ULTRA T2D

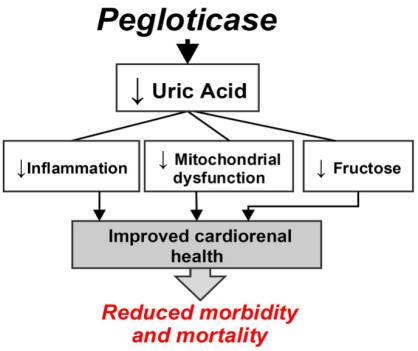
Protocol #: 18-1700
Project Title: ***ULTRA-T2D Study: Uric acid lowering trial in youth onset T2D***
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I. Hypotheses (H) and Specific Aims (SA):

Adolescents and young adults with youth-onset type 2 diabetes (T2D) are disproportionally impacted by hyperuricemia compared to non-diabetic peers and youth with type 1 diabetes (T1D)(1). In fact, 50% of males with youth-onset T2D have serum uric acid (SUA) greater than 6.8 mg/dl (1). We also recently demonstrated that higher SUA conferred greater odds of developing hypertension and diabetic kidney disease (DKD) in youth with T2D over 7 years follow-up. Elevated SUA is thought to lead to cardiovascular disease (CVD) and DKD by inflammation, mitochondrial dysfunction and deleterious effects on nephron mass (2-8). While there are studies demonstrating beneficial effects of uric acid (UA) lowering on vascular health in the general population, to our knowledge, there are no studies in youth-onset T2D. Youth-onset T2D carries a greater risk of DKD and CVD compared to adult-onset T2D and T1D (9).

Accordingly, a clinical trial evaluating UA lowering therapies is needed in youth-onset T2D. Krystexxa (pegloticase), a uricase, effectively lowers SUA and therefore holds promise as a novel therapy to impede the development of CVD and DKD in youth-onset T2D. This proposal describes a pilot and feasibility trial evaluating the effect of UA lowering by pegloticase on markers of CVD and DKD in ten (n=10) youth aged 18-35 with youth-onset T2D (diagnosed ≤25 years of age) over 7 days. Our overarching hypothesis is that pegloticase improves marker of cardiorenal health by lowering UA (**Figure 1**).

Fig 1 Proposed Effect of Pegloticase



Specific Aim 1: ***To evaluate the impact of UA lowering by a single dose of pegloticase on markers of early CVD in young adults with youth-onset T2D.***

Hypothesis 1: UA lowering following a single infusion dose of pegloticase will decrease blood pressure, improve central arterial stiffness and endothelial dysfunction.

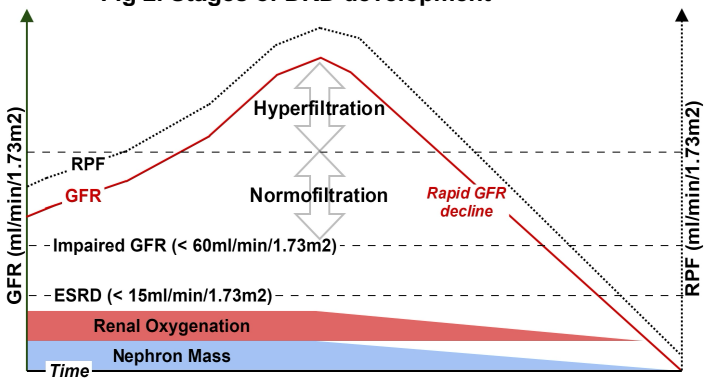
Specific Aim 2: ***To evaluate the impact of UA lowering by single dose of pegloticase on markers of early DKD in young adults with youth-onset T2D.***

Hypothesis 2: UA lowering following a single infusion dose of pegloticase will decrease albumin excretion, glomerular pressure and attenuate hyperfiltration.

In summary, youth-onset T2D have a lifetime risk for complications. Our preliminary data suggest that SUA is an important modifiable risk factor for DKD and CVD in youth-onset T2D. UA lowering trials in youth-onset T2D are therefore needed. Pegloticase, uricase, significantly lowers SUA and therefore holds promise as a novel therapy to impede the development of CVD and DKD. This pilot and feasibility study will provide preliminary data and set the platform for future development of a uric acid lowering trial in youth-onset T2D.

II. Background and Significance:
DKD remains a leading cause of morbidity and mortality in people with type 2 diabetes (T2D) (10-12). In the United States, almost half of patients with renal failure have DKD, and the majority (≥80%) have T2D (13). Current treatments are beneficial, but only partially protect against DKD in T2D. Therefore, identifying new therapeutic targets to slow the progression of DKD remains a public health priority. Youth-onset T2D carries a higher risk of progressive DKD compared to type 1 diabetes (T1D) and adult-onset T2D (14-24).

Fig 2. Stages of DKD development



Elevated serum uric acid (SUA) is increasingly recognized as an important risk factor for hypertension and DKD in adults with type 1 diabetes (T1D) (7) and T2D in addition to the general population (4, 5). The mechanisms whereby elevated SUA confers increased risk for CVD and DKD are incompletely defined. Proposed pathogenesis includes inflammation (25), insulin resistance (6), intrarenal hemodynamic dysfunction (26, 27), vascular, glomerular and tubular injuries (6, 28) and loss of nephron mass (2, 29). Renal hypoxia, stemming from a mismatch between renal oxygen (O_2) utilization and consumption, is increasingly proposed as a unifying pathway in the development of DKD (30). The kidneys are metabolically active and have a high energy requirement to sustain filtration, intrarenal hemodynamic function and tubular reabsorption (31). Hyperfiltration and hyperglycemia of T2D result in increased renal O_2 consumption (32). However, in T2D, emerging animal data suggest that the kidneys are not able to sufficiently compensate for the increased O_2 consumption due to the effects of diabetes on O_2 utilization (33-35). The less O_2-efficient fuel profile of T2D is thought to be secondary to insulin resistance (IR) (31, 33-35).

Scientific premise: Therapies lowering SUA have demonstrated attenuation of elevated albumin excretion and slowing of eGFR decline in adults with type 2 diabetes (36, 37), however to our knowledge there are no such trials yet in youth-onset T2D. In T1D, SUA lowering with febuxostat moderately lowered blood pressure without impacting the renin-angiotensin-aldosterone system, suggesting that elevated SUA may augment other hemodynamic or inflammatory mechanisms (38). Results from preventing early renal function loss (PERL) allopurinol study, an ongoing multicenter randomized clinical trial in adults with T1D, are still pending (39). It is also important to emphasize that in contrast to youth and adult with T2D, people with T1D tend to have lower SUA concentrations due to the uricosuric effect (6-8). Therefore, findings in T1D may not be generalizable to T2D and vice versa. Furthermore, trials on UA lowering have focused xanthine oxidase inhibitors, including allopurinol and febuxostat which limit the amount of UA produced. These agents can reduce the SUA by 3-4 mg/dl. In contrast, rasburicase and pegloticase are uricases and therefore break down UA rather than limiting UA production. Specifically, these agents metabolize UA to allantoin, which is up to 10-fold more water soluble than UA. Accordingly, these agents can lower SUA much more effectively. In contrast to rasburicase, pegloticase is pegylated which increases its half-life and also less immunogenic (40). Pegloticase is FDA approved in adults for treatment of chronic gout in adult patients who are refractory to conventional therapy. Pegloticase is an ideal agent to evaluate the acute effects of UA lowering on markers of renal and cardiovascular function. The present pilot and feasibility trial has the potential to transform our understanding of how acute UA lowering influence markers of early DKD and CVD.

Our overall hypothesis is that a single administration of pegloticase (8 mg IV in 250 mL 0.9% normal saline) will improve cardiorenal health markers over 7 days by lower SUA. To address this hypothesis, we propose a pilot and feasibility trial to determine the impact of SUA lowering in 10 young adults with youth-onset T2D with pegloticase over 7 days on blood pressure, GFR by iohexol clearance, renal plasma flow (RPF), aortic pulse wave velocity (PWV), wall shear stress (WSS) and strain metrics by 4D flow MRI.

Impact: Clinical trials in early DKD have largely yielded disappointing results for the past two decades. This trend may be explained in part 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 SUA lowering on markers of DKD and CVD in young adults with T2D.

III. Preliminary Studies/Progress Report:

Aim 1: To evaluate the impact of UA lowering by pegloticase on markers of early CVD in young adults with youth-onset T2D.

Hypothesis 1: UA lowering following a single infusion dose of pegloticase will decrease blood pressure, improve central arterial stiffness and endothelial dysfunction.

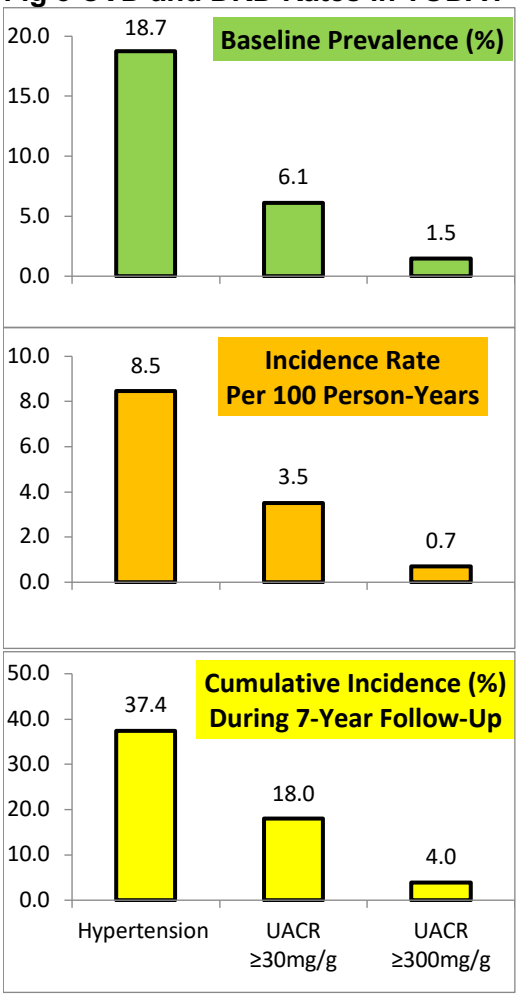
Aim 1 - Preliminary Data:

1.a Hyperuricemia is common in youth-onset T2D: In the TODAY study (n=539), we found that the mean SUA at baseline was 5.8±1.4 mg/dl with 26% of participants found to have hyperuricemia (**Table 1**). Compared to girls, boys had greater baseline SUA (5.4±1.2 vs. 6.6±1.4 mg/dl, p<0.0001) and hyperuricemia prevalence (12 vs. 50%, p<0.0001).

Table 1	Overall (n=539)	Female (n=344)	Male (n=195)	P-value
SUA (mg/dL)	5.8 ± 1.4	5.4 ± 1.2	6.6 ± 1.4	<.0001
Hyperuricemia (%)	26%	12%	50%	<.0001
SBP (mm Hg)	113 ± 11	111 ± 10	117 ± 11	<.0001
DBP (mm Hg)	66 ± 8	66 ± 8	67 ± 8	.03
UACR (µg/mg)	7 (5-13)	7 (5-15)	6 (4-12)	.04
eGFR (mL/min/1.73m^2)	117 ± 19	122 ± 19	108 ± 17	<.0001
ACEi/ARB therapy (%)	5%	5%	4%	ns

1.b Youth-onset T2D have greater risk of CVD and DKD compared to adult-onset T2D and T1D: In TODAY, at baseline, 18.7% of the participants had hypertension, 6.1% had UACR ≥ 30m/g and 1.5% had UACR ≥ 300mg/g. The incidence rates over 7 years, was 8.5 per 100 person-years for hypertension, 3.5 per 100 person-years for UACR ≥ 30m/g and 0.7 per 100 person-years for UACR ≥ 300mg/g. The cumulative incidence was 37.4% for hypertension, 18% for UACR ≥ 30m/g and 4% for UACR ≥ 300mg/g, when excluding baseline prevalence (**Fig 3**).

Fig 3 CVD and DKD Rates in TODAY



1.c Elevated SUA predicts hypertension: Higher baseline SUA correlated with increase in SBP (β±SE: 0.66±0.16, p<.0001) and DBP (0.37±0.15, p=0.01) over time in multivariable models. In cox proportional hazard models, for every 1 mg/dl increase in SUA at baseline there was greater risk of incident hypertension (HR: 1.2, 95% CI 1.1, 1.4, p=0.01) after adjusting for age, sex, race-ethnicity, BMI, eGFR, treatment group, HbA1c and hypertension medications.

1.d Innovative noninvasive MRI-based CVD methods: We have extensive experience with muscle(41, 42), hepatic(43), visceral(41, 43), aortic(44) and cardiac(45, 46) MRI. MRI is the single imaging modality that allows assessment of both the ventricle and the vasculature without radiation exposure and without limitations of inadequate acoustic windows encountered in echocardiography of obese subjects. MRI also allows segment-specific measures (47, 48) MRI is the reference standard for measuring ventricular volume, mass, function, and evidence of myocardial fibrosis. In adult studies, MRI is also now emerging as a technique for evaluating measures of vascular function, including flow patterns, flow velocity, wall shear stress (WSS), and recently, vorticity and helicity by 4-dimensional (4D) flow (49, 50). Mounting evidence implicates WSS, a mechanical force against the vessel endothelium, and flow dynamics in arterial stiffness and atherosclerosis(51, 52). Our novel MRI-based approaches (45, 46, 53-55) allow noninvasive assessment of aortic stiffness (pulse wave

velocity [PWV], distensibility), flow and WSS. In our youth with diabetes, MRI PWV was elevated and correlated with peak systolic and time-averaged WSS (56). In our EMERALD study of T1D youth, we found significant improvements in PWV and WSS following 3 months of treatment with metformin (Bjornstad and Nadeau et al. *Circulation 2018 in-press)*, showing evidence of MRI-detectable CVD and detectable change in CVD by MRI in youth with diabetes.

Aim 2: To evaluate the impact of UA lowering by pegloticase on markers of early DKD in young adults with youth-onset T2D.

Hypothesis 2: UA lowering following a single infusion dose of pegloticase will decrease albumin excretion, glomerular pressure and attenuate hyperfiltration.

2.a Elevated SUA predicts DKD: Higher baseline SUA correlated with increase in log UAE (0.05±0.02, p=0.01) over time in multivariable models. In cox proportional hazard models, for every 1 mg/dl increase in SUA at baseline there was greater risk of incident elevated albumin excretion (HR: 1.2, 95% CI 1.1, 1.5, p=0.02) after adjusting for age, sex, race-ethnicity, BMI, eGFR, treatment group, HbA1c and hypertension medications.

2.b Sex-specific interactions between SUA and DKD: We found sex-specific interactions between SUA and elevated albumin excretion (p<0.05). When stratified by sex, a relationship between SUA and DKD was only significant in boys with T2D (HR: 1.5, 95% CI 1.1, 1.9, p=0.003) after multivariable adjustments. This sex-specific interaction was not observed with hypertension.

*2.b Innovative noninvasive MRI-based renal methods (**Fig 4**):* Phase-contrast and 4D flow renal MRI allow us to quantify blood flow in the renal arteries and veins, in addition to PWV and WSS in the renal vessels. The major advantage of PC-MRI is in capturing flow hemodynamic waveform through the kidney hilum in single acquisition, and therefore acquiring flow through both arterial and venous renal vessels. Furthermore, the anatomical variability in renal vasculature (double or triple branch renal artery) will be then easily accounted for by processing of the same MRI data set, without further acquisition being needed.

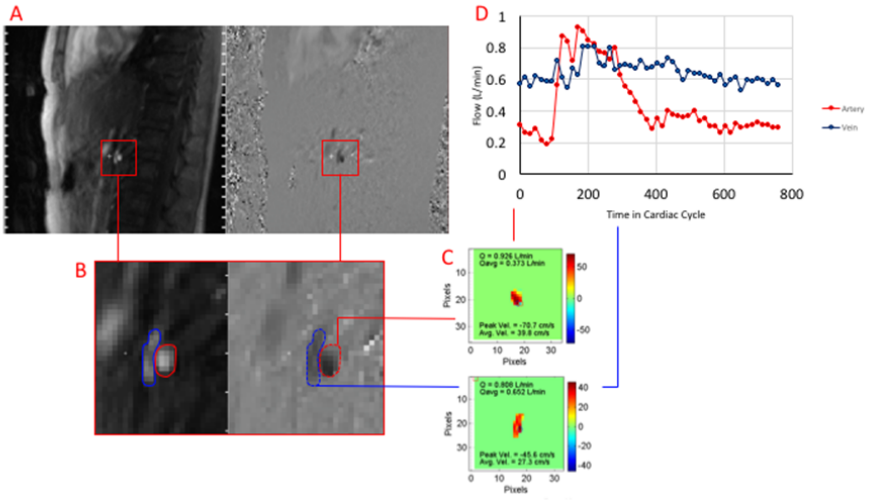


Figure 4. **A)** Sagittal oblique PC-MRI slicing through the right renal hilum of 12-year-old participant with magnitude images on the left and phase velocity encoding images on the right. **B)** Segmentation of the renal artery and vein to acquire delineated phase-velocity encoding fields. **C)** Velocity polar maps depicted at respective vessel systolic phase. **D)** Generated flow waveforms for both renal artery and vein. One can appreciate systolic pulsatile upstroke in renal artery, whereas flow through the renal vein portrays relatively steady flow profile.

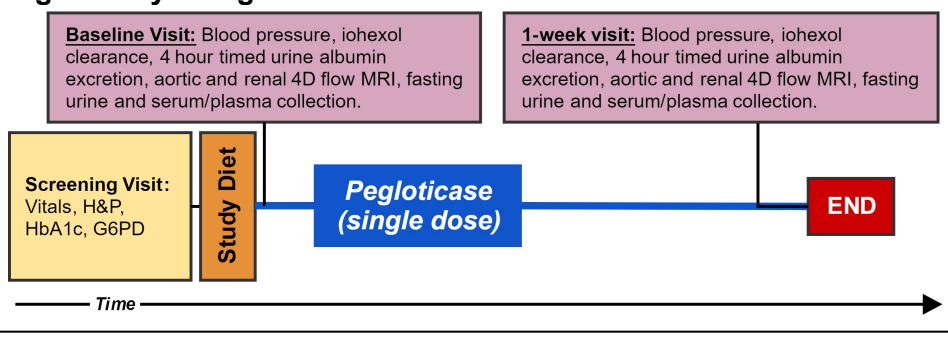
Our team is successfully quantifying these renal metrics by MRI in existing protocols (#17-7844 and #15-1309).

IV. Research Methods:

Overall Study Design for All Aims: We propose to address the study aims in an open-label non-placebo-controlled investigator-initiated pilot and feasibility trial with ten (n=10) 18-35-year-olds with youth-onset T2D (diagnosis ≤25years). Since men have higher burden of hyperuricemia, and there are important sex-specific interactions between SUA and DKD, we will limit this pilot study to men. Following a screening visit, eligible participants will be asked to refrain from strenuous physical activity for 3 days prior to admission (**Fig 5**) due to the impact of exercise on intrarenal hemodynamic function. They will also be provided with a 3-day diet instructions (55% carbohydrates,

30% fat, 15% protein and a goal of 3.45 g salt/day) to limit impacts of nutrients on cardiovascular and renal function, as previously described (57). Participants will present fasting (from midnight) to the Adult or Pediatric CTRC in the morning, where they will undergo fasting urine and blood collections, blood pressure measurements, iohexol clearance to measure GFR and aortic/cardiac MRI. These procedures will be followed by a single dose of pegloticase infusion (**Fig 5**), after which the participant will be observed for 1 hour and discharged home. The participant will return 7 days later and have all baseline measures repeated fasting (**Fig 5**).

Fig 5 Study Design



A Timeline Example of Study Procedures for the Baseline Visit	
7:30 to 08:30	
Aortic and renal MRI	Fasting
GFR by iohexol clearance	Fasting
4-hour urine collection (all urine collected over 4 hours) to quantify albumin excretion rate	Fasting
8:30-12:30	
Plasma/serum and spot urine collection	Fasting
GFR by iohexol clearance	Fasting
4-hour urine collection (all urine collected over 4 hours) to quantify albumin excretion rate	Fasting
12:30-13:00	
Lunch	Non-fasting
13:00-15:00	
Pegloticase infusion	Non-fasting

Recruitment: Ultimately 10 young men with youth-onset T2D will be enrolled (see power analysis). Recruitment will begin year 1 and will consist of patients with type 2 diabetes from the Barbara Davis Center for Childhood Diabetes (BDC), adult diabetes clinic at UCH and Type 2 Diabetes Clinic at Children's Hospital Colorado (CHCO) where principal investigator Dr. Bjornstad and co-investigator Dr. Nadeau are medical providers and have weekly clinics. Patients over the age of 18 are often still seen in the pediatric endocrine/lifestyle medicine clinics at Children's Hospital Colorado, due to existing relationships with providers. However, we anticipate that most patients will transition over their care to the adult endocrine clinics at University of Colorado hospital by the age of 25. As a result, we plan to recruit from both pediatric clinics at Children's Hospital, and adult clinics at UCH, in addition to patients receiving T2D care at the Barbara Davis Center. The investigator team has extensive experience successfully recruiting and retaining T2D patients. This recruitment strategy has been used successfully for our similarly powered individual ongoing studies.

Detailed Study Design: The study timeline is illustrated in **Fig 5**, and details from each study visit is summarized below.

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, glucose-6-phosphate dehydrogenase deficiency (GP6D) test and SUA). The screening visit will take approximately 1-2 hours.

Visit 2 (baseline visit and pegloticase infusion): Participants will fast from midnight the day prior. They will have a cardiac and renal MRI performed fasting at CHCO Radiology (where co-investigator Drs. Browne and Barker are faculty). Following the MRI scans, they will undergo fasting blood and urine collection, blood pressure measurements, and GFR assessment by iohexol clearance at Pediatric or Adult CTRC. The

participants will be provided with lunch followed by a pegloticase infusion. Total duration of visit will be approximately 8 hours.

Visit 2 (1-week visit): One week after the pegloticase infusion, participants will re-present fasting for repeat cardiac and renal MRI at CHCO Radiology, followed by fasting blood and urine collection, blood pressure measurements, GFR assessment by iohexol clearance at Pediatric or Adult CTRC. This concludes the study. Total duration of visit will be approximately 6 hours.

A. Outcome Measure(s):

Primary outcomes:

- SBP, DBP and mean arterial pressure (MAP)
- Pulse wave velocity (PWV) and wall shear stress (WSS) by aortic MRI and renal MRI (4D flow)
- Renal blood flow by 4D flow
- GFR by iohexol clearance in plasma
- Albumin excretion rate

Secondary outcomes:

- Calculated parameters of intrarenal hemodynamic function (afferent arteriolar resistance, efferent arteriolar resistance, glomerular pressure, oncotic pressure, filtration fraction and renal vascular resistance)
- Change in SUA

B. Description of Population to be Enrolled:

Inclusion Criteria	Exclusion Criteria
Youth-onset T2D (diagnosis ≤25years)	Uric acid lowering medications (e.g. allopurinol, febuxostat)*
Men	G6PD deficiency
Ages 18-35 years	Allergies to seafood or iodine
SUA ≥ 5 mg/dl	MRI contraindications (weight ≥450 lbs., non-MRI compatible implantable devices, severe claustrophobia)
	DKA or hyperglycemic hyperosmolar episode <1 month prior
	Congestive heart failure
	Pegvisomant, pegvaliase, peginterferon alfa 2b, peginterferon alfa 2a, pegfilgrastim, pegaspargase, pegaptanib, pegademase and certolizumab pegol (pegloticase will decrease concentrations of these drugs)
	A history of significant multiple and/or severe allergies or anaphylactic reactions
	Any other disease state or uncontrolled illness, which judged by the investigator, could interfere with trial participation or trial evaluation
	Participation in another investigational study within 2 weeks prior to study

*Participants already on uric acid lowering medications (e.g. allopurinol, febuxostat) will be allowed to complete a 2 week wash out period if they wish to participate in this study.

Study Design and Research Methods

Aim 1-2 – Specific Methods:

Diet instructions: The diet instructions will be composed of 55% carbohydrates, 30% fat, 15% protein, goal of 3.45g of salt/day and will have no caffeinated drinks (as in our previous studies) (58, 59)), as diet, activity (60) and circadian rhythms affect IR and renal function (58, 59).

Aim 1 - Specific Methods (Table 2):

Table 2 Aim 1 Primary Outcomes	Measurement Tool
Blood Pressure	Sphygmomanometer
Aortic stiffness (PWV)	Aortic MRI
Wall Shear Stress (WSS_{MAX} and WSS_{TA})	Aortic MRI
Secondary Outcomes	**Measurement Tool**
Aortic Distensibility (RAC)	Aortic MRI
Cardiac Structure	Left ventricular mass
Cardiac Function	Ejection fraction

1.a Blood Pressure Measurements: SBP and DBP will be measured after 5 minutes rest, in the sitting position with a Sphygmomanometer. MAP will be calculated as 2/3 DBP + 1/3 SBP.

1.b Aortic and Cardiac MRI: Cardiovascular MRI evaluation will occur with a 1.5T or 3.0T magnet system (Philips, Best, Netherlands) using a 32-channel coil. A free-breathing phase contrast sequence will be applied with Cartesian encoding and retrospective sorting (TR: 14–28 ms/30 to 50 phases, TE: 2.2–3.5 ms, matrix: 160 × 256, flip angle: 25°) with 100% k-space sampling and no further temporal interpolation. Depending on participant size and field of view (128-225 × 210-360 mm), the cross-sectional pixel resolution will range from ~ 0.82 × 0.82–1.56 ×1.56 mm^2 with a slice thickness of 5 mm. Phase contrast acquisition time for each plane varies per heart rate, between 2-3 min. Velocity-encoding values are adjusted according to the maximum velocities encountered during scout sequences to avoid aliasing artifact (typically 100-250 cm/s). Raw images are transferred to an off-line processing system, MatLab (Mathworks, Inc., Natick, MA), to determine arterial stiffness by pulse wave velocity (PWV), aortic distensibility by relative area change (RAC) and aortic wall shear stress (WSS). A gradient-echo ECG-gated sequence is applied to obtain tissue intensity and phase-velocity maps. A single plane of acquisition for flow hemodynamic evaluation is placed in an orthogonal fashion in the ascending aorta at approximately one centimeter above the sino-tubular junction, corresponding to the descending aorta on the identical plane 3-5 cm below the origin of the left subclavian. Using flow-area (dQ/dA) method, flow and area waveforms are generated from time-frame-segmented respective phase and magnitude images (44, 61). Flow-area diagrams asses regional PWV by computing dQ/dA slope from early systolic data points. Aortic distensibility is measured using the RAC ([maximum area-minimum area]/maximum area) × 100%). WSS is calculated from segmented phase and magnitude images as described previously using an 8-point model to calculate through-plane WSS from the shear curve, yielding maximum systolic WSS (WSS_{MAX}) and time-averaged WSS (WSS_{TA}). We will also obtain left ventricular mass and ejection fraction per standard cardiac MRI methods.

The assessment of intracardiac and vascular flow will be assessed using 4D MRI with complete volumetric coverage of the ventricles and aorta, extending from the transverse arch to the abdominal aorta. The sequence is performed during free breathing and will use the diaphragm movement and EKG to determine timing of image acquisition (respiratory navigation and prospective EKG gating). Velocity encoding sensitivity will be adjusted to eliminate aliasing (appx. < 150 cm/s); that is, we will adjust the velocity scale based on the estimated vascular velocity to avoid artifacts from the presence of higher velocity. Typical sequence parameters are estimated to be: flip angle 14-15degrees, SR: isotropic 1.5 – 2.0mm, TR: 36-42 ms, with time of acquisition 6 to 10 minutes depending on patient size, heart rate and navigator efficiency.

Raw 4D-Flow CMR data sets are imported for initial processing into Matlab (Mathworks) for standard noise filtering, background phase-offset corrections, and conversion into visualization and quantification mode software (Paraview, Kitware). Defined anatomical landmarks will be segmented from reconstructed PC images as shown previously using freely available software (ITK Snap, Kitware). Specific flow volumetric, spatially quantitative metrics, quantitative (Qp:Qs) and qualitative control (presence of aliasing and noise) will be performed within the segmentation delineated region. Vascular contours required for anatomical segmentation will be created by conversion of the 4D-flow MRI magnitude and phase specific images into the 3D phase contrast MRI domain as shown previously (62). Manual segmentation of the vascular compartments will be done from constructed phase-contrast MRI using freely available software (ITK_Snap, Kitware, Clifton, NY). The quantification of vorticity (ω) and helicity ($\mathcal{H}$ will be then conducted within the segmented vascular segments. Co-Investigators Drs. Browne, Schafer and Barker are expert in the proposed methods.

Aim 2 - Specific Methods:

We will determine the impact of SUA lowering by pegloticase on GFR, RPF and AER (primary outcomes) and calculated parameters of intrarenal hemodynamics (**Table 3**).

2.a GFR by iohexol: To control for the effect of acute glycemia on intrarenal hemodynamics, we will measure iohexol clearance fasting. A bolus of iohexol (5mL of 300 mg/ml [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 our local protocol (63, 64). Blood for iohexol clearance will be drawn at +120, +150, +180, +210, +240 min (63, 64) per our other protocols (#16-1752; #17-820; #18-0704)

Table 3 – Aim 2

Primary Outcomes	Measurement Tool
Measured glomerular filtration rate (GFR)	Iohexol clearance
Renal plasma flow (RPF)	Renal MRI
PWV and WSS	Renal MRI
Secondary Outcomes	**Measurement Tool**
Intrarenal hemodynamic parameters (see **Table 3**)	Gomez' equations
Albumin excretion	Urine AER

The iohexol period last 240 minutes. 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 clearance on a HPLC platform.

2.b Intrarenal hemodynamic parameters: We will use Gomez' equations to calculate parameters of intrarenal hemodynamics (65, 66) (**Table 4**).

Table 4 Intrarenal hemodynamics

Variables	Definition:
Filtration fraction (FF)	FF = GFR / RPF
Renal blood flow (RBF)	RBF = RPF (1-Hct)
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)	$\pi_G = 5 \times (C_M - 2)$
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

2.c Hyperfiltration: Hyperfiltration will be defined as measured GFR ≥ 135mL/min/1.73m^2 based on physiological data (42, 43).

2.d Albumin excretion rate (AER): Urine albumin and creatinine for AER will be collected over 4 hours to determine urine AER.

2.e Renal MRI: Renal vessel flow waveforms will be analyzed from time-frame segmented respective magnitude and phase-contrast images as previously described (Matlab Program; Mathworks, Inc., Natick, MA, USA). The MRI processing involves routinely applied software segmentation with identical post-processing algorithm. Specifically, each temporal phase will be segmented using semi-automatic level-set method (Segment, Medviso), where constructed algorithm applies active contour model which tracks the edges of the vessel wall initialized by a manual delineation in one time-frame. The software derived parameters will include 1) standard flow hemodynamic metrics (maximum flow, average flow, renal stroke volume, peak and average blood velocity, regurgitation fraction) and 2) advanced shear hemodynamic metrics (maximum systolic shear stress, time-averaged shear stress, oscillatory shear). All metrics will be collected in automated platform in graphical and tabular formatted. To avoid analytical bias, the MRI analysis will be conducted in blinded fashion, carrying only the label of the study with anonymized patient information.

All acquired MRI scans will be reviewed by an attending radiologist during scan acquisition and post data processing for potential confounding pathologies and reported in standard clinical fashion as per radiology protocol.

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 3 visits (~ 16 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 5: Data to be collected during study

Blood and Urine Samples	Blood pressures and HR
Questionnaires	Cardiac and renal MRI
Anthropometric measurements	Iohexol clearance

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. The explanation might also be conducted over approved tele-health telephone and/or video conference, should this option work best. 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. The participant will be provided a copy of the consent form for better understanding and record purposes. If telephone and/or video conference option is selected, the participant will have the option to receive the consent form by postal mail or electronic mail, print the document, sign, and send back to the study personnel via postal mail or electronic mail. The subject will receive the consent form prior to the discussion in order to follow along. An unaffiliated witness will be included in the process if tele-health telephone or video conferencing is used.

5. **Consent Plan:** Consent will be obtained from all participants in the study. Subjects below 18 years of age will not be eligible to participate. All subjects age 18 or older will sign the standard consent form.

6. **Participant Compensation:** Subjects will be paid $400 in greenphire or checks upon completion of all procedures in this study (**Table 6 below**). If the subject withdraws before the start of visit 2, they will still receive $25 completion of the screening visit. These payments are similar to the payments being made for each visit type in our currently ongoing adolescent studies.

Table 6. Subject payment schedule

Visit 1	Visit 2	Visit 3	Total
$25.00	*$175.00*	*$200.00*	**$400.00**

7. **Potential Risks to Subjects:**

Pegloticase Infusion: Pegloticase is a recombinant DNA-produced form of the porcine enzyme uricase that metabolizes urate to allantoin, an inert and water-soluble purine metabolite that, unlike uric acid, is rapidly cleared by the kidney. Infusions of pegloticase decrease SUA concentrations and can alleviate the symptoms and complications of hyperuricemia and gout. Pegloticase was approved in the United States in 2010 for use in patients with chronic symptomatic gout. It is available as a sterile concentrate of 8 mg in a single use vial. The recommended dose 8 mg given intravenously every two weeks. Common side effects of pegloticase include local and systemic infusion reactions, nausea, vomiting, constipation, chest pain and nasopharyngitis. Pegloticase infusions are associated with paradoxical flares of clinical gout during the first 3 months of therapy in up to 80% of patients. Severe adverse reactions may include anaphylaxis (in up to 7% of patients), severe infusion reactions, hemolysis and methemoglobinemia (in patients with G6PD deficiency) and congestive heart failure. Due to its immunogenicity, neutralizing antibodies against pegloticase form in some people, which is associated with loss of efficacy. These antibodies typically form 2 weeks after the administration.

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. The screening visit (visit 1) will include 6 ml or less (HbA1c, G6PD and SUA). The baseline visit (visit 2) and follow-up visit (visit 3) will include a total of 50mL of blood, which contains 18-20mL for the iohexol studies, 10mL for fasting labs and 20mL for biobanking.

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 nurses help to minimize these risks.

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 several current (#16-1752, #17-0820 and #18-0704) and past protocols (63) using iohexol clearance to calculate GFR Furthermore, collaborator Drs. Cherney and Nelson have at least 20-year experience with iohexol studies in both pediatrics and adults with and without 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.

Recommended 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 call study team or their diabetes provider with high or low blood sugars.

Magnetic Resonance Imaging (MRI): The MRI is a non-invasive scan of the heart and kidneys. There are no contrast agents used for the MRI modalities. 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.

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.

8. Plan to minimize risk / protection against risk:

Pegloticase infusion: Participants with congestive heart failure or G6PD will be excluded as they have higher risk of adverse reactions. Only men will be enrolled based on sex-specific interactions observed between SUA and DKD in preliminary studies, and pegloticase has pregnancy category C.

Pegloticase 8 mg IV in 250 mL 0.9% normal saline will be infused slowly over ≥ 120 minutes. In the event of an infusion reaction, the infusion will be slowed, or stopped and restarted at a slower rate. Patients will be pre-treated with diphenhydramine 25mg PO, acetaminophen 1000mg PO and hydrocortisone 200 mg IV. As steroids can increase blood sugar in patients, we will monitor blood glucose levels in participants prior to administration of hydrocortisone, as well as after the pegloticase infusion. The half-life of hydrocortisone is short (approx. 1.5 hours), so long-term effects on blood glucose levels should be minimal. However, patients will be asked to monitor their blood glucose levels once they return home as well, and will be instructed to contact the investigative team if high blood glucose levels persist.
The participant will also be monitored with vital sign checks 1 hour after completion of the infusion. Delayed type hypersensitivity has also been reported, and therefore the participants will be instructed to monitor for such symptoms at home, treat with over the counter Benadryl (diphenhydramine) and contact study team. Immunogenicity to pegloticase is established to manifest after 1-2 months of therapy in most patients (and rarely before 2 weeks), and therefore we will likely not encounter loss of efficacy issues related to immunogenicity with a single dose of pegloticase (67).

We have defined the following as AEs of special interest for the purposes of monitoring: any infusion reactions related to the pegloticase infusion. The PI and the investigative team will report 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. *We will submit an IND application to FDA to use pegloticase for this study (seeing as its current indications are symptomatic hyperuricemia and gout). The study will not start without permission from FDA to use pegloticase. The investigative team is experienced with the IND process, and hold active IND(s).*

IV placement, iohexol infusion and blood draws: The IV placements, blood draws and iohexol infusion are standard procedures used in several research studies and settings. Adverse events are uncommon when the procedures are 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 CTRC with iohexol 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 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: The investigator team has successfully recruited youth-onset T2D for multiple previous studies with more complex study designs including the NIDDK TODAY, RISE, IMPROVE-T2D and Renal-HEIR studies (59, 68-74) and we have over 300 youth-onset T2D patients from the Barbara Davis Center for Diabetes (BDC) and Children's Hospital Colorado as well as several established T2D cohorts to draw from. The study will be completed in just three visits (including the screening visit), limiting concerns for drop-out and retention. Drs. Bjornstad, Nadeau, Browne, Schafer and Barker have significant experience with advanced MR

imaging (41, 75-82). Drs. Bjornstad, Johnson and Cherney have extensive experience measuring GFR (by inulin and iohexol) (63, 66, 83-88). Finally, the principal investigator is the co-leader of the Renal Working Group of the TODAY study, and is skilled in DKD analyses, interpretation of renal physiology measures and statistics. Thus, we have the necessary expertise to perform the proposed study. Our proposal is based on strong preliminary data and a track record of successful collaborations and publications (12, 66, 68, 69, 83, 88-100).

Potential scientific problems: In any clinical study, unforeseen human factors provide challenges. We have experience with iohexol 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:

Aims 1 and 2 - Expected outcomes and limitations:
Based on our preliminary, we expect the SUA lowering following a single infusion of pegloticase to decrease SBP, DBP and MAP, in addition to aortic PWV and WSS (**Aim 1**). Furthermore, we anticipate a decrease in elevated GFR (attenuation of hyperfiltration), decrease in RPF and AER in response to pegloticase (**Aim 2**). Limitations of our pilot and feasibility trial includes the small sample size. The strict eligibility criteria may also narrow the applicability of our findings. Strengths of our study include gold-standard assessment of GFR, state-of-the-art MRI methods to quantify RPF, PWV and WSS. A major impediment in early DKD research is the reliance on eGFR to assess GFR in younger people, in whom eGFR is ineffective to ascertain early GFR change. While youth with T2D are a challenging population to recruit for translational studies, the investigator team has successfully recruited and enrolled participants with youth-onset T2D for RESISTANT, TODAY and RISE studies. We also have extensive experience with the MRI methods, and our research MRI is equipped with in-MRI movie-viewing with noise-cancelling headphones and thus is very tolerable. 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.

F. Potential Benefits:

Evidence of Direct Benefit to Subjects: This pilot and feasibility trial is designed to evaluate the effect of SUA lowering by pegloticase on markers of CVD and DKD in adult men with youth-onset T2D. This trial will allow us to determine whether aggressive SUA lowering can impede the development of cardio-renal disease to reduce future risk of cardiovascular morbidity and mortality. Since the trial only includes a single dose of pegloticase, participants' SUA levels will return to pre-study concentrations a couple of weeks after the conclusion of the study. Accordingly, participants are unlikely to gain a long-term medical benefit from participating.

Information gained by conducting this study is expected to yield novel, important findings regarding the relationship between SUA and the mechanisms underlying early DKD in youth-onset T2D. Participants will also benefit from being in close contact with study staff and learning more about their renal and cardiac 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 and CVD are very prevalent in youth-onset T2D 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. Youth-onset T2D carries a particularly high risk of DKD and CVD, significantly higher than adult-onset T2D and several-folds higher than youth with T1D. In the United States, almost half of patients with renal failure have DKD, and most of those (≥80%) have T2D. Despite the high prevalence and gravity of DKD in youth onset T2D, widely effective therapeutic options are lacking. The findings of this study will also apply to type 1 diabetes (T1D) and obesity, who like their T2D counterparts also demonstrate strong relationships between SUA, DKD and CVD, in addition to adult-onset T2D. Although data to guide care of DKD in adult-onset T2D 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. Our preliminary studies suggest that youth-onset with T2D are at uniquely high risk of DKD

and CVD. In our novel and innovative proposal, we postulate that SUA lowering with pegloticase can reduce early markers of CVD and DKD in adults with youth-onset T2D.

A better understanding of the pathophysiology underlying early DKD, including hyperfiltration, and its strong relationship with insulin resistance 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 insulin resistance in early DKD development in youth-onset T2D. The investigator team is also unique in being one the few groups skilled in performing both gold standard direct measures of GFR in addition to advanced MRI evaluation of the cardiac and renal system in youth-onset T2D.

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. We will consent up to 14 to allow for screen failures. Power calculations are based on paired *t*-tests. A sample size of 10 achieves ≥ 99% to detect a mean paired difference of 4.80 mg/dl with a standard deviation (SD) of 0.62 mg/dl for SUA. The sample size also provides 80% power to detect a mean paired difference of 4.92 mm Hg and 3.58 mm Hg for SBP and DBP, respectively. The anticipated effect size for the MRI vascular hemodynamic parameters are listed in the table below, assuming a correlation between pre and post-intervention measures of 0.9:

MRI-derived vascular markers	Effect size for N=10
AA WSS	1.03 dyne/cm^2
DA WSS	1.79 dyne /cm^2
AA PWV	0.58 m/s
DA PWV	0.72 m/s

We will determine if the following potential confounders impact treatment response: ethnicity, baseline SUA and change in SUA and baseline GFR and change in GFR. We will use paired *t*-test, in addition to multivariable linear models of the change in each outcome, adjusted for potential confounders.

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 acute effects of severe SUA lowering on markers of CVD and DKD in young adults with youth-onset T2D. Carefully designed human studies, like the one proposed in

this application, are needed to advance our understanding of the pathophysiology driving early DKD and CVD, and direct the development of new therapeutic strategies to impede the development of DKD. Finally, the findings of this project could also inform other conditions associated with elevated SUA (insulin resistance, metabolic syndrome, fatty liver disease and obesity). The results and techniques from this project will be used to direct future clinical trials.

I. Adverse Events and Serious Adverse Events:

Safety Reporting

The Sponsor and Sponsor-Investigator shall be solely responsible for ensuring the safety of each subject enrolled in the study, according to best international standards, and must keep the oversight on the safety within the respective trial.

The Sponsor shall carry out, and shall be solely responsible for all sponsors' obligations regarding the compliance with all safety reporting requirements in the country(ies)/regions where the Study is conducted.

a. Definition of Roles:

- **Sponsor: Petter Bjornstad, M.D.**
- **Site Principal Investigator: Petter Bjornstad, M.D.**
- **Licensed Independent Practitioner (LIP): Petter Bjornstad, M.D.**

b. Definition of Adverse Events (AE)

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related (21 CFR 312.32 (a))

c. Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the Site Principal Investigator or Sponsor, it results in any of the following outcomes:

- death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

d. Classification of an Adverse Event

i. Severity of Event:

A study team member, who is a Licensed Independent Provider (LIP) will be responsible for determining the severity of each AE.

For adverse events (AEs), the following guidelines will be used to describe severity:

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".]

ii. Relationship to Study Intervention:

A study team member, who is a Licensed Independent Provider (LIP) will be responsible for determining the relationship of each AE to the study intervention.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", or downgraded to …, as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the

iii. Expectedness:

A study team member, who is a Licensed Independent Provider (LIP) will be responsible for determining whether an AE is expected or unexpected.

An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

e. Time Period and Frequency for Event Assessment and Follow Up

21 CFR 312.64(b) requires that the Site Principal Investigator records non-serious adverse events and reports them to the Sponsor according to the timetable for reporting specified in the protocol.

A properly delegated study team member will record all adverse events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study intervention. All events will be followed for outcome information until resolution or stabilization.

All AEs occurring while on study will be documented regardless of their relationship to the study intervention, and whether or not they meet the definition of an SAE. AEs will be captured, reviewed and assessed by an LIP member of the study team. The outcome of this review and assessment will be documented by a properly delegated study team member on an Adverse Event Log. Information to be collected includes event

description, time of onset (start date) and time of resolution/stabilization of the event (end date), and the LIP's assessment of expectedness, severity and relationship to study intervention.

Any medical condition that is present at the time the participant is screened will be considered as baseline medical history, and will not be recorded as an AE. However, if the study participant's condition deteriorates at any time during the study, the event will be recorded as an AE.

Changes in the severity or frequency of an AE will be documented as separate events to allow an assessment of the duration of the event at each level of severity or frequency.

AEs characterized as intermittent will be documented with onset and duration of each episode.

At each study visit, a properly delegated study team member will document all occurrences of AEs and SAEs since the last visit. This information will be gathered through direct observation (e.g. rash), elicited from or spontaneously volunteered by the participant, documentation in Medical Records, laboratory or other clinical reports or notes, or identified in patient-reported outcome tools (e.g. diaries, surveys, questionnaires), or any other means.

f. Adverse Event Reporting

Adverse Event Reporting will be performed in accordance with the study protocol, any contractual agreement, and all IRB requirements.

21 CFR 312.64(b) requires that the Site Principal Investigator records non-serious adverse events and reports them to the Sponsor according to the timetable for reporting specified below.

Disease-Related Events (DREs):

The following disease-related events (DREs) are expected and common in the study population, and will not be reported per the standard process for reporting.

Hyperglycemia – we anticipate that our participants with type 2 diabetes will be hyperglycemic and will therefore not report this disease-related event.

Obesity – we anticipate that our participants with type 2 diabetes will be obese and will therefore not report this disease-related event.

These events will still be recorded and monitored in a separate log.

AEs will be reported as follows (check the arrangement to be used for this protocol):

i. To Safety Monitoring entity:

☒ Study Principal Investigator is an LIP and provides safety oversight

- Review of AEs and SAEs will be conducted in regularly occurring research team meetings that at scheduled at pre-determined frequency. The review and any follow-up action will be documented in meeting minutes.

ii. To IRB:

Within 5 days of becoming aware of them, a properly delegated study team member will report to the IRB any AE that meets the definition of an unanticipated problem (UAP), which means the event is are unexpected, related to the research procedures, and caused harm or risk of harm.

SAEs that do not meet the criteria for prompt reporting will be tabulated on an aggregate reporting form for submission at the time of continuing review.

> **Note**: If there is an independent safety monitor for the study (e.g., Safety Officer, DMC, or DSMB), aggregate reports do not need to be submitted at the time of continuing review. Instead, a summary report from the monitor should be submitted at the time of continuing review.

iii. To FDA:

A narrative or tabular summary showing the most frequent and most serious adverse events by body system will be provided by the Sponsor to the FDA with each annual report in compliance with 21 CFR 312.33(b)(1).

g. Serious Adverse Event Reporting

The Site Principal Investigator will immediately report to the Sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure, and will include the study team LIP's assessment of whether there is a reasonable possibility that the study intervention caused the event.

Study endpoints that are serious adverse events (e.g. all-cause mortality) will be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the Site Principal Investigator must immediately report the event to the Sponsor.

All SAEs will be followed until satisfactory resolution or until the study team's LIP deems the event to be chronic or the participant is stable.

Serious adverse events that are considered related (i.e., determined to be possibly, probably, or definitely related) to the investigational product by the Sponsor will be followed until the event resolves or stabilizes. Any SAE that occurs after treatment completion, and is considered by the Sponsor to be related to the investigational product, will be documented and reported as appropriate.

i. To FDA:

The Sponsor is responsible for notifying the Food and Drug Administration (FDA). Events meeting the following criteria will be submitted to the FDA as expedited IND Safety Reports according to the following guidance and timelines:

7 Calendar Day Telephone or Fax Report

The Sponsor will notify the appropriate review division at the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the Sponsor to be possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the investigational product's Investigator Brochure. Such reports are to be telephoned or faxed to the appropriate review division at FDA and to the manufacturer of the investigational product within 7 calendar days of first learning of the event.

The report will be completed using Form FDA 3500A (Mandatory Reporting).

15 Calendar Day Written Report

The Sponsor will notify the appropriate review division at the FDA, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the investigational product's investigator brochure.

Written IND Safety reports will include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed by the Sponsor with the IND concerning similar events will be analyzed and the significance of the new report in light of the previous, similar reports commented on.

Written IND safety reports with Analysis of Similar Events will be submitted to the appropriate review division at the FDA, and the manufacturer of the investigational product within 15 calendar days of first learning of the event, using Form FDA 3500A.

Written IND Safety Reports will be submitted to the IRB(s) of record in accordance with IRB Guidelines.

IND Annual Reports
The Sponsor will provide annual reports to the appropriate review division at the FDA within 60 days of the IND's anniversary date, until the IND is withdrawn or terminated.

i. Horizon Pharma:

1) The sponsor agrees to provide the following safety information to Horizon:
   a. A copy of any Serious Adverse Event (SAE) report and any follow up information until resolved or attained a stable outcome for an SAE within two working days (24 hours if fatal or life-threating) after becoming aware of the SAE/SAE follow up information. The SAE report shall include at a minimum:
      a. Study number,
      b. Subject number, sex and gender,
      c. The date of report,
      d. The study drug and any co-suspect drug(s),
      e. A description of the SAE (event, seriousness criteria),
      f. Causal relationship to the study drug and co-suspect drug(s)
   b. A complete copy of all submission documents for any adverse event that have been submitted to regulatory authorities within 24 hours after submission to the authorities
   c. Information on any pregnancy and its outcome occurring in a subject participating in the study within two working days after becoming aware of the information.

2) The Sponsor and Sponsor–Investigator is responsible for the safety monitoring of the study and agrees to immediately inform Horizon when new safety information is identified, which may alter the benefit risk balance of the study medication.

All safety communication to Horizon including the provision of SAEs shall be sent to:

Horizon Pharma, Inc.
Pharmacovigilance
Email: AdverseEvents@horizonpharma.com
Fax: 1-800-860-7836

ii. To Safety Monitoring entity:

SAEs will be reported to the safety monitoring entity at the next scheduled research team meeting or as defined in the Safety Monitoring Plan, which ever applies to this study.

2. Unanticipated Problems

a. Definition of Unanticipated Problems (UAP)

An unanticipated problem involving risk to participants or others includes any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

b. Unanticipated Problem Reporting

The Site Principal Investigator will report unanticipated problems (UAPs) to the reviewing Institutional Review Board (IRB) per IRB policy.

c. Reporting Unanticipated Problems to Participants

Any event which may relate to subjects' willingness to continue participation will be provided to the subjects, per IRB policy.

J. References:

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