# Evaluation of Coffee Therapy for Improvement in Renal Oxygenation

## Protocol and Statistical Analysis Plan

**COMIRB: 18-1874**

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*COMIRB Protocol*
**COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD**
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**Protocol #:** 18-1874
**Project Title:** ***COFFEE STUDY:*** ***C****offee, renal* ***o****xygenation, blood* ***f****low and glomerular* ***f****iltration rate in* ***e****arly diab****e****tic kidney disease.*
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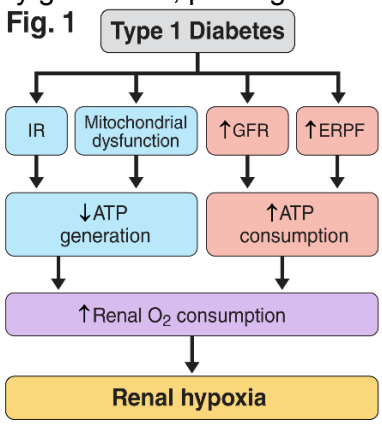
**I. Hypotheses (H) and Specific Aims (SA)**:

Type 1 diabetes (T1D) is a complex metabolic disorder with pathophysiological disturbances beyond β-cell injury, including insulin resistance (IR) and mitochondrial dysfunction which are causally related to the development of diabetic kidney disease (DKD) and contributes to reduced life expectancy (1-6). Current treatments, such as control of hyperglycemia and hypertension, are beneficial, but only partially protect against DKD. Finding new, safe and effective therapies to halt DKD has proven to be challenging (7-14). A potential explanation is the narrow focus on the clinical manifestations of disease, e.g. hyperglycemia and hypertension, rather than identifying and targeting the initial underlying metabolic derangements driving DKD (15). The kidneys are highly metabolically active and are second only to the heart with respect to oxygen ($O_2$) consumption per tissue mass. The high $O_2$ demand is necessary to maintain adequate adenosine triphosphate (ATP) production for the $Na^+/K^+$-ATPase, as 95% of the ATP produced in the kidney is through aerobic metabolism (16, 17). Therefore, the principal determinant of renal ATP consumption is tubular reabsorption of *filtered* sodium ($Na^+$). Experimental models suggest that T1D is associated with an environment that initially upregulates ATP consumption, due to: 1) increased $Na^+$ reabsorption mediated by glucosuria, prolonged exogenous supraphysiological insulin exposure (18-23) and elevated vasopressinergic and renin-angiotensin aldosterone system (RAAS) activity (24-27), and 2) increased *filtration* of $Na^+$ due to elevated glomerular filtration rate (GFR, i.e. hyperfiltration) which is observed in up to 50% of youth with T1D and predicts progressive DKD (28-30). Further, emerging animal data suggest that in diabetes the kidneys are unable to sufficiently compensate for the increased ATP consumption due to the effects of IR and mitochondrial dysfunction on substrate utilization (impaired ATP generation, **Fig 1**) (19-22). Finally, the resultant ATP deficit is followed by increased perfusion or effective renal plasma flow (ERPF) to deliver more $O_2$ to the kidney. However, in the kidneys increased ERPF leads to increased GFR, and thus a higher $Na^+$ load which further increases renal $O_2$ consumption.

**Fig. 1**




Caffeine, a methylxanthine, is known to alter kidney function by several mechanisms including natriuresis, hemodynamics and renin-angiotensin-aldosterone system (31). In contrast, to other natriuretic agents, caffeine is thought to fully inhibit the local tubuloglomerular feedback (TGF) response to increased distal sodium delivery. This observation has broad-ranging implications as caffeine can reduce renal oxygen ($O_2$) consumption without impairing effective renal plasma flow (ERPF) and glomerular filtration rate (GFR). There are also data suggesting that chemicals in coffee besides caffeine may provide important cardio-renal protection (32). Yet, there are no data examining the impact of coffee-induced natriuresis on intrarenal hemodynamic function and renal energetics in youth-onset T1D. Our overarching hypothesis in the proposed pilot and feasibility trial is that coffee drinking improves renal oxygenation by reducing renal $O_2$ consumption without impairing GFR and ERPF. To address these hypotheses, we will measure GFR, ERPF, renal perfusion and oxygenation in response to 7 days of cold brew coffee (one Starbucks® Cold brew 325ml bottle daily [205mg caffeine]) in an open-label pilot and feasibility trial in 10 adolescents with T1D already enrolled in CASPER Study (#17-0820, PI: Bjornstad).

**SA1: To evaluate the impact of 7 days of coffee drinking on renal perfusion and oxygenation in adolescents with T1D.**

*H1: Coffee drinking will improve renal oxygenation and perfusion in adolescents with T1D.*

**SA2: To evaluate the impact of 7 days of coffee drinking on intrarenal hemodynamic function and tubular injury markers in youth with T1D.**

*H2.1: Coffee drinking will not significantly change GFR and ERPF in youth with T1D.*

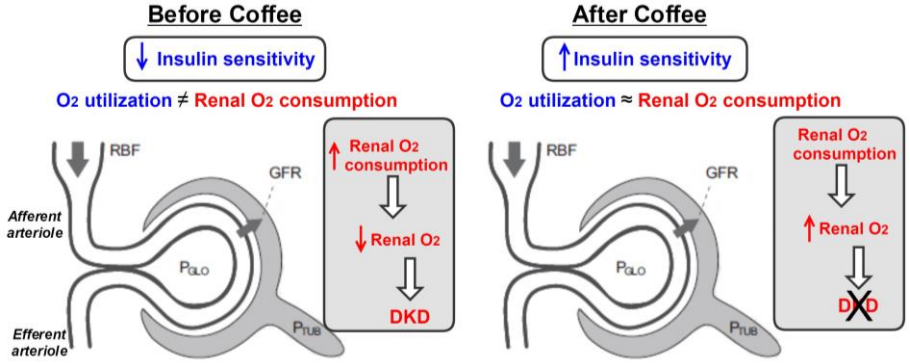
*H2.2: Coffee drinking will decrease concentrations of tubular injury markers in youth with T1D.*

**II. Background and Significance**: Over 1.25 million Americans have T1D, increasing risk for early death from cardiovascular disease (CVD) (33, 34). Despite advances in glycemic and blood pressure control, a child diagnosed with T1D is expected to live up to 17 years less than non-diabetic peers (1-4). The strongest risk factor for CVD and mortality in T1D is DKD (5, 6). Current treatments, such as control of hyperglycemia and hypertension, are beneficial, but only partially protect against DKD (7-14). This limited progress may relate to a narrow focus on clinical manifestations of disease, rather than on the initial metabolic derangements underlying the initiation of DKD (15). Renal hypoxia, stemming from a potential metabolic mismatch between increased renal energy expenditure and impaired substrate utilization, is increasingly proposed as a unifying early pathway in the development of DKD (21, 35, 36). T1D is impacted by several mechanisms which *increase renal ATP consumption* and *decrease ATP generation*.

Caffeine is known to modify renal function by several mechanisms including natriuresis, hemodynamics and renin-angiotensin-aldosterone system. Caffeine is also thought to inhibit the expression of the $Na^+/K^+$ ATP pump and isoform 3 of the $Na^+/H^+$ exchange; both are key proteins in the mechanism of active/passive tubular reabsorption (31). In contrast, to other natriuretic agents, caffeine is theorized to fully inhibit the local TGF response to increased distal $Na^+$ delivery. This observation has broad-ranging implications as caffeine can reduce renal $O_2$ consumption (by inhibiting $Na^+$ reabsorption which accounts for the majority of renal $O_2$ consumption) without impairing ERPF and GFR. Such mechanisms can provide protection from acute and chronic tubular and glomerular injuries. There are also important non-renal mechanisms hypothesized to explain the potential protective effect of coffee drinking on mortality including reduced inflammation, improved insulin sensitivity, and endothelial function (37-39).

*Rigor of the prior research:* To our knowledge, there are no studies examining the impact of coffee or caffeine-induced natriuresis on intrarenal hemodynamic function and renal energetics in youth with T1D. Our preliminary data suggest that coffee intake protects against acute kidney injury (see below), and recent findings from the UK Biobank reports an inverse relationship between caffeinated and non-caffeinated coffee drinking and mortality (32). Therefore, the UK Biobank data strongly support the importance of noncaffeine constituents in the coffee-mortality association (32). Consistent with these data, recently published data also demonstrate that daily coffee intake is associated with decreased risk of development of chronic kidney disease {Jhee, 2018 #15851}. Accordingly, it is important to evaluate the effect of coffee rather than simply caffeine on intrarenal hemodynamic function and energetics (oxygenation). In addition, brews are thought to contain a higher number of bioactive compounds including polyphenols than instant coffee (40). Further, cold brews are proposed to retain the bioactive compounds better through their slower brewing process compared to hot brews (41).

**Fig 2 Proposed Mechanism of Coffee Drinking on Renal Function**




*Our overall hypothesis* in this pilot and feasibility trial is that cold brew coffee drinking for 7 days improves renal oxygenation by reducing renal $O_2$ consumption, without impairing GFR and ERPF (**Fig 2**).

*Impact:* Clinical trials in DKD have yielded disappointing results for the past two decades. This trend may be explained in part by the lack of understanding around what initiates early DKD, and by failing to intervene at the initial stage of the disease when benefit may be maximally achieved. This proposal seeks to investigate the effect of coffee on intrarenal hemodynamics, renal perfusion and oxygenation in youth-onset T1D. Given the global diabetes and obesity epidemic, the ability to potentially reduce renal $O_2$ consumption and increase renal oxygenation without the TGF-mediated reduction in ERPF and GFR, provides a strategic approach to reduce the huge burden of DKD, a major cause of morbidity and premature mortality. This pilot and feasibility study will provide preliminary data and direct a larger clinical trial.

**III**. **Preliminary Studies/Progress Report:**

*Hyperfiltration is common in youth and adults with T1D, and predicts rapid GFR decline and impaired GFR:* We demonstrated that the prevalence of hyperfiltration in youth and young adults with T1D can exceed 50% when GFR is measured by inulin clearance (28), and between 13-31 % when GFR is estimated by serum creatinine and serum cystatin C (42, 43). The discrepancy in prevalence is likely attributed to the inaccuracy of estimated GFR in the normal to elevated GFR range (44), hence we propose to measure GFR in this study. In adults with T1D (n=646) in CACTI, we also demonstrated that hyperfiltration predicted greater odds of rapid GFR decline over 6-years (OR: 5.00, p<0.0001), when adjusting for traditional risk factors including HbA1c, systolic blood pressure (SBP), LDL-C, sex, duration and albumin-to-creatinine ratio (ACR) (44). Furthermore, over 12-year follow-up in CACTI, adults with T1D who experienced rapid GFR decline (annual GFR loss > 3mL/min/1.73m$^2$) were more likely to demonstrate hyperfiltration at baseline (OR: 32.9, p<0.0001) and impaired GFR at 12-year follow-up (OR: 11.6, p=0.0003) (unpublished data). In a parallel analysis we conducted in CACTI and the Pittsburgh Epidemiology of Diabetes Complications Study (EDC), elevated GFR at baseline was associated with a greater odds of rapid GFR decline over 6 and 8-years respectively (CACTI: OR 2.62, p<0.0001, EDC: OR 2.16, p=0.0003) (45).

*BOLD and ASL MRI demonstrate decreased renal oxygenation in chronic kidney disease (CKD):* Our collaborator, Dr. Prasad demonstrated that adults with CKD have lower renal cortical oxygenation compared to controls (18.0±1.62 *vs*. 20.6±3.4 s$^{-1}$, p<0.0001) by BOLD MRI (46). Furthermore, renal perfusion measured by ASL MRI correlated strongly with eGFR (r=0.67, p<0.0001) (47).

*Coffee consumption is associated with lower risk of acute kidney injury (AKI):* Unpublished data from Atherosclerosis Risk in Communities Study (ARIC, Dr. Parikh) found an inverse relationship between any coffee consumption and AKI risk (**Table 1**). Furthermore, there was a stepwise relationship between number of cups of coffee per day and AKI risk (≥ 3 cups / day: 0.72 [0.61, 0.86], 2-3 cups/day: 0.75 [0.63, 0.90], 1-2 cups/day: 0.86 [0.75, 0.99], <1 cup/day: 0.83 [0.71, 0.96] compared to never: 1.00 [reference group]).

**Table 1**

| Acute Kidney Injury | Never (n=2,746) | Any Coffee (n=11,461) |
|---|---|---|
| **# events** | 347 | 1,347 |
| **Incidence rate per 1,000 p-y** | 6.1 | 5.5 |
| **RR:** | 1 (ref.) | 0.81 (0.78-91) |

**IV. Research Methods:**

**Overall Study Design for All Aims:** This study will capitalize on the structure of our JDRF-funded *CASPER Study (CTRC #17-0820)*. We plan to recruit a subset of *CASPER Study* enrolled subjects (n=10) to participate in this pilot and feasibility trial. The *CASPER Study* visits will serve as the baseline visit for this trial. The pilot and feasibility trial will be a prospective, open label, non-randomized interventional study.

***(1) Pre-coffee visit (Casper Study #17-0820):*** As per the *CASPER Study* protocol, following a screening visit, eligible participants (**Table 2, page 7**) will be asked to refrain from strenuous physical activity for 3 days prior to admission (**Fig 6**) due to the impact of exercise on IR and intrarenal hemodynamic function. They will also be provided with a 3-day diet instructions (45% carbohydrates, 30% fat, 25% protein, a goal of 3.45 g salt/day and asked to abstain from caffeine and decaffeinated coffee beverages), as previously described (48). Participants will be admitted to Children's Hospital Colorado CTRC inpatient, have fasting urine and blood collected, undergo fasting renal BOLD/ASL MRI at Brain Imaging Center, followed by quantification of GFR and ERPF by iohexol and PAH clearance studies during clamped mild hyperglycemia. Upon completion of GFR and ERPF measurements, participants will be provided with lunch after which they undergo dual-energy x-ray absorptiometry (DXA), which concludes the study.

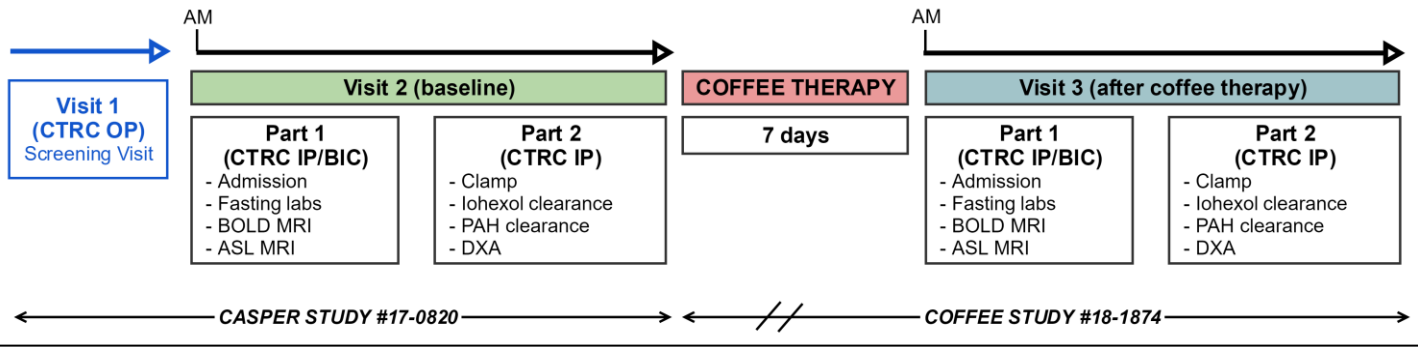
***(2) Post-coffee visit (COFFEE Study #18-1874):*** Ten participants recruited from the *CASPER Study*, will be enrolled in the *COFFEE Study*. These participants will be provided with Starbucks® Cold brew 325ml bottles and instructed to drink one (1) bottle every morning between 6 and 9 am for 6 days. They will be asked to abstain from other caffeinated beverages and also decaffeinated coffee-based beverages for the duration of the study. During this time, they will also be instructed to follow the diet instructions (45% carbohydrates, 30% fat, 25% protein, a goal of 3.45 g salt/day). Six (6) days later, the participants will be return to CTRC for their follow-up (post-intervention) visit. The procedures of the post-intervention visit will match the pre-intervention visit. After obtaining fasting urine and blood, the participant will be asked to drink 1 bottle of Starbucks® Cold brew, after which they will undergo renal BOLD/ASL MRI at Brain Imaging Center, followed by quantification of GFR and ERPF by iohexol and PAH clearance studies during clamped mild hyperglycemia. Upon completion of GFR and ERPF measurements, participants will be provided with lunch, which concludes the study (we will not repeat DXA since it is unlikely that it will significantly change in 7-30 days, and it is not a study objective for this pilot and feasibility trial). Preferably participants will complete the post-intervention study 7 days after the pre-intervention study, but to facilitate recruitment we will allow participants to start the 7 days of coffee therapy followed by the post-intervention study within 30 days of the pre-intervention study.

**Intervention:** Starbucks® Cold brew 325ml bottles daily [205mg caffeine] will be provided to the participants. Participants will be instructed to drink 1 bottle every morning between 6 and 9 am for 6 days prior to the post-intervention visit. The 7$^{th}$ day is the post-intervention visit, and participants will be asked to drink 1 bottle the morning of the study visit. We decided on cold brews as it contains a high number of bioactive compounds including polyphenols (40) through their slower brewing process (41) which are thought to have protective effects beyond caffeine (32). The daily caffeine dose of 205 mg (1 bottle) is consistent with quantities used in previous studies, typically ranging between 200-400 mg (49-55). Caffeine dosing less than 400mg/day considered safe in non-pregnant subjects and not associated with adverse effects in terms of acute toxicity, cardiovascular toxicity, bone and calcium effects, behavior, and development and reproduction (53-56). This is roughly the amount of caffeine in four cups of brewed coffee. We will also exclude caffeine-naïve participants to reduce the likelihood of withdrawal effects and exclude participants with prior adverse effects to caffeine, on other stimulants for ADHD, tremors, tics, Tourette's, arrythmias, insomnia and overactive bladder. Prior caffeine intake will also be assessed with a self-reported retrospective log of weekly caffeine consumption that quantifies the use of coffee, tea, cola, chocolate and other caffeine-containing drinks, dietary supplements and over the counter medications (57).

**Recruitment:** Ultimately 10 adolescents with T1D (~50% male) will be recruited and enrolled from the *CASPER study*. The recruitment plan for the *CASPER study* is described in detail in its protocol. In 3 months, we successfully recruited and enrolled 41 of the 50 participants needed in the *CASPER Study,* and are above our target enrollment. Accordingly, we have an ample pool of participants to recruit from and do not foresee any difficulties with recruitment.

**Detailed Study Design:**
The study timeline is illustrated in **Fig 3**, and details from each study visit is summarized below. Visit 1 is the screening visit, and Parts 1 and 2 of visit 2 and 3 will take place consecutively.

**Fig 3**



***Pre-coffee visit (CASPER Study #17-0820):***

**Visit 1 (Screening visit):** Prescreening will be done by phone or in conjunction with a clinical diabetes visit. The full screening visit will include the consent process, history, allergies confirmed, physical exam, and screening labs (HbA1c, hemoglobin and hematocrit [H&H], serum creatinine, urine microalbumin to creatinine ratio). All females will also have a pregnancy test and detailed menstrual history. The screening visit will take approximately 2 hours.

**Visit 2, part 1:** Participants will present the evening before the study visit, or present fasting to the Pediatric CTRC in the morning, and have IV(s) placed. Fasting blood will be drawn for serum copeptin, lipids, insulin, cystatin c, creatinine, sodium, hematocrit and total protein prior to the clamp. Participants will be escorted to the Brain Imaging Center or CHC Radiology for a BOLD and ASL MRI per local protocol. BOLD MRI measurements will be performed at baseline and following administration of a small dose of furosemide (20 mg IV) via the PIV. A study physician or nurse will be responsible for the furosemide administration. Total duration of part 1 will be approximately 1.5 hours.

**Visit 2, part 2:** Participants return to pediatric CTRC and will have their blood glucose concentrations clamped in the hyperglycemic range. Participants will also undergo iohexol and PAH clearance studies, in addition to 4-hour urine collection for album excretion rate (AER) and albumin to creatinine ratio (ACR). Upon completion of visit 2, participants will be provided with a study meal (lunch). After lunch, participants will undergo DXA scan for quantification of body composition. This concludes the study. Total duration 6 hours.

***Post-coffee visit (COFFEE Study):***

**Visit 3, Part 1:** Participants will present the evening before the study visit, or present fasting to the Pediatric CTRC in the morning, and have IV(s) placed. Fasting blood will be drawn for serum copeptin, lipids, insulin, cystatin c, creatinine, sodium, hematocrit and total protein prior to the clamp. Participants will be asked to drink 1 bottle of Starbucks® Cold brew before being escorted to the Brain Imaging Center or CHC Radiology for a BOLD and ASL MRI per local protocol. BOLD MRI measurements will be performed at baseline and following administration of a small dose of furosemide (20 mg IV) via the PIV. A study physician or nurse will be responsible for the furosemide administration. Total duration of part 1 will be approximately 1.5 hours.

**Visit 3, part 2:** Participants return to pediatric CTRC and will have their blood glucose concentrations clamped in the hyperglycemic range. Participants will also undergo iohexol and PAH clearance studies, in addition to 4-hour urine collection for album excretion rate (AER) and albumin to creatinine ratio (ACR). Upon completion of visit 2, participants will be provided with a study meal (lunch). This concludes the study. Total duration 6 hours.

**A. Outcome Measure(s):**

**Aim 1:**

- Renal oxygenation (R2*) by BOLD-MRI
  - Before and after Lasix
- Renal perfusion by pCASL MRI

**Aim 2:**

- GFR by iohexol clearance
- ERPF by PAH clearance
- Tubular injury markers (e.g. NGAL, KIM-1, IL-18, MCP-1, YKL-40)

**Table 2: Eligibility Criteria**

| Inclusion criteria | Exclusion criteria |
|---|---|
| Antibody+ T1D with <10 yr duration | Severe illness, recent DKA |
| Age 12-21 years | eGFR <60ml/min/1.73m$^2$ or creatinine > 1.5mg/dl or history of ACR≥300mg/g |
| BMI ≥ 5%ile | |
| Weight<350 lbs and > 57 lbs. | ACE inhibitors, angiotensin receptor blockers (ARB), diuretics, sodium-glucose co-transport (SGLT) 2 or 1 blockers, daily NSAIDs or aspirin, sulfonamides, procaine, thiazolesulfone or probenecid, atypical antipsychotics and steroids |
| Previous exposure to caffeine | Tachyarrhythmias, ADHD, tremors, tics, Tourette's, arrythmias, insomnia and overactive bladder |
| | Anemia or allergy to shellfish or iodine |
| HbA1c <12% | Pregnancy or nursing |
| | MRI scanning contraindications (claustrophobia, implantable devices, >350 lbs) |

## B. Description of Population to be Enrolled:

### Study Design and Research Methods

*Study Diet:* Participants will be instructed to follow a study diet 3 days prior to visit 2 (CASPER #17-0820), and between visit 2 and visit 3 (COFFEE #18-1874). The diet will be composed of 45% carbohydrates, 30% fat, 25% protein, goal of 3.45g of salt/day and will have no caffeine (besides what is provided in the cold brew), no decaffeinated coffee, regular soda or syrup, no candy as in our previous studies (58, 59)), as diet affects renal function.

*Cold Brew Coffee:* Starbucks® Cold brew 325ml bottles daily [205mg caffeine] will be provided to the participants. Participants will be instructed to drink 1 bottle every morning between 6 and 9 am for 6 days prior to the post-intervention visit. The 7$^{th}$ day is the post-intervention visit, and participants will be asked to drink 1 bottle the morning of the hyperglycemic clamp and renal clearance studies.

*Renal oxygenation and renal perfusion by MRI:* We will perform BOLD MRI (74) to quantify renal oxygenation, whereas renal perfusion will be measured by ASL (47). These studies will be performed at the UCD Research Imaging Center (3T Siemens Skyra scanner) which already has an active renal BOLD-MRI protocol as part of the multi-center COMBINE (CKD Optimal Management with BInders and NicotinamidE) study (**Table 3**) (46), for which our collaborator Dr. Prasad directs the CORE for trouble-shooting and analyzing the renal MRIs. Dr. Prasad is an international leader in advanced MR research and will provide the techniques for BOLD and ASL MRI acquisition and analyses.

| **Table 3. Renal MRI protocol 3T Skyra** | | | | | |
|---|---|---|---|---|---|
| **Series** | **Localizer** | | | **BOLD** | **ASL** |
| Plane | 2 Plane | Coronal | Axial | Coronal | Coronal |
| Pulse sequence | gre | gre | gre | gre | Custom |
| TR/ms | 7 | 7 | 7 | 61 | 3000 |
| TE/ms | 2.66 | 2.66 | 2.66 | 4.92, 9.84, 14.76, 19.68, 24.6, 29.52, 34.44, 39.36 | 1.92 |
| Read out mode | n/a | n/a | n/a | Bipolar | n/a |
| In line mapping | n/a | n/a | n/a | T2* (VB 17)/R2* (VD 13) Map | n/a |
| BW Hz/pixel | 290 | 290 | 290 | 260 | 501 |
| FOV mm | 400 | 400 | 400 | 400 | 400 |
| Slices | 3 ax, 3 cor | 5 | 5 | 5 | 1 |
| Concatenation | 6 | 5 | 5 | 5 | 1 |
| Slice Thickness / mm | 8 | 8 | 8 | 5 | 8 |
| Skip | 50% | 50% | 50% | 0 | n/a |
| Fat suppressed | No | No | No | Fat Sat | n/a |
| Parallel Imaging acceleration factor | None | None | None | None | None |
| Diffusion mode | n/a | n/a | n/a | n/a | n/a |
| B value s/mm2 | n/a | n/a | n/a | n/a | n/a |
| Diff. gradients | n/a | n/a | n/a | n/a | n/a |
| NEX | 2 | 2 | 2 | 1 | 50 |
| Acq-matrix | 154x256 | 154x256 | 154x256 | 192x256 | 128x128 |
| Flip angle | 20 | 20 | 20 | 30 | 60 |
| Typ. Scan time m:s | 0:14 | 0:07 | 0:07 | 0:30-1:00 (5 slices) | 0:05 |
| Options/comments | Site preference | | Instruct subjects to not to take deep breaths. | Changing FOV and/or matrix size not preferred | Instruct subjects to not to take deep breaths. |

**Abbreviations:** BOLD: Blood oxygen level dependent, SSFP: steady state free precession, VLA: vertical long axis view, LVOT: left-ventricular outflow tract view, GRE: gradient recalled echo.

Hyperglycemic insulin clamp: One IV will be placed in each arm to clamp the blood glucose concentrations between 170-190 mg/dL. One IV will be used to administer the boluses of iohexol and PAH in addition to a variable infusion of 20% dextrose and insulin, and the other IV will be used for blood draws. Blood glucoses will be measured every 10 minutes at the bedside, and glycemic control will be achieved using the modified and simplified insulin clamp methodology to remove glycemic effects on GFR and ERPF. The rate of insulin is adjusted based on blood specimens drawn every 10 minutes (example algorithm). Patient blood glucose (BG) levels will be checked at 8am, and adjusted to reach and maintain mild hyperglycemia up until 10am. If participant BG <70mg/dl, we will give 4 oz juice and re-test BG in 15 min. If still <70mg/dl, another 4 oz juice will be given, and BG will be re-tested 15 min later. If still <70 mg/dl, study MD will be called, and IV D20 will be started at 100ml/hr. For BG between 70-180 mg/dL, a variable D20 infusion will be given on a sliding scale based on participant current BG and weight, as used in previous and current studies (COMIRB #13-0122, #16-1752, #17-0820, #18-0704). Once BG > 190mg/dl, D20 will be discontinued. If BG is not responding to changes within 2 hours or if increases above 200 mg/dl, a study physician will be contacted. Each time blood is drawn during the clamp, blood will be drawn to a clear line in the syringe to ensure there will be no IV fluid dilution and will be re-infused into the subjects after obtaining the blood sample to minimize blood loss. A blood glucose range of 170-190mg/dL (mild hyperglycemia) was chosen to limit the acute effects of severe hyperglycemia on GFR and ERPF reproducibility, while allowing us to assess renal function in a glycemic milieu similar to the participants native pathophysiology. Furthermore, the clamp maintains an easily achieved and safe range of glycemia.

**Insulin Algorithm**

| BG | Insulin dose |
|---|---|
| <180 | -- |
| 181-190 | 0.01 u/kg/hr |
| 191 - 200 | 0.015 u/kg/hr |
| 201 - 250 | 0.03 u/kg/hr |
| 251 - 300 | 0.045 u/kg/hr |
| >301 | 0.06 u/kg/hr |

Iohexol clearance (GFR) and PAH clearance (ERPF): A bolus of iohexol (5mL of 300 mg/ml iohexol [Omnipaque 300, GE Healthcare]) will be given slowly over 2 min followed by a 10 ml normal saline flush, which will be time 0 for the iohexol clearance. 120 min will be allowed for equilibration of iohexol per or local protocol (62, 63). Blood for iohexol clearance will be drawn at +120, +150, +180, +210 and +240 min (62, 63). PAH (weight/75 x 4.2 ml) [University of Minnesota] will be given slowly over 5 minutes followed by a 10 ml normal saline flush. An infusion mixture of 8 mL of PAH and 42 mL of normal saline will be prepared in a 60mL syringe and infused in Syringe Pump at a rate of 24mL/hr, which will be time 0 of the PAH clearance. Ninety min will be allowed for equilibration of PAH per co-investigator Dr. Cherney's protocol (62, 63). Blood for PAH clearance will be drawn at +90 and +120 min (33-36). We will use Gomez' equations to calculate parameters of intrarenal hemodynamics (56, 64) (Table 2, pg. 5). Hyperfiltration by measured GFR (iohexol) will be defined as ≥135ml/min/1.73m$^2$ (23, 65-67). The iohexol and PAH infusion period lasts 240 minutes and will occur concurrently with the mildly hyperglycemic clamp during which time the subject will rest in bed in the pediatric CTRC. During the entire 240-minute glucose/insulin infusion period, a pediatric CTRC nurse will remain at the bedside, and a Study Physician or Pediatric Nurse Practitioner or Pediatric Physician Assistant will also remain at the bedside to minimize any risks associated with iohexol and PAH infusion. The IV site will be continuously monitored to minimize risk of IV infiltration. De-identified coded blood samples will be sent to collaborator Dr. Robert G. Nelson's lab at NIH/NIDDK who will run iohexol and PAH clearance on a HPLC platform (as per #16-1752, #17-0820, #18-0704).

RVR and Gomez equations (**Table 4**): RVR will be calculated as RVR = MAP/RBF. To derive additional information about intrarenal hemodynamic function we will use mathematical equations by Gomez et al (55). By using measurements of GFR, RBF, ERPF, renal vascular resistance, hematocrit and serum protein, we can calculate afferent and efferent arteriolar resistance ($R_A$ and $R_E$), glomerular pressure ($P_{GLO}$) and filtration pressure (**Table 4**).

| Table 4 Measures | Definition: |
|---|---|
| Filtration fraction (FF) | FF = GFR / RPF (renal plasma flow) |
| Renal blood flow (RBF) | RBF = RPF (1-Hct) |
| Renal vascular resistance (RVR) | RVR = MAP/RBF |
| Filtration pressure $\Delta P_F$ | $\Delta P_F$ = GFR/$K_{FG}$, $K_{FG}$: filtration coefficient |
| Mean plasma protein ($C_M$) | $C_M$ = total protein / FF x natural log (1/1-FF) |
| Glomerular oncotic pressure ($\pi_G$) | $\pi_G$ = 5 x ($C_M$ – 2) |
| Glomerular pressure ($P_{GLO}$) | $P_{GLO}$ = $\Delta P_F$ + $P_{BOW}$ + $\pi_G$, $P_{BOW}$ is 10 mmHg |
| Afferent arteriolar resistance ($R_A$) | $R_A$ = [(MAP-$P_{GLO}$)/RBF] x 1328 |
| Efferent arteriolar resistance ($R_E$) | $R_E$ = [(GFR/$K_{FG}$ x (RBF-GFR))] x 1328 |

Blood pressure: After participants have been laying supine for a minimum of 5 minutes, blood pressure measurements will be obtained using an automatic blood pressure machine and 3 measurements will be averaged. MAP will be calculated from the blood pressure readings.

Albumin excretion: ACR and AER be determined from 4 hr. urine collection.

Plasma tubular injury markers: NGAL and KIM-1 will be measured from fasting plasma samples on a Meso Scale Discovery Electrochemiluminescence (MSD-ECL) platform at Dr. Prarikh's lab at John Hopkins, Baltimore, Maryland.

Fractional excretion of sodium (FeNa): We will measure serum and urine sodium and creatinine to calculate fractional excretion of sodium.

DXA: Participants will also undergo DXA by standard methods on a Hologic device (Waltham, MA) to determine lean and fat mass (72).

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

1. **Study Duration:** The anticipated duration for this add-on study is 2 years

2. **Duration of Participation for Each Subject:** 7 days

3. **Sources of Research Material:** The patient's medical record will be reviewed for diabetes diagnosis, medications, allergies and other diagnoses that may disqualify patient from participation.

| **Table 6: Data to be collected during study** | |
|---|---|
| Blood and Urine Samples | Blood pressures and HR |
| Questionnaires | Hyperglycemic clamp |
| DXA | Iohexol clearance |
| Anthropometric measurements | PAH clearance |
| ASL and BOLD MRI | |

4. **Informed Consent Plan:** Appropriately qualified and informed personnel who have completed the COMIRB and HIPPA course requirements will fully explain the study protocol and consent form to the subject and guardian verbally in the language they understand. The explanation will be conducted in a quiet environment with adequate time given for the subject and guardian to review the study procedure before the commencement of the study. Asking the subject to explain the study in their own words will assess the subject's understanding. If non-English speaking subjects are enrolled in the study, the investigators will adhere to Section 10C of the COMIRB Instructions for Clinical Investigators regarding the consent of these subjects. The qualified personnel mentioned above will then obtain written consent from the guardian and assent from the subject, co-signed on the consent form, or in subjects who are 18 years or older, direct consent. The subject and guardian will be provided a copy of the consent form for better understanding and record purposes.

5. **Special Consent/Assent Plan:** Consent will be obtained from all participants in the study. Following explanation, all subjects below 18 years old will co-sign the consent form in addition to the parents signing the consent form. All subjects age 18 or older will sign the standard consent form.

6. **Participant Compensation:** Subjects will be paid $400 upon completion of all procedures in this add-on study (**Table 7 below**). If the subject begins the overnight fast and needs to stop for any reason, the

subject will be given $75 and will be paid for all subsequent completed visits as outlined below. These payments are similar to the payments being made for each visit type in *Renal HEIR, IMPROVE-T2D and CASPER,* our currently ongoing adolescent studies.

**Table 7 Subject payment schedule**

| Visit 3, part 1 | Visit 3, part 2 | Total |
|---|---|---|
| *$200.00* | *$200.00* | **$400.00** |

## 7. Potential Risks to Subjects:

Blood Samples: The collection of blood samples may result in temporary discomfort, bruising, bleeding, and on rare occasions, infection. EMLA cream helps to prevent discomfort, and sterile technique helps to minimize these risks. Nationally, the NIH Clinical Center has a guideline of 9mL/kg in 6-8 weeks for pediatric studies. Certain studies at our institution do draws over 7mL/kg in 6 weeks, or up to 7 mL/kg in a single draw but include iron supplementation. The screening visit (visit 1) will include 5 ml or less of blood (1 tsp) (HbA1c, H/E). The clamp and renal infusion studies (visit 2) includes 66mL of blood, which includes 48 mL from the clamp and 18 mL from the iohexol and PAH clearance studies. Thus, the clamp and renal infusion studies are within the more conservative pediatric CTRC guidelines of 2.5mL/kg for a single draw for pediatric studies for subjects 26 kg or greater. This is highly unlikely to exclude many subjects, as we are recruiting adolescents between ages 12 and 18 and excluding, and subjects with BMI<5% (due to potential undiagnosed illness), those groups likely to be the lightest in weight. Therefore, we will limit recruitment to subjects 26 kg or greater. In addition, by study design, subjects <5% for weight are excluded as are subjects with anemia, screened by our baseline H/H, further increasing the safety of the study regarding blood draws.

IV risks: There is temporary discomfort when the needle goes in and 10% of the time there is a small amount of bleeding under the skin that may produce a bruise. Rarely, there is a risk of a blood clot formation, infection or infiltration. EMLA cream helps to prevent discomfort and sterile technique by experienced pediatric nurses help to minimize these risks.

Hyperglycemic Clamp: To minimize the risk of any complications with the clamp, the clamp is only performed in the inpatient pediatric Clinical Translational Research Center (CTRC), by experienced personnel. To minimize this risk further, IV access is obtained prior to glucose and insulin administration, and blood sugars are monitored approximately every 10 minutes throughout the procedure and maintained at 190 mg/dL. Two IV's will be in place, in case there is a problem with one IV. If the blood glucose level is decreasing, dextrose will be given to stabilize and increase blood glucose levels. IV access will also be left in place until blood glucose values are stable after the study is completed. During the clamp, as with any IV fluids, there is also a risk of infiltration of the IV solution, which could lead to skin burn or tissue damage. During the entire clamp procedure, a pediatric CTRC nurse will remain at the bedside, and a Pediatric Study Physician or Pediatric Nurse Practitioner will also remain at the bedside. The IV site will be continuously monitored, and the IV integrity will be noted on a check sheet.

- *Glucose injection and infusion:* Irritation of a vein resulting in phlebitis can occur with administration of concentrated glucose solutions. The risk will be decreased by minimizing the use of concentrated solutions, monitoring the veins closely during the infusion and selecting large veins for infusion of solutions.

Iohexol infusion: Iohexol (Omnipaque 300, GE Healthcare, Chicago, IL) is a nonionic, low osmolar contrast agent with a history of extensive use in Radiology practice (for contrast x-rays) that shares many qualities of an ideal GFR marker, like inulin, such as not being absorbed, metabolized, or secreted by the kidney. Low toxicity is reported in Radiology practice in children and adults in which doses that are 10-50 times higher than for GFR determination are used. In fact, for over 20 years, publications have argued that iohexol is a gold-standard measure of GFR. In contrast to some agents used to measure kidney function it

is not radioactive (e.g. iothalamate renography at CHCO). The dose to be used in this study is 10-50 times less than that used in radiology studies. Our protocol has been adapted from that used by the RASS study where they reported only minor allergic reactions in over 1,200 iohexol studies performed in over 200 adults with type 1 diabetes in that study. Principal investigator, Dr. Bjornstad, has performed iohexol studies in adolescents with type 1 diabetes at Barbara Davis Center for Diabetes (62) which was well tolerated without any reactions. Furthermore, collaborator Dr. Cherney has at least 10-year experience with iohexol studies in both pediatrics and adults with and without type 1 diabetes. To further limit risk of allergic reactions, patients with an allergy to iodine, seafood or iohexol will not be allowed to participate. Rare adverse effects associated with iohexol in radiology studies include cardiac arrhythmias (an irregular heartbeat), headaches, blurred vision, and nausea. To reduce risk of contamination, single use vials will be used.

PAH infusion: PAH (University of Minnesota) has been used to measure RPF in human research for decades and is very well tolerated and generally recognized as safe with low toxicity. Collaborator, Dr. Cherney has infused PAH in children and adults with and without T1D for the last 10 years without any significant reactions (38, 55, 60, 62, 64, 79-81). PAH was previously approved by the FDA as the gold standard to quantify RPF. As the prior manufacturer, Merck, no longer supplies PAH, Dr. Bjornstad has submitted an IND application to use PAH produced by University of Minnesota in this study as an investigational product (IND #140129). To minimize risk, a sterile preparation is used and prepared by the pharmacy using sterile techniques. Furthermore, single use vials will be used to reduce risk of contamination. However, as with any infusion, there is the possibility of infection, and the possibility of an allergic reaction. In the event of an allergic reaction, the study will be discontinued, and the participant will be treated.

Coffee therapy: The caffeine dose of 205 mg (1 bottle) during the 6 days prior to the post-intervention visit is consistent with quantities used in previous studies, typically ranging between 200-400 mg (49-55). During the post-intervention visit, the 7$^{th}$ day, the participants will also receive 1 bottle of Starbucks® Cold brew. These doses are less than the 400mg/day considered safe in non-pregnant subjects and not associated with adverse effects in terms of acute toxicity, cardiovascular toxicity, bone and calcium effects, behavior, and development and reproduction (53-56).

While caffeine at the doses used in this study is considered safe for most people, we will warn participants of the following possible side-effects including insomnia, nervousness and restlessness, stomach irritation, nausea and vomiting, increased heart rate and respiration, headache, anxiety, agitation, chest pain, and ringing in the ears. If the participants develop any of these symptoms we will ask them to only drink ½ bottle, and if symptoms persistent we will withdraw them from the study. We will further reduce the risk of these side effects based on the dosing schedule in the morning and avoiding higher doses which increases risk for unpleasant side effects. Too much caffeine may worsen arrhythmias, insomnia, tremors, tics, generalized anxiety disorder, bipolar disease, panic attacks, Tourette's, epilepsy or overactive bladder. Participants with these conditions will be excluded. Participants with previous adverse reactions to coffee will also be excluded. Participants naïve to coffee and caffeine will also be excluded to prevent withdraw symptoms upon study completion. While caffeine is considered safe in pregnant and breast-feeding women when used daily amounts are less than 200-300 mg, we will exclude pregnant or nursing mothers due to the other procedures being performed in this study.

Study Diet: There is a small risk that blood sugars will be higher or lower than normal in subjects with diabetes at home while eating the recommended study diet as it may differ from the subject's usual diet. To avoid this risk, subjects will be asked to monitor their blood sugars frequently at home and will be called daily to review blood sugars. Carbohydrate content will also be provided for each food item to assist with carbohydrate counting.

DXA Scan: There is no pain with this procedure. For females, a urine pregnancy test will be checked prior to the test to avoid any x-ray exposure to a pregnant female. This procedure involves the use of X-ray radiography. The amount of radiation exposure during the DXA test is approximately 15 mSv which is 7

times the level of background radiation in Colorado or approximately equal to the amount of radiation a subject would receive being outdoors in Denver for one day.

Magnetic Resonance Imaging (MRI): The MRI is a non-invasive scan of the renal arteries. MRI uses a magnet and there is no radiation involved with the MRI. The scan may be loud; therefore, the subject is provided with audio protection and optional television. Due to the magnet, subjects with implanted metal devices will be excluded. Some people feel claustrophobic in small spaces, and if this occurs the MRI will be stopped. Incidental findings on MRI will be relayed to ordering physician and/or PCP.

A small single dose of furosemide will be injected intravenously to obtain BOLD MRI before and after injection. The dose of furosemide (20mg) is below the typical 1-2 mg/kg dose used clinically in pediatrics. Potential side effects include excessive diuresis (increased discharge of urine), low potassium (leading to symptoms like dry mouth, excessive thirst, weak or irregular heartbeat, muscle pain or cramps), stomach upset, dizziness, muscle weakness, low blood pressure with change of position, hyperglycemia, jaundice, rash, sensitivity to the light, ringing or buzzing noises in the ears. All these while considered possible are very rare following a single low dose of 20 mg. After the Furosemide injection, participants will receive IV fluids during the clamp and will remain well hydrated. All participants will be asked to drink 500mL of water with their study lunch, and therefore ensure adequate hydration of all subjects. A study physician and/or nurse will be present during the injection.

Physical Exam, urine samples and anthropomorphic measures: There are no known risks associated with the physical exam, urine samples, or anthropomorphic measures.

Confidentiality: Violation of privacy and loss of confidentiality are both risks to which research participants are exposed. The possibility of these risks increases when protected health information is collected. Every effort will be made to decrease this risk by limiting access to protected health information, storing this information in a password protected database, and identifying subjects only by a unique identifier that is kept in a separate location in a locked container, traceable only by study personnel. All tests involve risk of identifying asymptomatic/subclinical abnormalities. The study may also include risks that are unknown at this time of examination.

**8. Plan to minimize risk / protection against risk:**

The hyperglycemic clamps, iohexol and PAH infusions are standard procedures used in several research studies and settings, including our RENAL-HEIR protocol (COMIRB #16-1752), CASPER protocol (COMIRB #17-0820), IMPROVE-T2D protocol (COMIRB #18-0704). Adverse events are uncommon when the procedure is done by experienced personnel in an appropriate setting. There have been no Serious Adverse Events (SAE) in the research group's experience in the Pediatric Clinical and Translational Research Center with insulin clamps, iohexol or PAH clearance studies. Therefore, we do not anticipate encountering SAEs. However, we have defined the following as possible SAEs for the purposes of monitoring**:** infection related to blood draw or IV placement. In addition, allergic reactions to iohexol or PAH requiring intervention (e.g. oral Benadryl) would also be considered a SAE. The PI will report serious adverse events, and any decision to suspend or halt the protocol to the CTRC and COMIRB immediately. The subject will also be instructed to report the event to their PCP. Caffeine dosing less than 400mg/day considered safe in non-pregnant subjects and not associated with adverse effects in terms of acute toxicity, cardiovascular toxicity, bone and calcium effects, behavior, and development and reproduction (53-56). This is roughly the amount of caffeine in four cups of brewed coffee. We will also exclude caffeine-naïve participants to reduce the likelihood of withdrawal effects and exclude participants with prior adverse effects to caffeine, on other stimulants for ADHD, tremors, tics, Tourette's, arrythmias, insomnia and overactive bladder.

All investigators are currently certified in the Colorado IRB and HIPAA regulatory courses required to perform human subject research at the University of Colorado Denver and the Children's Hospital Colorado and will be required to maintain such certification throughout the study. No protected health information will

be collected until the appropriate HIPAA forms are completed. This information will be accessible only by the study investigators, Federal agencies overseeing human subject research, the Colorado Multiple Institutional Review Board, regulatory officials from the institution where the research is being conducted to monitor safety and compliance with policies. Every effort will be made to decrease the risk of loss of confidentiality by limiting access to protected health information, storing this information in a password protected database, and identifying subjects only by a unique identifier that is kept in a separate location in a locked container, traceable only by study personnel. Electronic Data will be stored in a password protected database and file servers. Paper files will be stored in a locked cabinet in the office of the Principal Investigator, accessible only to study investigators.

## E. Potential Scientific Problems and Feasibility:

**Overall feasibility:** This is pilot and feasibility study is an add-on study to CASPER study (#17-0820). Recruitment and enrollment for CASPER study has successful. In fact, in 3 months, we successfully recruited and enrolled 41 of the 50 participants needed in the *CASPER Study,* and are above our target enrollment. Furthermore, the study procedures are identical to the ones in the *CASPER* study and are other active studies. Accordingly, we do not foresee any difficulties with enrollment or study visit completions. Our investigative team has substantial experience with hyperglycemic, hyperinsulinemic-euglycemic clamps in T1D, T2D, obese and lean youth (59, 65, 66, 84), functional MR imaging (46, 47, 74, 86-91), and renal physiology studies (68, 70, 75-80). Our proposal is based on strong preliminary data and a track record of successful collaborations and publications (14, 30, 65, 66, 70, 75, 80, 92-102).

**Potential scientific problems:** In any clinical study, unforeseen human factors provide challenges. We have experience with hyperglycemic clamps, iohexol and PAH clearance studies and MRI methods in youth with T1D and have already obtained preliminary data. We anticipate an increase in renal oxygenation due to decreased renal oxygen consumption with preserved GFR and ERPF in response to coffee therapy. In addition, we expect decreased plasma concentration of tubular injury markers in response to coffee therapy. Clearance of PAH is the gold standard method to measure ERPF, and GFR by iohexol clearance is more accurate and precise than eGFR. Importantly, iohexol and PAH clearances do not interfere with the assessment of insulin sensitivity during a hyperglycemic clamp. Mild hyperglycemia (~ 190mg/dl) during the GFR measurements and a salt replete and protein controlled diet are critical strengths as severe hyperglycemia acutely increases GFR, and salt and protein intake influence intrarenal hemodynamics (97). Furthermore, mild hyperglycemia allows us to quantify intrarenal hemodynamic function in a glycemic milieu similar to their native pathophysiology. In contrast, a hyperinsulinemic-euglycemic clamp would render the participants completely normoglycemic, which does not reflect their usual glycemic environment, and expose them to high concentrations of insulin which may interfere with the assessment of their intrarenal hemodynamic function. Based on our preliminary work, we expect the 2 renal sequences (BOLD and ASL) to be completed within 60 min. In our other studies we have had adolescents with T1D, T2D and without diabetes undergo similar MRI procedures lasting 120 min, indicating that recruiting and tolerability in youth for such procedures are feasible. Our research MRI is equipped with in-MRI movie-viewing with noise-cancelling headphones and thus is very tolerable to youth. The participant will be monitored by heart rate, blood pressure, and oxygen saturation measurements, and will be able to communicate with the MRI technician and investigator immediately outside of the scanner. As BOLD is potentially influenced by $O_2$ delivery (ERPF) and $O_2$ consumption (tubular Na transport accounts a majority of renal $O_2$ consumption (103)) we will control for ERPF and fractional excretion of sodium (FeNa). We will perform the MRI after the clamp to remove the effects of acute variations in glycemia on RPF. Participants on medications that might alter $O_2$ delivery and/or consumption, e.g. angiotensin-converting enzyme inhibitors (ACE-I), angiotensin receptor blockers (ARBs) and diuretics will be excluded.

## F. Potential Benefits:

**Evidence of Direct Benefit to Subjects:** This pilot study is designed to learn more about renal energetic and intrarenal hemodynamic effects of coffee consumption in adolescents and young adults with T1D. Data

obtained from this study will be used to direct a larger study and identify non-caffeine bioactive compounds in coffee to impede the development of DKD to reduce future risk of cardiovascular morbidity and mortality. The participants will receive a daily dose of caffeine equivalent to four cups of coffee which may have beneficial effects on their renal health. However, this is a short study, and the medical benefits of participating is likely modest. Information gained by conducting this study is expected to yield novel, important findings regarding the mechanisms underlying early DKD in youth with T1D, and how it responds to coffee. Participants will also benefit from being in close contact with study staff, blood sugar monitoring and learning more about their renal health. All lab and procedure results with clinical relevance obtained as part of the study will be shared with participants.

**Evidence of Benefit to Society:** The importance of the knowledge gained from this protocol is high, since DKD is very prevalent in T1D and increases the risk of death from CVD and shortens lifespan. In fact, T2D in youth is increasing in prevalence in parallel with the obesity epidemic. In the United States, almost half of patients with renal failure have DKD. Despite the high prevalence and gravity of DKD in youth onset T1D, widely effective therapeutic options are lacking. Diabetes mellitus is the third most prevalent severe chronic disease of childhood. DKD remains a leading cause of morbidity and mortality, and a major risk factor for CVD in diabetes. Although data to guide care of DKD in adults with diabetes are limited, even less data exist regarding the antecedents of DKD in youth with diabetes. Yet, these antecedents of adult DKD are present in children. As DKD is one of the leading causes of death in people with diabetes and the antecedents develop in childhood, data to inform clinicians as to treatment in this high-risk population are of great public health importance. In our novel and innovative proposal, we postulate that coffee will decrease renal oxygen consumption, thereby improving renal oxygenation without impairing GFR and ERPF. Knowledge gained from this proposal will provide new information about the effects of coffee on renal energetics and intrarenal hemodynamic function in T1D. The investigative team is also unique in being one the few groups skilled in performing both gold standard renal physiology studies and advanced MRI evaluation in youth with T1D.

## G. Data Analysis Plan (formulated with biostatistician Laura Pyle PhD):

***Aim 1-2 Power calculations, statistical analysis, rigor and reproducibility:*** For this initial pilot and feasibility trial, we plan to recruit 10 youth. Power calculations are based on paired *t*-tests. A sample size of 10 provides 80% power to detect a mean paired difference of 0.44 SD for renal oxygenation and tubular injury markers. The following variables will be considered for inclusion in models as confounders: response: age, sex, ethnicity, T2D duration, HbA1c, BP, LDL-C and FeNa. We will use paired *t*-test and McNemar's test in addition to multivariable linear models of the change in each outcome, adjusted for potential confounders. This is a pilot and feasibility study and the data should be considered hypothesis generating. We acknowledge that we may be underpowered for multivariable modeling, and our primary analyses will be unadjusted.

*Renal Volume:* Using 3D Slicer (SPL, Harvard Medical School, MA), the semi-automated segmentation of kidneys will be conducted initially with a thresholding procedure. Manual editing will then be performed to refine the segmentation of bilateral kidneys by placing straight lines at the convex level to separate the right kidney from liver and to remove bilateral ureters from the kidneys. This editing process will need to be performed on few contiguous slices with limited human interaction. The kidney volume will then be computed as the number of voxels within one kidney multiplied by the voxel size.

*BOLD MRI:* Regions of interest (ROI) analysis for BOLD MRI will be performed on a Leonardo Workstation (Siemens Medical Systems, Germany). Typically, 1 to 3 regions in each, cortex and medulla, per kidney per slice will be defined leading to a total of about 10 ROIs per region (cortex and medulla) per subject. The mean and standard deviation of these 10 measurements will be used a $R_2$* measurement for the region, for the subject and for that time point. Additionally, two $\Delta R_2$*s will be calculated as defined below:

$\Delta R_2$*(medulla, furosemide) = $R_2$* (medulla, pre-furosemide) - $R_2$* (medulla, post-furosemide);

$\Delta R_2$*(cortex, medulla) = Baseline $R_2$* (medulla) – Baseline $R_2$* (cortex).

*ASL MRI:* ROI analysis will be used to estimate $\Delta M$ (difference in signal intensity between non-selective and selective inversion images). Using the same ROI, $M_0$ will be estimated from the proton density image. $T_1$ measurements from the same ROI will be obtained by fitting the signal intensity *vs.* inversion time data as described previously (104) using XLFit (ID Business Solutions Ltd., UK) or $T_1$ maps created using MRI Mapper (Beth Israel Deaconess Medical Center, Boston). Partition coefficient will be assumed to be 0.8 ml/gm (105, 106). These values will then be used to estimate regional blood flow.

For data analyses, all MRI markers will be measured for each kidney (left and right) for each subject and these two measurements for each specific MRI marker would be averaged to generate a summary measurement per subject for further statistical analysis. BOLD MRI measurements will be performed before and after administration of furosemide and the relative value (pre minus post) will be reported. The final averaged value will be analyzed statistically.

*Summary of MRI Parameters:*

| | |
|---|---|
| **Renal Volume:** | Left kidney volume (LKV), Right kidney volume (RKV) |
| **Diffusion Imaging:** | $ADC_{Left}$ (cortex), $ADC_{Left}$ (medulla), $ADC_{Right}$ (cortex), $ADC_{Right}$ (medulla) |
| **BOLD MRI:** | $R_2$*(cortex)$^{pre}_{Left}$, $R_2$*(medulla)$^{pre}_{Left}$, $R_2$*(cortex)$^{pre}_{Right}$, $R_2$*(medulla)$^{pre}_{Right}$<br>$R_2$*(cortex)$^{pos}_{Left}$, $R_2$*(medulla)$^{pos}_{Left}$, $R_2$*(cortex)$^{pos}_{Right}$, $R_2$*(medulla)$^{pos}_{Right}$<br>Pre – pre-furosemide; pos – post-furosemide |
| **ASL MRI:** | $flow_{Left}$ (cortex); $flow_{Right}$(cortex) |

***Data Management:***

**Data Entry**
Data will be entered from paper forms. Once forms are completed, verified and corrected for inconsistencies, they will be manually entered using a HIPAA compliant, Red Cap database system.

**Edit Checks**
Computerized data validation routines will be used to enhance data quality and verify the accuracy of data within predefined value ranges. These checks include but are not limited to: (a) initial screening of data, using logic and range checks built into data entry screens; (b) cross-form functional and consistency checks; and (c) edits assessing the serial integrity of data.

**Disaster Recovery**
Routine data backup will occur on data in conjunction with the children's hospital secure server and Redcaps.

**Security and Confidentiality**
All hard copy forms will be de-identified with a study number and filed in a locked cabinet, to which only the investigators will have access. Standard protection against computer hackers is implemented. Recovery from natural disasters (water, fire, or electrical) can occur through the ability to reconstruct both the database management system and the data from nightly backups.

**H. Summarize Knowledge to be Gained:**
The innovative and novel pilot and feasibility trial aims to define the effects of coffee therapy on renal energetics and intrarenal hemodynamic function in youth with T1D. Carefully designed human studies, like the one proposed in this application, are needed to advance our understanding of the pathophysiology driving early DKD and direct the development of new therapeutic strategies to impede the development of DKD. Finally, the findings of this project could also inform other acute and chronic kidney disease conditions. The data obtained from this project will help delineate which renal processes are influenced by coffee consumption. The results and techniques from this project will be used to direct future collaborative clinical trials between Drs. Bjornstad and Parikh.

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