Copeptin in Adolescent Participants with Type 1 Diabetes and Early Renal Hemodynamic Function

Protocol and Statistical Analysis Plan

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COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
CAMPUS BOX F-490 TELEPHONE: 303-724-1055 Fax: 303-724-0990

Protocol #: 17-0820
Project Title: ***CASPER Study: C**opeptin in **A**dole**s**cent **P**articipants with Type 1 Diabetes and **E**arly **R**enal Hemodynamic Function*

Principal Investigators: Petter Bjornstad, M.D.
Co-Investigators: Kristen J. Nadeau, M.D., M.S., Richard J. Johnson, M.D., Marian J. Rewers, M.D., Ph.D., Bruce A. Perkins, M.D., M.P.H. and David Z. Cherney M.D., Ph.D.

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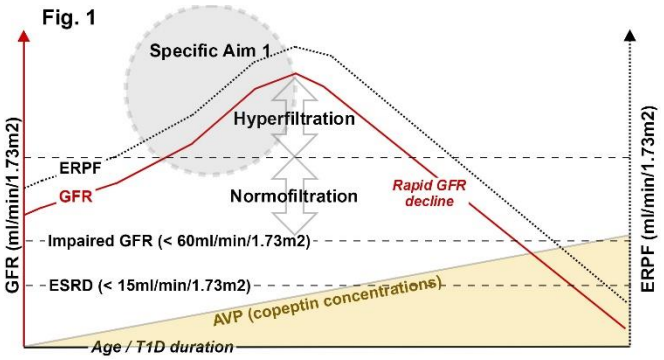
I. Hypotheses (H) and Specific Aims (SA):

Over 1.25 million Americans have type 1 diabetes (T1D), increasing risk for early death from cardiorenal disease (1, 2). 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 (3-6). The strongest risk factor for cardiovascular disease (CVD) and mortality in T1D is diabetic kidney disease (DKD) (7, 8). Current treatments, such as control of hyperglycemia and hypertension, are beneficial, but only partially protect against DKD. Clinical trials in DKD have yielded disappointing results (9-16), potentially due to the lack of interventions at early stages of disease when the benefit is most likely. In fact, the recently published results from the AdDIT trial demonstrated that use of angiotensin converting enzyme inhibitor (ACEi) and statin failed to change albumin excretion over time in youth with T1D (17). Thus, identifying new therapies to impede DKD remains a public health priority.

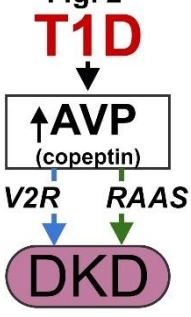
Fig. 1



Hyperfiltration is common in youth with T1D (18, 19), and predicts progressive DKD. Hyperfiltration is also associated with early changes in intrarenal hemodynamic function, including increased effective renal plasma flow (ERPF) and glomerular pressure. **Figure 1** summarizes the changes in glomerular filtration rate (GFR) and ERPF, that we propose underlie the natural history of DKD in T1D. Intrarenal hemodynamic function is strongly influenced by the renin–angiotensin–aldosterone system (RAAS) system, which is also considered a key player in the pathogenesis of DKD. In particular, angiotensin II (Ang II), shows increased activity during DKD (20, 21). Our preliminary data demonstrate differences in intrarenal hemodynamic function and RAAS activation in early and advanced DKD in T1D (22,23). The pathophysiology contributing to the differences observed in RAAS activation and intrarenal hemodynamic function in T1D are poorly defined.

Animal research demonstrates that arginine vasopressin (AVP) acts directly at the V2 receptor (V2R) to modify intrarenal hemodynamic function (24), but also indirectly by activating RAAS (22) (**Fig 2**). Our preliminary data suggest that elevated copeptin, a marker of AVP, predicts DKD in T1D adults, independently of other risk factors (25, 26). However, no human studies to date have examined how copeptin relates to intrarenal hemodynamic function in early DKD in T1D. A better understanding of this relationship is critical to inform development of new therapies targeting the AVP system in T1D. Accordingly, in this application, we propose to define the relationship between copeptin and intrarenal hemodynamics in early stages of DKD, by studying youth aged 12-21 y with T1D duration < 10 y (**Fig. 1**).

Fig. 2

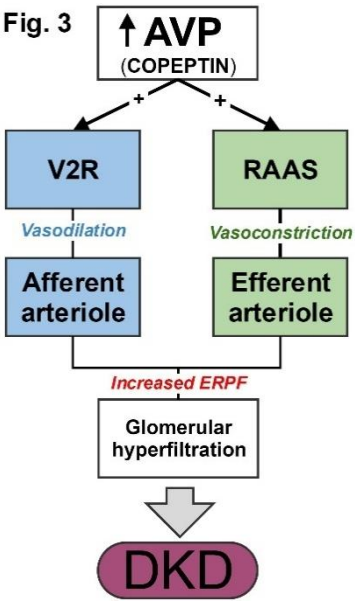


SA1: Define the relationship between copeptin and intrarenal hemodynamic function in T1D youth.
H1.1: Copeptin will be higher in youth with T1D and hyperfiltration vs. those without hyperfiltration.
H1.2: Copeptin will *positively* correlate with GFR, ERPF and intrarenal hemodynamics in short-standing T1D.

SA2: To understand the effects of copeptin on renal oxygenation and perfusion in T1D youth.
H2.1: Copeptin will correlate positively with renal oxygenation consumption and perfusion in T1D youth.

II. **Background and Significance**: Prior bench and translational research into the mechanisms of hyperglycemic injury and its modifiers (27) has to date not successfully translated into adjuvant therapeutics to supplement intensive insulin therapy in the prevention of DKD. Accordingly, there is an urgent need for novel modifiable risk factors. AVP has diverse actions in physiology which include direct effects on vascular hemodynamics, RAAS, volume, and osmolality regulation (28, 29). The actions of AVP are mediated by at least three distinct receptor subtypes: V1a, V1b, and V2. Experimental data strongly support a causal and direct role of AVP in the pathogenesis of kidney disease through V2R activation (30, 31) (**Fig 3**). In laboratory animals, elevated AVP directly leads to afferent arteriolar vasodilation by V2R stimulation (24) and indirectly to efferent arteriolar constriction by RAAS activation (28, 29). The net effects are increased ERPF with glomerular hypertension and hyperfiltration (**Fig 3**), in addition to albuminuria and tubulopathy (32-34). Supraphysiologic increases in ERPF, glomerular pressure and GFR are hallmarks of hyperfiltration (35), an early phenotype of DKD which predicts rapid GFR decline and impaired GFR in adults with T1D (36). Measuring AVP is cumbersome due to its small size and short half-life. Copeptin, a more stable peptide derived from the same precursor molecule as AVP, is thus recently recognized as a better method to determine AVP activity and hence will be used in this proposal (33).

Fig. 3



Scientific Premise: The relationships we and others have demonstrated between copeptin, DKD in T1D are based on epidemiologic data and limited to adults with advanced DKD (25, 26, 37). These studies have established that copeptin is significantly higher in adults with T1D, and that elevated levels of copeptin are associated with cardiorenal disease. Yet no human studies have examined the mechanisms whereby elevated AVP activity contributes to DKD, nor whether this relationship is different in early DKD. Intrarenal hemodynamic function is compromised in both early and advanced DKD (35, 38-40), and recent studies have also provided compelling evidence that intrarenal hemodynamic dysfunction can be partially restored with therapeutic agents in T1D (41-51). However, no data exist on the interaction between copeptin, intrarenal hemodynamic function in early DKD in T1D. Until this gap is addressed, our understanding of AVP as a risk factor and therapeutic target in DKD is limited.

Our overall hypothesis is that elevated copeptin in T1D is associated with distinct changes in intrarenal hemodynamic function in early vs. advanced DKD, which includes increased ERPF and GFR in early DKD (e.g. hyperfiltration) and reduced ERPF and GFR in established DKD (e.g. impaired GFR). To test these hypotheses, we will carry out a cross-sectional study in youth with short-standing T1D as well as take advantage of unique mechanistic data already collected in adults with longstanding T1D, with and without advanced DKD (DKD and DKD Resistors) from the JDRF-funded *Canadian Study of Longevity in Diabetes (Longevity)*.

Impact: This proposal seeks to investigate the roles of AVP on intrarenal hemodynamic function in youth and adults with early and advanced DKD. By understanding the interplay between AVP and intrarenal hemodynamic function in T1D, we will provide a foundation for future mechanistic studies and clinical trials targeting the AVP system. Renal physiology studies are required to advance DKD research and direct the development of new therapies to improve cardiorenal health and mortality for the growing number of people afflicted by T1D (52).

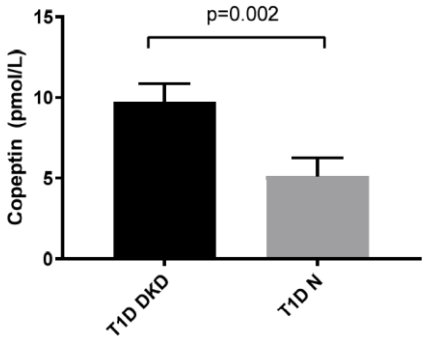
Our proposal is innovative because:
1. We move beyond the traditional determinants of DKD (ABC risk factors; Hb**A**1c, **B**lood pressure and LDL-**C**holesterol), to focus on elevated AVP activity.
2. We can validate our epidemiologic findings in CACTI and the T1DX with mechanistic renal physiology data.
3. Hyperfiltration is understudied. It is important to examine early changes in GFR, because albuminuria does not reliably reflect progressive DKD and commonly reverts to normoalbuminuria (53). Hyperfiltration is now recognized to be a common phenotype of early DKD in young adults with T1D (18, 19). Hyperfiltration

predisposes to progressive nephron injury by increasing glomerular pressure (54). We have shown that hyperfiltration precedes albuminuria and predicts rapid GFR decline and chronic DKD in adults (36).
4. We propose innovative methods by a highly skilled, multi-disciplinary team to define intrarenal hemodynamic function, including gold standard measurements of GFR by iohexol clearance, ERPF by para-aminohippurate (PAH) clearance and calculations of intrarenal hemodynamic parameters by Gomez' equations (55).
5. There is the potential to translate the findings of these studies to a rational intervention strategy in T1D using selective V2R blockers (e.g. vaptans), which are FDA approved in adults with clinical hyponatremia.

III. Preliminary Studies/Progress Report:

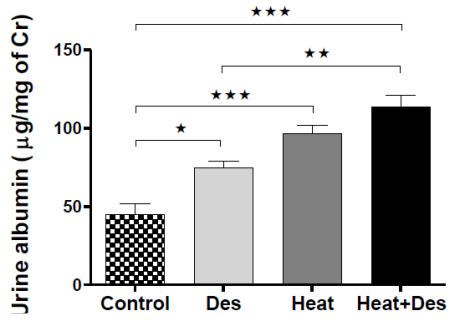
1. Copeptin concentrations are greater in adults with T1D compared to non-diabetic controls: Copeptin was greater in participants with T1D compared to those without (geometric mean, 95% CI: 3.5 [2.3-3.8] vs. 2.8 [2.7-3.1] pmol/L, p=0.003), and the difference remained significant after adjusting for age, sex and eGFR (p=0.0008) (26).

Fig 4. Copeptin is higher in DKD



2. Men with T1D and albuminuria have 3-fold higher copeptin levels than men with T1D and normoalbuminuria: In a case-control study from the T1DX Biobank registry, men with T1D and albuminuria (T1D DKD) had greater copeptin levels than men with normoalbuminuria (T1D N). The difference between T1D DKD and T1D N remained significant after adjusting for HbA1c, eGFR and ACEi / angiotensin II receptor blockers (ARB) use (Fig 4). Higher copeptin levels conferred greater odds of impaired GFR, independent of other important risk factors. In a generalized linear mixed model, one standard deviation (SD) increase in copeptin predicted greater odds of impaired GFR (OR: 2.94, 95% CI 1.39-6.21), and remained significant after multivariable adjustments (T1D duration, ACEi/ARB use, HbA1c and case/control status) (25).

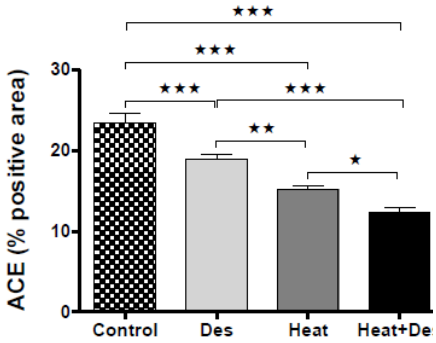
Fig 5. V2R Stimulation - Glomerulopathy



3. Copeptin is associated with cardio-renal disease in adults with T1D: In adults with T1D in in the CACTI study, elevated copeptin (>13pmol/L [>97.5th percentile for healthy adults]) was associated with greater odds of impaired eGFR (OR: 18.52, 95% CI 4.03-85.02), albuminuria (OR: 10.55, 95% CI 2.24-49.62), and CAC scores ≥300 (OR: 6.24, 1.51-25.90) adjusting for age, sex, HbA1c, systolic BP and LDL-C (26).

4. Stimulation of the V2R with desmopressin is associated with glomerular and tubular injury: We showed that the administration of desmopressin (des), a V2 agonist, to mice resulted in glomerular and tubular damage. Urine albumin concentration was greater in mice exposed to des (Fig 5). Urinary NGAL excretion was also highest in groups receiving des (30). We next stained tissues for angiotensin converting enzyme (ACE) as a marker of the proximal tubular injury. A progressive loss of ACE was associated with the addition of des (Fig 6). These data support glomerular and tubular injury in response to V2R stimulation (30).

Fig 6. V2 stimulation - Tubulopathy



5. 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 with T1D can exceed 50% when GFR is measured by inulin clearance (56), and between 13-31 % when GFR is estimated by serum creatinine and serum cystatin C (19, 57). 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, SBP, LDL-C,

sex, duration and albumin-to-creatinine ratio (ACR) (58). 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 Pittsburgh Epidemiology of Diabetes Complications Study (EDC), elevated GFR at baseline was associated with 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) (59).

6. Unique intrarenal hemodynamic dysfunction in early vs. established DKD:

a. Early DKD in young adults: We evaluated intrarenal hemodynamic function in healthy controls (n=30, mean age 27) and in normotensive, normoalbuminuric participants with T1D and either normofiltration (n=36, mean age 24, GFR 90–134 ml/min/1.73m^2) or hyperfiltration (n=32, mean age 23, GFR≥135 ml/min/1.73m^2) during euglycemic and hyperglycemic conditions. We used Gomez's equations to derive renal blood flow (RBF), efferent (R_E) and afferent (R_A) arteriolar resistances, glomerular hydrostatic pressure (P_{GLO}) from inulin (GFR) and PAH (ERPF) clearances, plasma protein and estimated ultrafiltration coefficients (K_{FG}). Participants with T1D and hyperfiltration (T1D-H), had significantly greater GFR, ERPF, RBF, and lower R_A and higher P_{GLO} compared to healthy controls (HC) (60) (Fig 7). These observations illustrate the potential of applying Gomez' equations in DKD research.

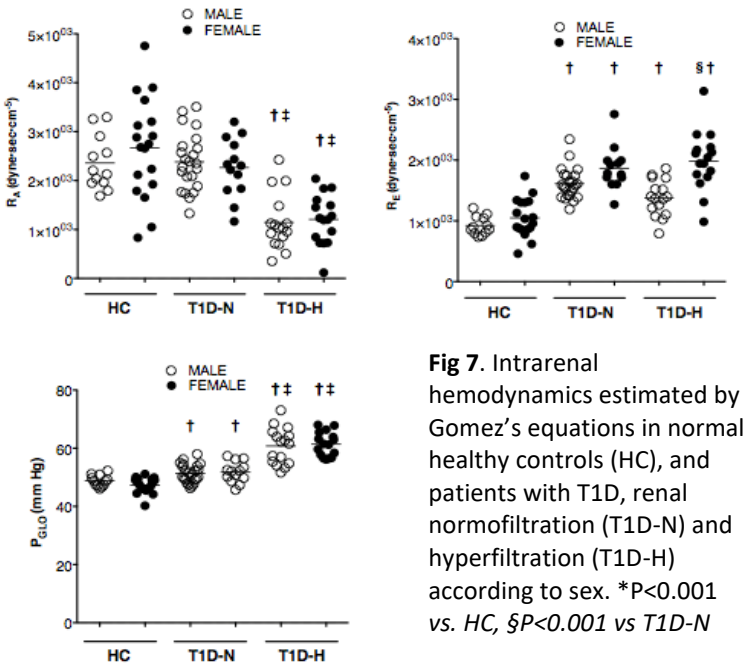


Fig 7. Intrarenal hemodynamics estimated by Gomez's equations in normal healthy controls (HC), and patients with T1D, renal normofiltration (T1D-N) and hyperfiltration (T1D-H) according to sex. *P<0.001 *vs. HC, §P<0.001 vs T1D-N*

In summary, our preliminary epidemiologic data suggest that: 1) copeptin is higher in adults with T1D compared to their non-diabetic peers; 2) elevated copeptin is associated with DKD in the form of albuminuria and impaired GFR, as well as with coronary artery calcification (CAC) in adults with T1D; 3) men with T1D and albuminuria have 3-fold greater copeptin levels than men with T1D with normoalbuminuria; 4) stimulation of V2R with desmopressin (vasopressin agonist) induces tubular and glomerular injuries in murine models; 5) hyperfiltration is common in youth and adults with T1D and predicts rapid GFR decline and impaired GFR; 6) there are unique intrarenal hemodynamic dysfunction profiles in early DKD. We also present experimental data showing unique intrarenal hemodynamic profiles in early and advanced DKD in people with T1D, arguing for dedicated studies at different time points in the natural history of DKD. To understand the pathogenesis whereby elevated AVP activity contributes to DKD, we need carefully designed translational studies, like the one proposed in this application, to define the interplay between AVP activity and intrarenal hemodynamic dysfunction in early DKD. The proposed study is feasible in the current environment: 1) the Barbara Davis Center for Diabetes (BDC) and the Anschutz Medical Campus (AMC) provide ample study participants for a study in youth with short-standing T1D; 2) the experience and expertise of the investigator and collaborator team will ensure the success of the planned research.

Table 1: Eligibility Criteria

Inclusion criteria	Exclusion criteria
Antibody+ T1D with <10 yr duration	Severe illness, recent DKA
Age 12-21 years	ACEi, ARB, diuretics, SGLT 2/1 blockers, atypical antipsychotics, steroids
BMI ≥ 5%ile	Macroalbuminuria (>300mg/g) or eGFR <60ml/min/1.73m^2
Weight<300 lbs.	Pregnancy, nursing
HbA1c <12%	Anemia or allergy to shellfish or iodine
BP < 140/90 mm Hg	

IV. Research Methods:

Study design and study population: We propose to address SA1 and SA2 with a cross-sectional observational study with 50 youth with short-standing T1D (50% female, 12-21 yr, <10 yr duration). The participants will be recruited from the BDC Clinic (>4000 pediatric patients). Following a screening visit, eligible participants (**Table 1**) will be asked to refrain from strenuous physical activity for 3 days prior to admission due to the impact of exercise on

intrarenal hemodynamic function. They will also be provided with a 3-day diet instructions (45% carbohydrates, 30% fat, 25% protein and a goal of 3.45 g salt/day), with females studied in the follicular phase of the menstrual cycle where possible to limit impacts of nutrient and menstrual hormone variations on IR and renal function, as previously described (61). Participants will ask to administer their long acting insulin (e.g. Lantus or Levemir) like they normally would. Patients on insulin pumps can remain on their basal rate throughout the study. If on a hybrid-closed loop (artificial pancreas), participants will be asked to open their loop for the duration of the study. The morning of the study, participants will be asked to check their blood sugar prior to arriving at the CTRC. If their blood sugar is >= 250 mg/dl, they will be asked to administer a bolus of short acting insulin using their home correction factor with a blood sugar target of 200 mg/dl. Participants will arrive the evening before, or present fasting in the morning and have 2 IVs placed and have their blood glucose concentrations clamped in the mild hyperglycemic range (details below) (**Fig 8**). Baseline blood will be drawn for serum copeptin, lipids, insulin, cystatin c, creatinine, sodium, hematocrit and total protein, and iohexol and PAH boluses given via IV (see below for details). GFR and ERPF will be measured by iohexol and PAH clearance in plasma. A 4 hr urine sample will be collected to calculate ACR and AER.

Figure 8 Study Protocol for SA 1

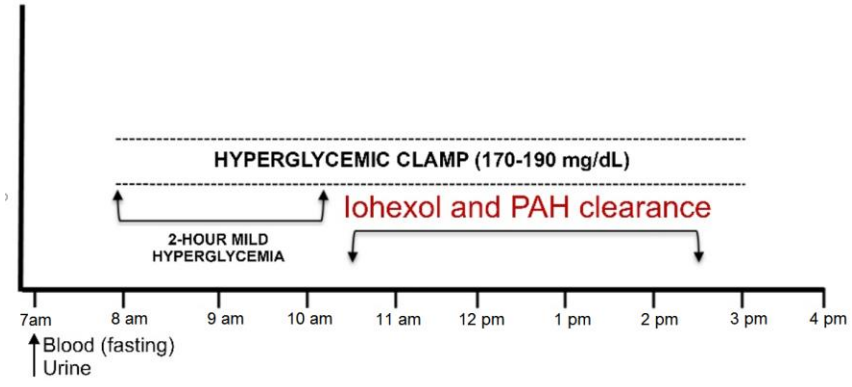


Table 2 – Aims 1 and 2	
Primary Outcomes	**Measurement Tool**
Copeptin	ThermoFisher Kryptor Plus
GFR	Iohexol clearance
ERPF	PAH clearance
Renal O_2	BOLD-MRI
Renal perf.	ASL-MRI
Secondary Outcomes	
RVR	MAP/RBF (**Table 3**)
eGFR	Creatinine, cystatin C
Albumin excretion	Albumin-to-creatinine ratio (ACR)

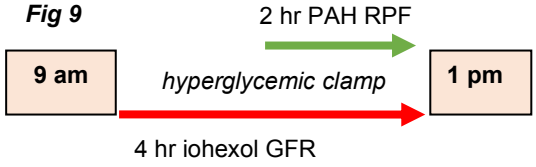
Specific methods: Outcomes are summarized in **Table 2**. Detailed methods are listed below:

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, #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

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

pathophysiology. Furthermore, the clamp maintains an easily achieved and safe range of glycemia. Co-I Dr. Nadeau has extensive experience with normoglycemic, hyperinsulinemic, hyperglycemic and hypoglycemic clamps in T1D youth, which are well tolerated in the proposed ages.

Fig 9



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) (**Fig 9**). 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).

Table 3 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 ΔP_F	ΔP_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 (π_G)	π_G = 5 x (C_M – 2)
Glomerular pressure (P_{GLO})	P_{GLO} = ΔP_F + P_{BOW} + π_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

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.

RVR and Gomez equations (**Table 3**): 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 3**).

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

Copeptin: Copeptin will be measured by ultrasensitive assays on KRYPTOR Compact Plus analyzers using the commercial sandwich immunoluminometric assays (Thermo Fisher Scientific, Waltham, MA). The copeptin assay has a lower limit of detection of 0.9 pmol/L, and a sensitivity of <2pmol/L. Elevated copeptin will be defined as >13pmol/L, which is >97.5th percentile for healthy adults (68).

Estimated GFR: As an exploratory aim, we will evaluate the agreement between measured GFR (iohexol) with estimated GFR by serum creatinine and cystatin C. Due to the expected normal to elevated GFR for youth with T1D, we will calculate eGFR by Zappitelli equation (eGFR = 25.38 * (1/serum cystatin C)$^{0.331}$ * (1/serum creatinine)$^{0.602}$ * (1.88 * height)) (69-71).

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.

Metabolomics: We will measure serum metabolomics by Q Exactive UHPLC-MS Analysis at the Metabolomics Core Facility Services at University of Colorado (Dr. Angelo D'Alessandro, PhD).

Urine and serum osmolality: We will directly measure urine and serum osmolality using freezing point method with Micro-Osomometer Model 3300 (Advanced Instruments, Massachusetts, USA) at Johnson's Lab.

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).

Renal oxygenation and renal perfusion by MRI: The primary outcomes of Aim 2 are renal oxygenation and perfusion (**Table 2**). We will perform BOLD MRI (73) to quantify renal oxygenation, whereas renal perfusion will be measured by ASL (74)(**Table 2**). 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 4**) (75), 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 4. 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/degrees	20	20	20	30	60
Typ. Scan time m:s	0:14	0:07	0:07	0:30-1:00 (5 slices)	0:05

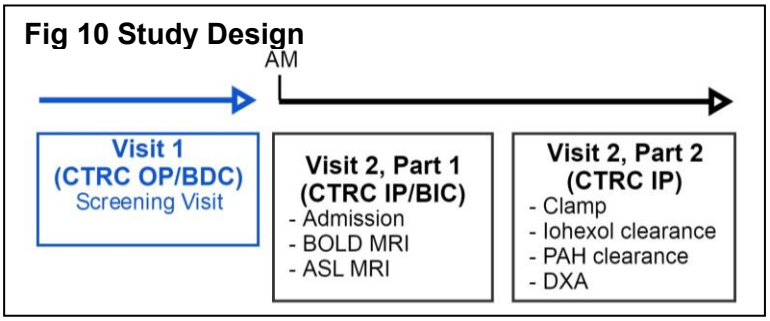
Options/comments	Site preference	Instruct subjects to not to take deep breaths.	Changing FOV and/or matrix size not preferred	Instruct subjects to not to take deep breaths.

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.

Recruitment: Ultimately 50 youth with T1D (50% male) will be enrolled (see power analysis). Recruitment will begin year 1 and will consist of adolescent patients with type 1 diabetes from the Barbara Davis Center for Childhood Diabetes (BDC), where principal investigator Dr. Bjornstad and co-investigator Drs. Nadeau and Rewers are medical providers and have weekly clinics. The investigator team has excelled when recruiting adolescent T1D patients. There are almost 4000 pediatric patients actively followed at BDC, and we do not foresee any difficulties with recruitment.

Detailed Study Visit Descriptions: The study timeline for is illustrated in **Fig 10**, and details from each study visit is summarized below. Visit 1 is the screening visit and visit 2 and 3 will occur consecutively.

Fig 10 Study Design



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.

A. Outcome Measure(s):

Aim 1: Measured GFR by iohexol clearance and RPF by PAH clearance

Aim 2: Renal oxygenation by BOLD-MRI and renal perfusion by ASL-MRI

B. Description of Population to be Enrolled:

Table 1: 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	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
Weight<350 lbs and > 57 lbs.	
No anemia	Anemia or allergy to shellfish or iodine
HbA1c <12%	Pregnancy or nursing
	MRI scanning contraindications (claustrophobia, implantable devices, >350 lbs)

* Shortly after arrival to radiology unit, a MRI technician will meet with patient and/or guardian to provide preparation for the scan. This conversation will include a thorough overview including what the scanner looks like, a sample of the MRI sounds, the scan set up and the importance of lying still for the scan. The metal prescreening precaution procedure will be conducted for both patient and guardian.

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

1. **Study Duration:** The anticipated duration for the study is 2 years

2. **Duration of Participation for Each Subject:** Subject participation consists of 2 visits (~ 11 hours)

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 clamps
DXA	Iohexol clearance
Anthropometric measurements	PAH clearance
ASL and BOLD MRI	Metabolomics

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 $350 upon completion of all procedures in this study (**Table 7 below**). If the subject withdraws before the start of visit 2 (and all successive visit parts), they will still receive a $25 gift card for completion of the screening visit. Subjects will be paid for all subsequent completed visit parts as outlined below. These payments are similar to the payments being made for each visit type in our currently ongoing adolescent studies.

Table 7 Subject payment schedule

Visit 1	Visit 2, Part 1Part 1	Visit 2, Part 2Part 2	Total
$25.00	*$175.00*	*$150.00*	**$350.00**

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.

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.

Metabolomics: Metabolomics is run on serum. The samples will be obtained off the IV so this assumes the same risk as the collection of blood samples: may result in temporary discomfort, bruising, bleeding and on rare occasions, infection.

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) and 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.

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:

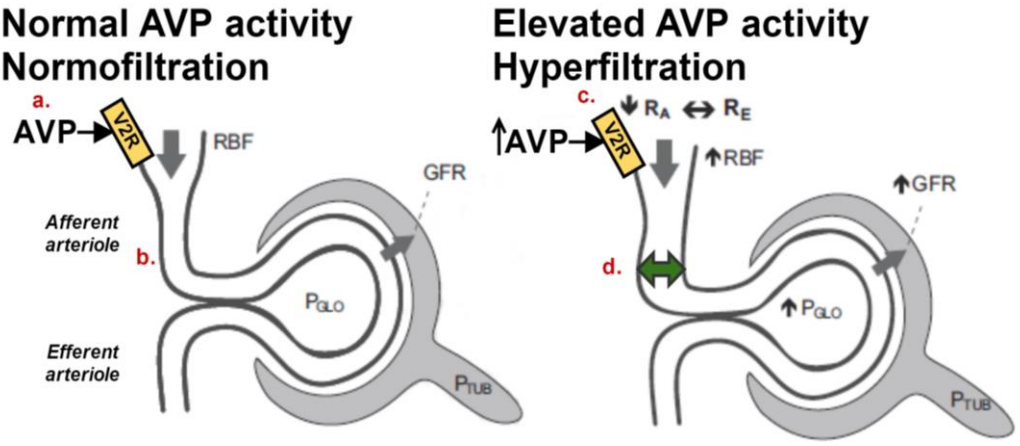
Overall feasibility: While an ambitious study, PI and co-investigators have performed numerous observational studies and clinical trials in youth and adults with T1D (41, 82, 83) and T2D (84), including the NIH TODAY (85, 86), RISE (87), and JDRF studies in T1D youth and adults, including Canadian Study of Longevity in Diabetes (Longevity), Coronary Artery Calcification in Type 1 Diabetes Study (CACTI), and Bromocriptine Quick Release (BCQR) as Adjunct Therapy in Type 1 Diabetes (NCT02544321) with renal measures similar to those now proposed (36, 41, 88-101). The proposed study in this application will be completed in a single research visit, limiting concerns for drop-out and retention. By recruiting adolescents (12-18 y) we expect that 30-50% will have hyperfiltration at baseline per our previous studies in this age group (23, 54, 65, 66, 81, 102-104). Drs. Nadeau and Prasad have extensive experience with MR imaging in youth (73-75, 105-110). Drs. Bjornstad, Johnson and Cherney are experienced in renal physiology measures (38, 55, 60, 62, 64, 79-81). Dr. Prasad is an international leader in renal MRI BOLD and ASL. Finally, our proposal is based on strong preliminary data (41, 55, 64, 111-113) and a track record of successful collaborations (16, 36, 55, 60, 86, 92, 93, 95, 111, 112, 114-120). We are therefore the ideal group to perform the important research proposed.

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 T2D, and have already obtained preliminary data. Expected outcomes and limitations for each aim are summarized below:

Aim 1 - Expected outcomes and limitations:

We expect copeptin to be greater in T1D youth with hyperfiltration vs. those without hyperfiltration. In youth with short-standing T1D and hyperfiltration, we anticipate that copeptin concentrations will positively correlate with GFR, ERPF, RVR, glomerular pressure and albumin excretion. We theorize that elevated AVP results in afferent arteriolar vasodilation with resultant increased ERPF and glomerular pressure which leads to hyperfiltration (Fig 10). Furthermore, a mild hyperglycemic clamp 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. Most studies focus on the classical definition of hyperfiltration which disregards ERPF and P_{GLO}. Our proposed project addresses these concerns and others in regard to scientific rigor, by also examining hyperfiltration defined as FF (GFR/ERPF) > 17.7% and calculating P_{GLO} by Gomez' equations (67, 121). The sustained hypermetabolism of hyperfiltration is thought to initiate the development of nephropathy as the energy profile of T1D cannot accommodate the increased energy demand which leads to progressive nephron loss (122-127). We hypothesize that the elevated AVP activity in T1D may play a role in initiating hyperfiltration through vasodilation of the afferent arterioles with increased ERPF and glomerular hypertension (Fig 10).

Fig 10. Proposed Mechanism of Vasopressin Over-activity and Hyperfiltration



Normal AVP activity at V2R at the afferent arteriole in T1D (a) is associated with normal tone (b), glomerular pressure and GFR. Elevated AVP activity at V2R at the afferent arteriole in T1D (a) leads to vasodilation (b) with increased RBF, increased glomerular pressure and hyperfiltration.

Control for potential confounders influencing intrarenal hemodynamic function includes: renal measures obtained during mild hyperglycemia, macronutrient and salt-controlled diet, limiting strenuous exercise for 3 days prior to visit, and control for stage of menstrual cycle. Our preliminary data demonstrate the feasibility of the proposal in young adults with T1D and our unique ability to perform the highly specialized intrarenal hemodynamic measures.

Aim 2 - Expected outcomes and limitations:
We expect increased renal perfusion and renal O_2 consumption in T1D youth with hyperfiltration. We also anticipate a strong correlation between copeptin, renal perfusion and renal O_2. Based on our preliminary work, we expect the 2 renal sequences (BOLD and ALS) to be completed within 60 min. Dr. Nadeau's NIH R56, JDRF and ADA studies 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 (128)) we will control for ERPF and fractional excretion of sodium (FeNa). We will perform the MRI fasting in the morning to remove the effects of acute glycemic variations on RPF and the impact of changes in hydration status of renal oxygenation. 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.

Expected outcomes and limitations:

F. Potential Benefits:

Evidence of Direct Benefit to Subjects: This study is designed to learn more about the vasopressinergic system (copeptin) and how it relates to intrarenal hemodynamic function and renal energetics in early DKD in T1D. The goal is to identify strategies that can be implemented to impede the development of DKD to reduce future risk of cardiovascular morbidity and mortality. No intervention is being proposed in this study, therefore participants will not directly gain a medical benefit from participating.

Information gained by conducting this study is expected to yield novel, important findings regarding the mechanisms underlying early DKD in T1D. These data will contribute to better understanding of the role of vasopressinergic system in the development of DKD. 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 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 the inappropriate vasopressin activity (elevated copeptin) results in hypermetabolism of hyperfiltration with consequent increased oxygen consumption which cannot be met with the energy inefficiency of diabetes. A better understanding of the pathophysiology underlying early DKD, including hyperfiltration, and its strong relationship with the vasopressinergic system is critical to inform development of new therapeutics. Knowledge gained from this proposal will provide new information about the intrarenal hemodynamic changes associated with hyperfiltration, and the role of elevated vasopressin in early DKD development in T1D. The investigator team is also unique in being one the few groups skilled in performing both gold standard direct measures of GFR, RPF and advanced MRI evaluation in youth with T1D.

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

Table 8 – Aim 1	
Primary Outcomes:	**Groups:**
GFR and ERPF	T1D w/ hyperfiltration vs. T2D w/o hyperfiltration

Aim 1 - Power calculations, statistical analysis, rigor and reproducibility: Primary outcomes for Aim 1 and study groups to be compared are summarized in **Table 8**. Based on our and others preliminary data we expect that 40% of youth with T1D (12-21 y) will demonstrate hyperfiltration (GFR ≥135ml/min/1.73m^2). Thus, a sample size of 50 youth with T1D provides 80% power to detect difference of 6.4 pmol/L in copeptin concentration between youth with T1D with and without hyperfiltration (H1.1), and 80% power to detect a correlation of 0.38 between copeptin, GFR and ERPF in youth with T1D (H1.2). Continuous variables will be checked for the distributional assumption of normality using plots. Variables that are skewed will be natural log-transformed for analyses. Descriptive statistics will be used to summarize baseline participant characteristics. Univariable and multivariable linear regression models will be built to examine the relationships between copeptin, GFR, ERPF and calculated intrarenal hemodynamic parameters (derived by Gomez' equations). Variables considered for inclusion in the multivariable models will include: age, sex, ethnicity, pubertal staging, BMI and HbA1c. A P-value < 0.05 will be considered statistically significant. Analyses will be performed in SAS (v9.4; SAS Institute, Cary, NC).

Table 9 – Aims 2	
Primary Outcomes:	**Groups:**
Renal O_2 and perfusion	T1D w/ hyperfiltration vs. T1D w/o hyperfiltration

Aim 2 – Power calculations, statistical analysis, rigor and reproducibility: Primary outcome for Aim 2 and study groups to be compared are summarized in **Table 9**. Our sample size (n=50, with 40% [n=20] anticipated to demonstrate hyperfiltration) will provide 80% power to detect an effect size of 0.8 SD for renal oxygenation and perfusion between participants with and without hyperfiltration. The analytic approach is as described in Aim 1. In our analyses, we will consider the following potential confounders for renal oxygenation and perfusion; age, sex, T1D duration, HbA1c, BP, LDL-C and FeNa. To avoid overadjusting our models, we will employ pared down models (e.g. model 1: univariable, model 2: age, sex, model 3: variables in model 2 + HbA1c, BP and LDL-C, model 4: variables in model 3 + FeNa). We will evaluate for collinearity before including both age and duration in the same model.

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 ΔR_2*s will be calculated as defined below:

ΔR_2*(medulla, furosemide) = R_2* (medulla, pre-furosemide) - R_2* (medulla, post-furosemide);

ΔR_2*(cortex, medulla) = Baseline R_2* (medulla) – Baseline R_2* (cortex).

ASL MRI: ROI analysis will be used to estimate ΔM (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 (129) 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 (130, 131). 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)$^{pre}_{Right}$
	R_2*(cortex)$^{pos}_{Left}$, R_2*(medulla)$^{pos}_{Left}$, R_2*(cortex)$^{pos}_{Right}$, R_2*(medulla)$^{pos}_{Right}$
	Pre – pre-furosemide; pos – post-furosemide
ASL MRI:	$flow_{Left}$ (cortex); $flow_{Right}$(cortex)

Aims 1-2 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 proposal aims to define the intrarenal hemodynamic function and renal oxygenation in early DKD in adolescents with T1D and define how these parameters relate to vasopressin. 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.

DKD outcomes have not changed significantly over the last couple of decades and continue to be a major concern for providers caring for patients with T1D (8, 132). Managing DKD in patients with T1D has been further complicated by disappointing results of clinical trials (9, 11-14). It is therefore important to identify novel and modifiable risk factors that contribute to the development and progression of early DKD (133).

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