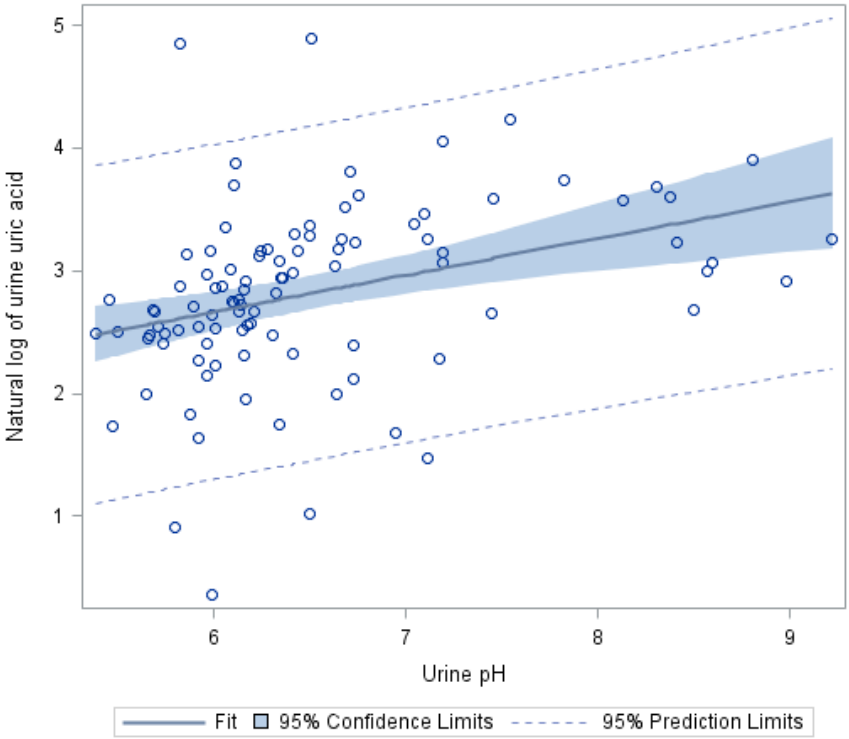
Serum uric acid (SUA) is lower in adolescents and adults with type 1 diabetes compared to non-diabetic peers (4, 5). Despite lower levels SUA remains an important risk factor for diabetic nephropathy in type 1 diabetes (6), with a large clinical trial underway examining the ability of allopurinol to prevent early renal loss (7). Several mechanisms have been proposed to explain the lower levels of SUA in type 1 diabetes including glucosuria induced uricosuria leading to spilling of urine uric acid (UUA) and lowering of SUA (8), and the notion that intracellular uric acid (IUA) and/ or UUA rather than SUA may be responsible for the development of complications. Animal studies have demonstrated that blocking uric acid production protects the kidney from tubulointerstitial injury, which suggests a causal role for uric acid in the development of diabetic tubular injury (9, 10). Relative dehydration, secondary to glucosuria, exercise or inadequate liquid intake, may lead to concentrated and acidic urine, which may cause UUA to precipitate and crystallize in type 1 diabetes (11-14). The UUA precipitation and crystallization is thought to induce inflammation and injury of the tubules with possible retrograde glomerular injury (11-14). Moreover, it was recently shown that UUA promoted apoptosis in human proximal tubular cells by oxidative stress and activation of NADPH Oxidase NOX 4 (15).

Oral alkali replacements are readily available, safe and include the following formulations sodium bicarbonate, BiCitra (sodium citrate and citric acid), PolyCitra (citric acid, sodium citrate, and potassium citrate), polycitra-K (potassium citrate and citric acid). In contrast to sodium bicarbonate, citrate is converted to bicarbonate in the liver and thus this conversion is affected by liver disease. Usual adult doses for urinary alkalinization are 325 to 2000 mg orally 1 to 4 times a day. One gram provides 12 mEq (mmoL) each of sodium and bicarbonate, and is titrated to a goal of urine pH of 8.0. In a prospective open-label trial 4 g of sodium bicarbonate was administered orally 3 times daily to 9 healthy volunteers for 24 hours, and after 10 hours all participants had a urine pH ≥ 7 and after 20 hours all participants had urine pH ≥ 8. No adverse effects or abnormal blood results were documented during the 24-hour follow-up (16). Urinary alkalinization should solubilize UUA thereby increasing the concentration of uric acid in urine and decreasing precipitation and crystallization of UUA. It is unknown whether alkalinization of urine reduces UUA precipitation and crystallization in type 1 diabetes.

With diabetic nephropathy being the leading cause of end-stage renal disease in the Western world. Novel therapeutic targets could help halt the progression of kidney disease in people with type 1 diabetes, thereby improving morbidity and mortality, and lessen the public health burden. While the currently available therapies focus on the glomerular injury of diabetic kidney disease, urine uric acid crystallization is associated with tubular injury, and is thought to precede the onset of glomerular disease in type 1 diabetes. Earlier interventions could decrease the rate of renal function loss and prolong the time to development of end stage renal disease. UUA is a particularly attractive therapeutic target due to the potential to reduce tubular injury with sodium bicarbonate. Furthermore, the ongoing Prevent Early Renal Loss (PERL) study with $27 million in NIH funding will determine if lowering uric acid with allopurinol slows decline in renal function, and sodium bicarbonate to alkalize urine could be a complementary, safe and cheap treatment. Accordingly, we propose a pilot experimental study examining the effect of urine alkalinization with oral sodium bicarbonate on UUA precipitation and crystallization in adults with type 1 diabetes.

III. Preliminary Studies/Progress Report:

Unpublished data in adolescent with type 1 diabetes demonstrated a positive relationship between urine pH and urine uric acid (r=0.48, p<0.0001, n=99, **Figure 1**), suggesting increased solubility of urine uric acid with more alkaline urine.



Preliminary data from adolescents with type 1 diabetes in the *Determinants of Macrovascular Disease* cohort.

However, no data exist on whether urinary alkalinization by sodium bicarbonate can increase solubility of urine uric acid and reduce precipitation and crystallization thereby preventing tubular injury.

IV. Research Methods

A. Outcome Measure(s):

Urine uric acid concentration, precipitation and crystallization measured before and after sodium bicarbonate treatment to increase urine pH.

B. Description of Population to be Enrolled:

Inclusion criteria: In this study we will aim to recruit 78 adults, both males and females aged 18-60 years with type 1 diabetes. Assuming 80% of subjects approached will be eligible to participate and 60% of eligible subjects will consent to participate, this provides at least 47 subjects which is needed for power of 0.8 to detect effect size of 20% in change in urine uric acid.

- Participants must be able to be fasting prior to study visit #2 and #3 at CTRC
- Participants must be able to give informed consent.

Visit #1 will take place at Barbara Davis Center in association with the participants' regular diabetes visit. We plan to meet them before or after their regular visit to discuss study

details including the pre-study diet, review medical history to determine study eligibility and consent eligible and interested participants. This visit will determine eligible prior to diet, urine and blood collection and thereby minimize risk and participant burden. We will also obtain consent from eligible participants during this visit.

Exclusion Criteria:

- Non-type 1 diabetes
- History of eGFR <60 ml/min/1.73m^2 or albuminuria
- History of hypocalcemia or at risk of hypocalcemia (metabolic alkalosis increases binding of calcium to albumin causing a low ionized calcium which can in rare cases cause laryngospasms, paresthesia and seizures in those with already low calcium)
- Taking allopurinol or uric acid altering medications
- Ketogenic diet (secretory mechanisms for uric acid may be inhibited by lactate, betahydroxubutyrate and acetoacetate)
- Ketonuria (secretory mechanisms for uric acid may be inhibited by lactate, betahydroxubutyrate and acetoacetate)
- Taking phosphorus binders (e.g. sevelamer)
- Taking blood pressure medications
- Taking SGLT2 inhibitors
- Pregnant or breastfeeding
- Taking the following medications which may interact with sodium bicarbonate (phentermine, pseudoephedrine, antifungal medication, cephalosporin antibiotics [e.g. Keflex], tetracycline antibiotics [e.g. doxycycline], steroids or lithium)

C. Study Design and Research Methods

Participants will be recruited from Diabetes Clinic at Barbara Davis Center. Informed consent will be obtained following standard COMIRB approved methods by the PI and/or members of the research team.

Sodium Bicarbonate Dose

- 3900mg (2x doses 1950 mg) of Sodium Bicarbonate over 24 hours was decided on based on the following:
    - Usual adult daily doses for Sodium Bicarbonate are 1,200-8,000mg with 1 gram providing 12 mEq (mmoL) each of sodium and bicarbonate.
    - For urinary alkalinization (with a goal urine pH of 8.0) doses tend to be greater and up to 12,000 mg daily (16).
    - We do not aim to alkalinize the urine of our participants (urine pH > 8.0) but rather normalize the relatively acidic urine found in participants with type 1 diabetes, by increasing the urine pH by 0.5 – 1.0.
    - Extrapolating from published urinary alkalinization data (16) and discussing with Prof. Richard Johnson we decided on approximately 4,000mg divided BID over 24 hours. Sodium Bicarbonate comes in 650mg tablet dosing which led to 1,950mg BID (3,900mg).

Recruitment

Participants will be recruited from Adult Diabetes Clinic at Barbara Davis Center by phone. We will discuss study details including the pre-study diet and fasting instructions. Interested participants will be mailed:

- Fasting instructions (for visit #1 and visit #2)– Patients will be asked to fast after midnight and will be asked to omit caffeine and smoking prior to the visit and
    - Fasting and insulin dosing instructions will be provided for the participants to reduce risk of hypoglycemia (these instructions are similar to what is provided once patients with type 1 diabetes come in for fasting labs)

- Participants will fast for only 8 hours
- Participants on insulin pump will be informed to not give themselves a bolus in the morning since they are fasting
- Participants on taking insulin by injections will be informed to not give themselves a bolus in the morning since they are fasting

- Diet instructions (for visit #1 and visit #1) – Patients will be asked to maintain a moderate protein (1.5g/kg of weight), and high sodium diet (3,450 mg of sodium per day) for one week prior to the study visit #1, and between study visit #1 and #2.
- Copy of patient information and consent form to review

Study Visit #1

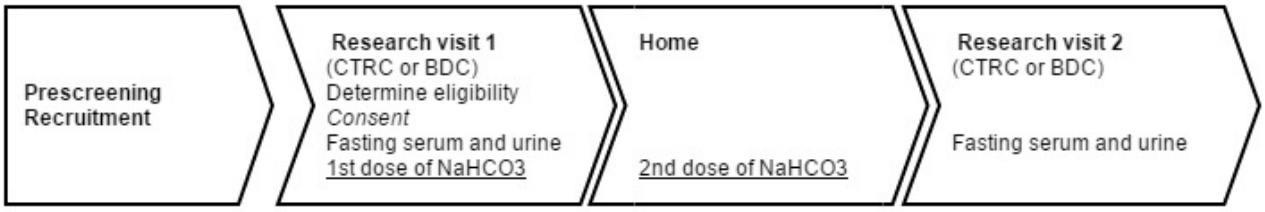
Visit #1 will take place at Adult CTRC or Barbara Davis Center. Medical history will be reviewed and eligibility determined. . Informed consent will be obtained from eligible participants following standard COMIRB approved methods by the PI and/or members of the research team.

At this visit, participants will present fasting from midnight, height, weight and blood pressure will be measured. Urine will be collected and blood will be obtained via venipuncture by RN or venipuncture-trained PRA. The baseline blood will be drawn for serum uric acid, serum glucose, serum creatinine and serum cystatin C. Urine will be collected for urine pH, urine ketones, urine glucose, urine creatinine and urine uric acid. The urine will also be examined for precipitation and crystallization of urine uric acid. The participant will take 1950mg of sodium bicarbonate (3 x 650mg tablets) with water. We estimate that the study visit will take 60 minutes. After completion of the study visit, the participant will receive the second dose of sodium bicarbonate to take approximately 12 hours after the first dose (1950mg of sodium bicarbonate [3 x 650mg tablets]).

The participants will need to remain on the moderate protein and high sodium diet until completion of study visit #2. We will have provide protein bars, or participants may bring their own high protein snack.

Study Visit #2:

Visit #2 will take place at Adult CTRC or Barbara Davis Center. Participants will present fasting from midnight. Urine will be collected and blood will be obtained via venipuncture by RN or venipuncture-trained PRA. Blood will be drawn for serum uric acid, serum glucose, serum creatinine and serum cystatin C. Urine will be collected for urine pH, urine ketones, urine glucose, urine creatinine and urine uric acid. The urine will also be examined for precipitation and crystallization of urine uric acid.



D. Description, Risks and Justification of Procedures and Data Collection Tools:

Labs methods: Serum creatinine, serum cystatin C, serum uric acid and serum glucose will be determined using standard methods in the CTRC core lab and Rick Johnson's lab. Urine uric acid, urine glucose, urine creatinine and urine pH will be measured at Rick Johnson's lab. Urine ketones will be checked at BDC.

Sample banking: Residual blood and urine volume will be stored for future research, in particular to use when new and promising urinary and serum biomarkers become available.

Sodium bicarbonate: Three tablets of 650mg of sodium bicarbonate (1950mg) will be given twice a day to all participants. Dr. Sam Ellis Pharm.D. will arrange and dispense medications. Prescriptions will be written for the dose to be taken by the participants at home.

Other forms: Standard medical history will be obtained (see accompanying forms in submission packet).

Estimation of GFR: Various equations have been developed to estimate GFR based on either serum creatinine or cystatin C plus other variables. We will employ the state of the art CKD-EPI combined creatinine and cystatin C to calculate estimated GFR in our participants.

CKD-EPI combined creatinine and cystatin C eGFR = eGFR = 135 x min(serum creatinine/k, 1)$^{-a}$ x max(serum creatinine/k, 1)$^{-0.601}$ x min(serum cystatin C/0.8, 1)$^{-0.375}$ x max(serum cystatin C/0.8, 1)$^{-0.711}$ x 0.995^{age} [x 0.969 if female] [x 1.08 if black]

Compliance:

To improve compliance, the first dose of sodium bicarbonate (1950 mg) will be administered at the visit #2 at CTRC. Moreover, participants will be asked to set an alarm on their phone for the second and final dose (1950mg) at home.

Plan to minimize risk:

1. *Fasting and hypoglycemia:*
Fasting will be limited to 8 hours, and detailed fasting and insulin instructions will be given to participants.

2. *Venipuncture to draw blood for lab tests:*
Venipuncture will be performed by research personnel with experience with venipuncture to reduce risk of pain. Gloves will be worn and the area of skin cleaned to prevent risk of infection.

3. *Sodium bicarbonate administration:*
The study participant will be monitored for a minimum of an hour after the administration of the first sodium bicarbonate dose to check for any reaction. The study participant will be told to call with any issues or concerns at home. Participants taking medications known to interact with sodium bicarbonate will be excluded from participating.

E. Potential Scientific Problems:

In any clinical study unforeseen human factors provide challenges. The current study is based upon a previous study with sodium bicarbonate administration in adults with no reported adverse effects. Furthermore, we aim to use lower doses of sodium bicarbonate in this study. We have also performed careful power analyses to allow meaningful analyses.

F. Data Analysis Plan:

(A HIPAA compliant, Red Cap database system will be used for this study.)

Table 1 Power calculations:

Outcome	Effect size	SD pre and post	Correlation of pre with post	SD of change	Power	N
Urine pH	0.5*	0.42	0.5	0.42	>99	20
	1.0*	0.42	0.5	0.42	>99	10
Urine uric acid	10%	16.48	0.95	5.21	80	47
	20%	16.48	0.80	10.42	80	47

* Units on the pH scale

Per our power analysis, with a sample size of 50 we will have power of 0.8 to detect effect size of 20% change in urine uric acid following 24-hours of $NaHCO_3$. Paired t-test will be employed to evaluate whether the change in urine uric acid pre- and post NaHCO3 administration is statistically significant. We will also adjust for covariates with ANCOVA or linear regression models [ANCOVA vs. linear regression models will be decided based on the variation of urine uric acid at baseline in our participants].

We will also calculate fractionated excretion of uric acid (FeUA = [(UUA * SCr / SUA * UCr)*100]), and regress FeUA, SUA and UUA on eGFR calculated by CKD-EPI creatinine and cystatin C. Furthermore, we will also regress blood glucose and urine glucose on FeUA, SUA and UUA.

G. Summarize Knowledge to be Gained:

Diabetic nephropathy remains the leading cause of end-stage renal disease and dialysis in the US. While diabetic glomerulopathy has received significant attention from researchers and clinicians, determinants of tubulointerstitial injury in diabetes are less well examined. Compared to glomerular injury, tubular injury is known to associate better with renal function in diabetes (2). In fact, tubular proteinuria may precede microalbuminuria with type 1 diabetes (3), suggesting that tubular damage may be induced earlier than glomerular injury in the course of diabetic nephropathy. Animal studies have demonstrated that blocking uric acid production protects the kidney from tubulointerstitial injury, which suggests a causal role for uric acid in the development of diabetic tubular injury (9, 10). Acidification of urine is associated with urine uric acid crystallization which may lead to tubular injury (11, 12). For that reason, urine alkalinization with sodium bicarbonate is a safe and potentially effective therapy to increase solubility of urine uric acid and prevent crystallization and associated tubular injury. Urine alkalinization could potentially prevent or halt progression of diabetic nephropathy in type 1 diabetes.

H. References:

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