Figure S1. Pre- and Post-Treatment Changes in Absolute GFR **(A)** and BSA-Standardized GFR **(B)**

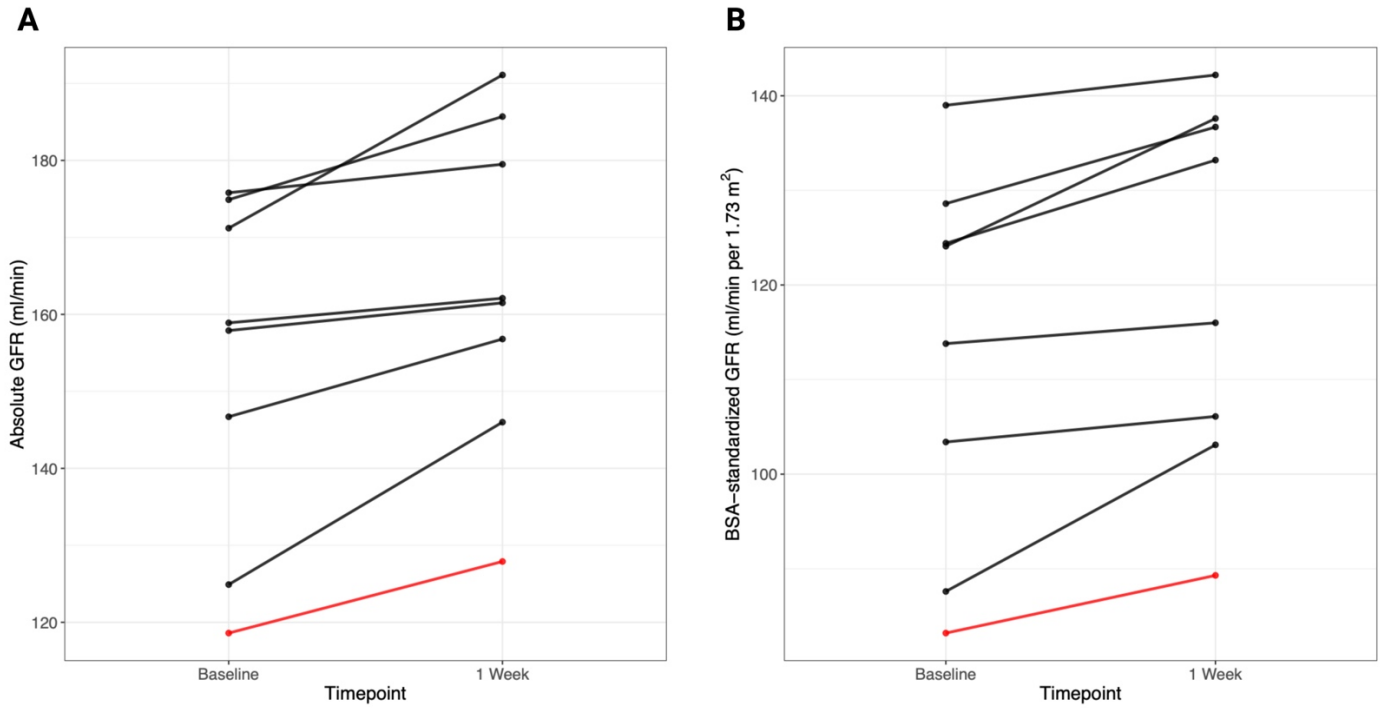


Figure S2. Schematic of cardiac MRI analyses. A. **(A)** LV endo- and epi-cardial contours and RV endo-cardial contour were semi-automatically drawn, and 3D volumetric information was reconstructed. Global function pre- and post-treatment were subsequently calculated from the results of end-systole and end-diastole. **(B)** The magnitude of peak strain was also obtained using time-resolved strain curves. Color shadows indicate the minimum and maximum values of each strain within the overall group.

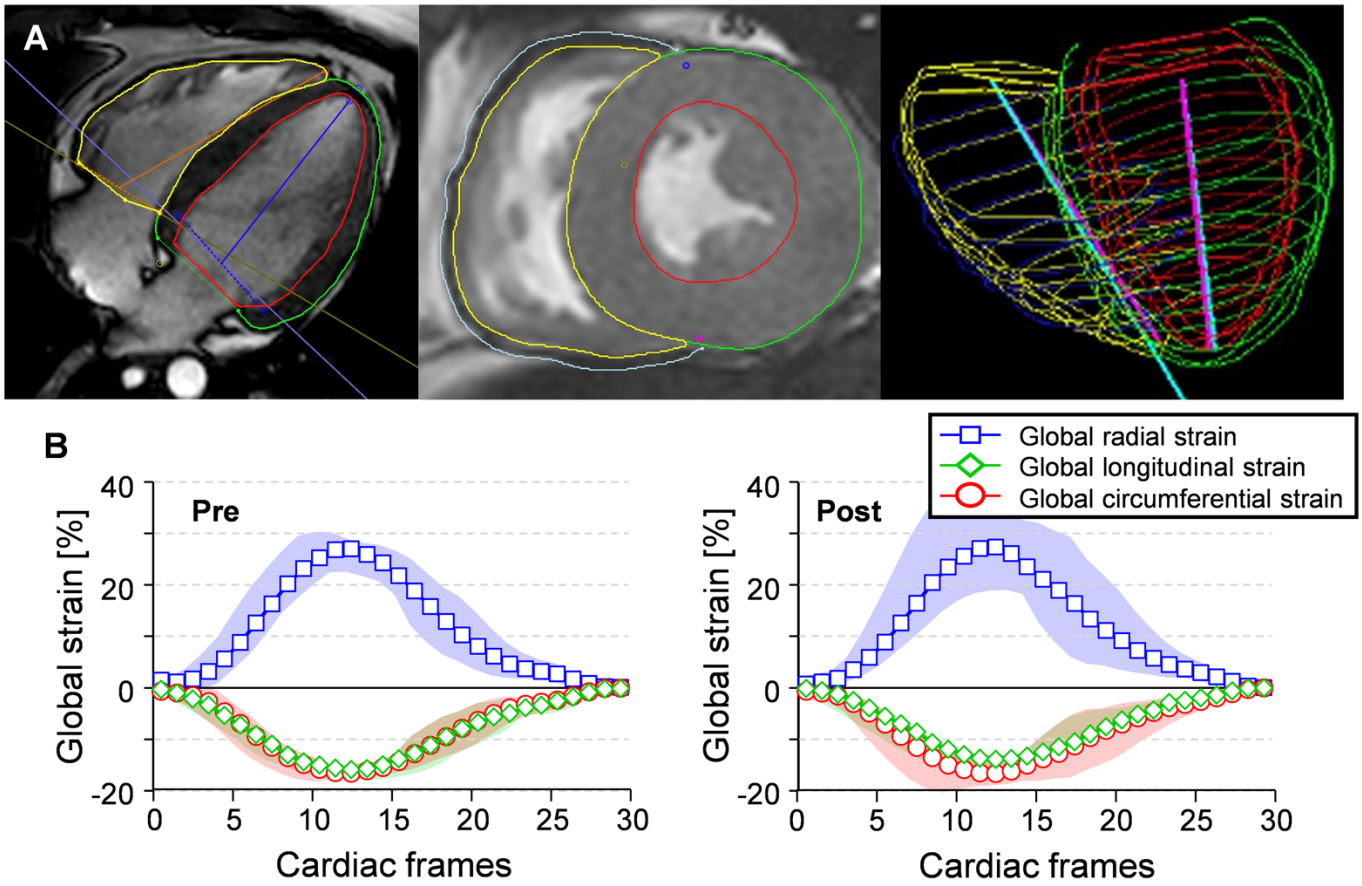


Figure S3. Schematic of kidney MRI analyses. **(A)** Contours were manually drawn based on the time-resolved phase and magnitude images. **(B)** Total flow volumes were extracted from the left renal vein and artery, and the right renal vein and artery. **(C)** An example of the time-resolved flow rate from the left renal vein.

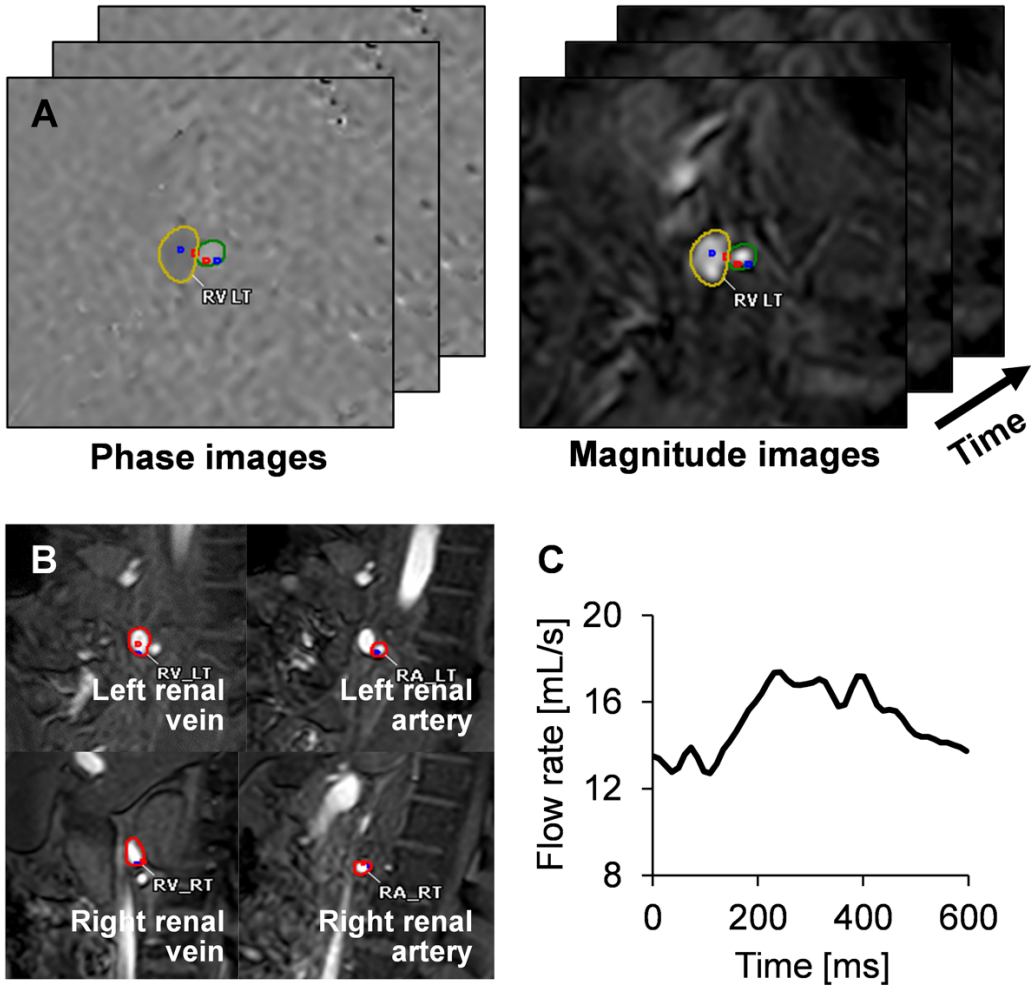
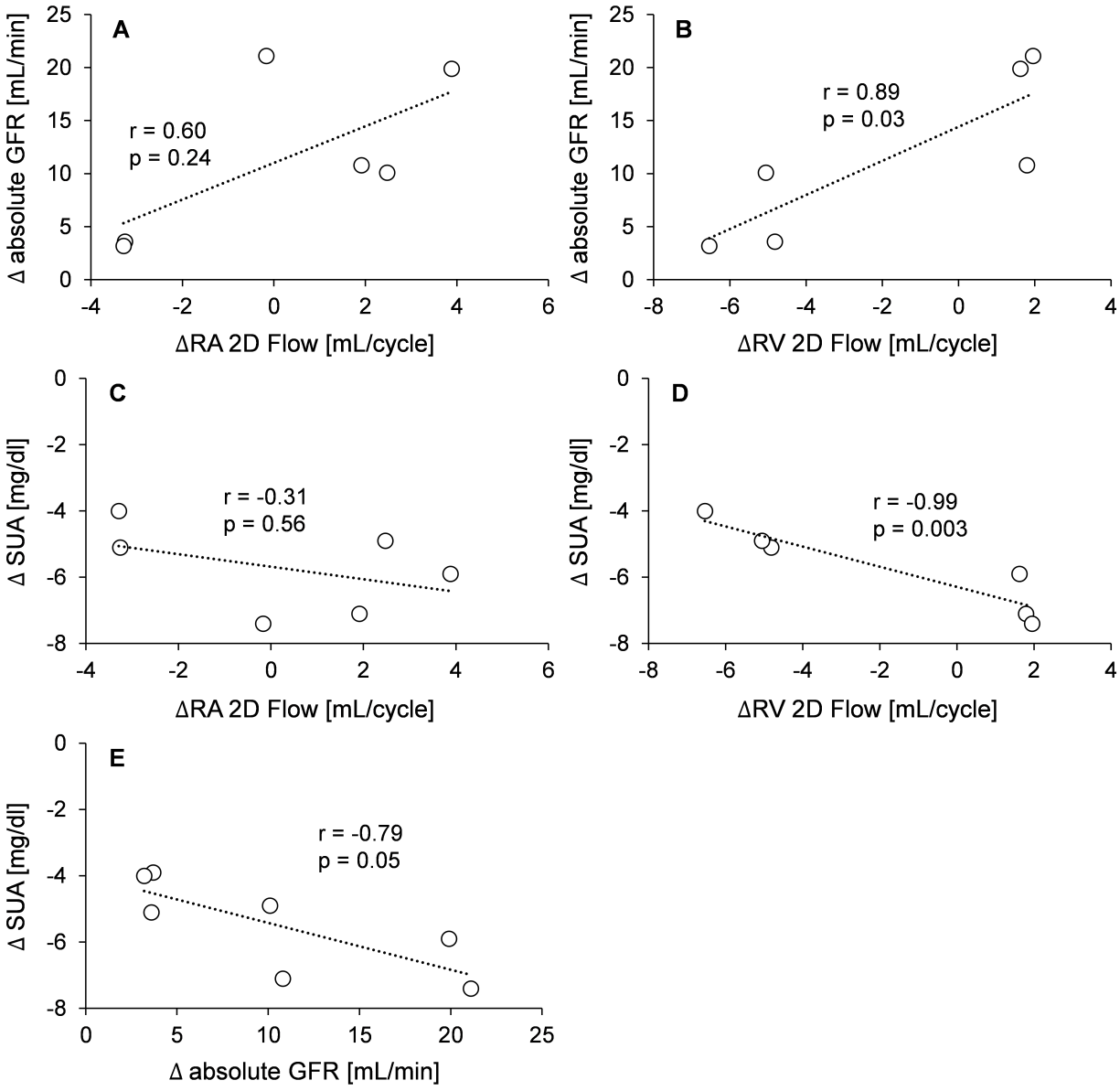


Figure S4. Correlation between **(A)** changes in renal artery blood flow obtained from 2D PC-MRI flow measurement and changes in absolute GFR **(B)** changes in renal vein blood flow obtained from 2D PC-MRI flow measurement and changes in absolute GFR **(C)** changes in renal artery blood flow obtained from 2D PC-MRI flow measurement and changes in SUA **(D)** changes in renal vein blood flow obtained from 2D PC-MRI flow measurement and changes in SUA **(E)** changes in SUA and changes in absolute GFR, in participants with a reduction in SUA of at least 3 mg/dL after pegloticase infusion. *Note:* One post-infusion 2D PC kidney MRI was excluded from analysis due to the presence of image artifact affecting the reliability of the measurement, resulting in fewer than 7 scatter points being displayed in panels A-D.



Item S1: Methods

Trial design

We conducted an open-label, non-placebo-controlled, investigator-initiated pilot and feasibility trial, recruiting young adult men from diabetes clinics at the University of Colorado Hospital (UCH) and Children's Hospital Colorado (CHCO). The protocol was registered with ClinicalTrials.gov (NCT03899883). This trial was approved by the Colorado Multiple Institutional Review Board. All participants provided written informed consent.

Participants

We limited enrollment to adult men, since they have higher burden of hyperuricemia, and because we previously found in TODAY that SUA was predictive of elevated urinary albumin excretion in males, not females.[1] The inclusion criteria were male adults aged 18 to 35 with T2D diagnosed before the age of 25, along with documented SUA levels ≥5 mg/dL. This criterion was selected based on its similar use in the PERL study, and the average SUA observed in young persons with T2D in TODAY.[1,2] Additionally, we aimed to include participants whose characteristics are generalizable to most youth with T2D. By setting this threshold, we ensured that our study cohort would be representative of a broader population, thus enhancing the applicability and relevance of our findings. Participants currently using other uric acid-lowering medications, such as allopurinol or febuxostat, were not eligible for the study. Additional key exclusion criteria included individuals with documented glucose-6-phosphate dehydrogenase (G6PD) deficiency, recent occurrences of diabetic ketoacidosis, or hyperglycemic hyperosmolar episodes within one month prior to enrollment. The full inclusion and exclusion criteria are listed in the **Table S1.**

Trial procedures

Eligible participants were asked to refrain from strenuous physical activity for 3 days prior to admission due to the impact of acute bouts of exercise on cardiac and kidney hemodynamic function. They were also instructed to follow a 3-day fixed diet (55% carbohydrates, 30% fat, 15% protein, goal of 3.45 g salt/day, with no caffeinated drinks) to limit impacts of nutrients on cardiovascular and kidney function.[3] Participants presented fasting (from midnight) to the University of Colorado Clinical and Translational Research Center (CTRC) in the morning, where they underwent fasting urine and blood collections, blood pressure measurements, iohexol clearance to directly measure GFR, and aortic, cardiac and kidney magnetic resonance imaging (MRI). These procedures were followed by a single dose intravenous pegloticase infusion (8 mg), after which the participants were observed for at least 1-hour post-infusion and discharged home. The participants returned 7 days later under the same conditions as the first visit and had all baseline measures repeated while fasting.

All laboratory assays were performed by University of Colorado CTRC.

Blood pressure measurement

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after 5 minutes rest, in the sitting position with an automatic sphygmomanometer. Mean arterial pressure (MAP) was calculated as 2/3 DBP + 1/3 SBP.

GFR by iohexol

An intravenous bolus of iohexol (5mL of 300 mg/mL [Omnipaque 300, GE Healthcare]) was administered slowly over 2 min followed by a 10 ml normal saline flush, which was time 0 for the iohexol clearance. Blood for iohexol clearance was drawn at +10, +30, +120, +150, +180, +210, +240 min.[4,5] Because the Brøchner-Mortensen equation underestimates high values of GFR , expected to be common in young participants with T2D, the Jødal-Brøchner-Mortensen (JBM) equation was used to calculate the GFR.[6] Our study did not use glucose clamping during the iohexol GFR measurements, which could result in variability in GFR estimates due to fluctuations in blood glucose levels.

Cardiac MRI and kidney MRI

Cardiac MRI and kidney MRI were acquired prior to pegloticase infusion and one week after the infusion using a 3T Philips Ingenia MRI system (Philips Healthcare, Best, Netherlands). For cardiac MRI, balanced steady-state free precession cine sequences of short-axis and 4-chamber views were performed with ECG gating using a 32-channel torso coil. The cardiac MRI parameters were as follows: repetition time (TR)/echo time (TE) = 2.6–3.2 ms/1.3–1.6 ms, acquisition matrix = 151–196×134–195×3–13, flip angle = 25–45°, voxel size = 0.9–1.3×0.9–1.3×8–10 mm^3, temporal resolution = 20–32 ms. Global function and peak values of magnitude of strain changes were analyzed using the short-axis and 4-chamber views using Circle CVI42 (version 5.9.3, Calgary, Canada) to compare the effect of pegloticase on cardiac structural and functional characteristics. Out of 9 participants, two baseline cardiac MRIs were excluded due to stringent quality control measures, and one similarly excluded post-infusion. In addition, one MRI data set for both before and after the infusion was further excluded due to the inability to

obtain cardiac MRI images, leaving 6 cardiac MRIs pre-infusion and 7 MRI's post-infusion available for analysis.

The whole heart time-resolved three-dimensional CMR (4D flow MRI) scans were performed with retrospective ECG gating and a free-breathing phase contrast sequence in a sagittal 3D volume with the following imaging parameters: TR/TE = 3.9–4.1 ms/2.3–2.4 ms, acquisition matrix = 114–151×114–151×36–55, flip angle = 7°, voxel size = 1.8–2.4×1.8–2.4×2.5–2.8 mm^3, temporal resolution = 35–49 ms. Velocity-encoding sensitivity was selected as 150 cm/s to avoid aliasing artifact. The obtained phase images were corrected through velocity anti-aliasing, noise filtering, and eddy current correction using an in-house MATLAB code (Mathworks, Inc., Natick, MA) to enhance image quality. A deep learning-based auto segmentation algorithm, which can greatly reduce pre-processing time (1.01 s/patient), was subsequently utilized to segment the blood pool and vessels.[7] As previously described, central aortic stiffness from ascending aorta to descending aorta was estimated by calculating pulse wave velocity (PWV) via a cross-correlation algorithm.[8] Mean wall shear stress (WSS) and the average of the top 5% of maximum WSS were also measured across the thoracic aorta. Out of 9 participants, two 4D flow MRI datasets were excluded due to imaging artifacts which affected the reliability of the measurement.

Kidney MRI included balanced-SSFP TRiggered ANgiography non-Contrast Enhanced (B-TRANCE) imaging to position ECG-gated breath-held 2D phase contrast (PC) MRI flow measurements (1.2×1.2×6 mm^3). PC-MRI was positioned orthogonal to the left and right renal artery at the takeoff of the descending aorta. When venous renal flow was visible, additional images were acquired of the venous return. In all cases, velocity sensitivity (venc≈100 cm/s) was adjusted to avoid velocity aliasing. Bilateral, time-resolved arterial and venous renal blood flow

were measured using region of interests drawn in Circle CVI42. Right, left, and total arterial and venous net flow was computed. Total bilateral blood flow was computed as the sum of the left and right kidney vessels. Out of 9 participants, one post-infusion 2D PC kidney MRI was excluded due to imaging artifacts which affected the reliability of the measurement.

Pegloticase infusion

Pegloticase 8 mg intravenous in 250 mL 0.9% normal saline was infused over ≥ 120 minutes. Participants were pre-treated with oral diphenhydramine 25 mg, oral acetaminophen 1000 mg and intravenous hydrocortisone 200 mg. As steroids can worsen hyperglycemia, we monitored blood glucose concentrations in participants prior to administration of hydrocortisone, as well as after the pegloticase infusion. Additionally, participants' vital signs were monitored for at least 1 hour after completion of the infusion.

Endpoints

The primary endpoints in this pilot trial were the change in SUA and iohexol-based GFR after 1 week after pegloticase infusion. Secondary outcomes were the following: the change of systolic and diastolic blood pressure, mean arterial pressure, albumin excretion rate, change of renal artery and renal vein blood flow, and change of cardiovascular structure and function after 1 week of single intravenous dose of pegloticase administration (in the form of global function and strain).

Power calculation and statistical analysis

For this initial pilot and feasibility trial, we planned to recruit 10 participants. Power calculations were based on paired *t*-tests. Published data from the Treatment Options in type 2 Diabetes in Adolescents and Youth Study was used as an estimate for the baseline value of SUA levels (mean [±SD] 5.8±1.4 mg/dL).[1] Prior research suggested that pegloticase infusion would reduce SUA levels to approximately 1 mg/dL.[9,10] Using the cross-sectional standard deviation, we estimated the standard deviation of the change in SUA, taking into account the correlation between pre- and post-infusion measures. Based on these estimates, a sample size of 10 participants was calculated to provide ≥99% power to detect a mean paired difference of 4.80 mg/dL in SUA, with a standard deviation of 0.62 mg/dL following pegloticase infusion.

Screening characteristics for participants who completed both baseline and 1-week visits were summarized as mean ± SD or as median [25th percentile, 75th percentile] for continuous variables; categorical variables were described as a count and percentage. Weight, metabolic, renal, and cardiovascular measures at baseline and 1-week were reported as either mean ± SD or median [25th percentile, 75th percentile] based on inspection of the measure's distribution. Baseline and 1-week measures were compared using paired *t*-tests when means were presented and Wilcoxon signed rank testing when medians were instead reported. All analyses were based on a pairwise complete-case approach. Pre- and post-treatment individual-level trends in absolute GFR and BSA-Standardized GFR were visualized with line plots. Baseline characteristics at screening visit, as well as comparisons of baseline and 1-week post-infusion data, were reported for the subset of participants with at least a 3 mg/dL reduction in SUA between timepoints, which was considered a sensitivity analysis for pegloticase responders. This threshold was defined based on the average level of SUA reduction observed in previous clinical trials

involving uric-lowering agents.[2,11,12] Spearman correlation coefficients for delta GFR and delta SUA, delta GFR and delta renal artery blood flow, in addition to delta GFR and delta renal venous blood flow were computed for this subset of responder participants; delta values were calculated based on the difference in 1-week and baseline measures. Spearman correlation coefficients for delta GFR and delta SUA for all participants were also reported. A significance level of 0.05 was assumed for hypothesis testing. Analyses were conducted using R v.4.2.3 and GraphPad Prism v10.2.0.

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Table S1. Inclusion and Exclusion Criteria

Inclusion Criteria	**Exclusion Criteria**
T2D diagnosis at ≤25 years of age	Uric acid lowering medications (e.g. allopurinol, febuxostat)*
Male	G6PD deficiency
Age 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
	Use of 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.

Table S2. Baseline Characteristics at Screening Visit

	All Participants (N=9)[1]	**Pegloticase Responders (N=7)[1]**
Age at Consent (years)	22 ± 5	21 ± 3
Weight (kg)	123 ± 35	113 ± 28
BMI (kg/m^2)	40.1 ± 11.0	36.9 ± 9.2
Obesity	7 (78)	5 (71)
Serum Uric Acid (mg/dL)	6.7 ± 1.1	6.8 ± 1.2
Positive G6PD Test Results	0	0
Race		
Black or African American	1 (11)	1 (14)
White	5 (56)	3 (43)
Other	3 (33)	3 (43)
Ethnicity		
Hispanic	7 (78)	5 (71)
Non-Hispanic	2 (22)	2 (29)
Diabetes Background		
Length of Diabetes Diagnosis (years)	3.2 [1.8,5.3]	3.2 [1.5,9.5]
Age at Diabetes Diagnosis (years)	17 ± 2	18 ± 3
Screening HbA1c (%)	8.6 ± 3.3	8.1 ± 3.0
Anti-diabetic Medications		
Metformin	5 (56)	5 (71)
Insulin	4 (44)	4 (57)
Pioglitazone	3 (33)	3 (43)
GLP-1 agonists	5 (56)	4 (57)
SGLT-2 inhibitors	0	0
No Diabetes Medication	1 (11)	0
Cardiovascular Disease Background		
Systolic Blood Pressure (mmHg)	132 ± 11	130 ± 9
Diastolic Blood Pressure (mmHg)	78 ± 12	75 ± 11

Mean Arterial Pressure (mmHg)	96 ± 10	93 ± 8
Pulse rate (beat per min)	94 ± 18	92 ± 20
Dyslipidemia	5 (56)	5 (71)
Sleep Apnea	2 (22)	1 (14)
History of Hypertension	3 (33)	1 (14)
History of Cardiovascular Disease	0	0
Anti-hypertensive Medication Use	3 (33)	1 (14)
Angiotensin Converting Enzyme (ACE) Inhibitors	2 (22)	1 (14)
Calcium Channel Blockers	1 (11)	0

[1] Continuous variables are presented as mean ± SD or median [p25,p75]; categorical variables are shown as counts (percentage)

Table S3. Safety Outcomes of Special Interest

Outcome	**Overall (N=10)**
Dizziness	1 (10%)
Nausea or vomiting	1 (10%)
Abdominal pain	1 (10%)
Muscle pain	2 (20%)
Chest discomfort	1 (10%)
Respiratory distress	2 (20%)
Nasopharyngitis	0 (0%)
Paradoxical flares of clinical gout	0 (0%)
Anaphylaxis	0 (0%)
Hemolysis	0 (0%)
Methemoglobinemia	0 (0%)
Congestive heart failure	0 (0%)