# Effect of Moderate Caloric Restriction on Glomerular Growth After Kidney Transplantation

# NCT03607500

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## OBJECTIVE:

To conduct a pilot randomized study to assess the impact of modest caloric intake reduction in kidney transplant recipients on the degree of post transplant glomerular hypertrophy.

## ABSTRACT

Weight gain after kidney transplantation is a common clinical problem affecting majority of kidney transplant recipients. Besides diabetes and hypertension, post transplant weight gain is associated with lower allograft survival, through yet poorly understood mechanisms. Accumulating evidence points to the role of glomerular hypertrophy, in accelerating podocyte detachment thereby leading to progressive glomerulosclerosis and eventual end stage kidney disease. Kidney transplants undergo glomerular hypertrophy early after transplantation, accompanied by loss of podocytes that is on average are about 6-fold higher than native controls. Previous simulation studies have demonstrated that changes in podocyte density after transplantation may limit allograft lifespan as well explain the strong donor age effect on allograft outcomes. The glomerular hypertrophy accompanying transplantation likely results from a combination of processes including the transition of the kidney from a 2 kidney state in the donor to a 1 kidney state in the recipient as well as post transplant weight gain. Studies of caloric restriction in experimental animals has shown that even small reductions in caloric intake can reduce glomerular enlargement, podocyte hypertrophic stress, proteinuria, glomerulosclerosis and kidney failure. With these concepts in mind we hypothesize that a modest caloric intake reduction in kidney transplant recipients will reduce post transplant glomerular hypertrophy. In this randomized pilot and feasibility trial of modest caloric intake reduction we will assess differences in markers of glomerular growth as well as quantitatively assess urinary podocyte detachment rates and markers of podocyte “hypertrophic stress” between the two arms.

**SPECIFIC AIMS**

Weight gain after kidney transplantation affects 50-90% of kidney allograft recipients. By one-year post transplantation, on average recipients gain about 10% of their pre-transplant body weight and 50% of all recipients are obese (BMI>30 kg/$m^2$)(1, 2). Increased weight gain after transplantation is associated with reduced allograft survival by poorly understood mechanisms (3). In model systems we have shown that weight gain is associated with increased glomerular volume (i.e. filtration surface area) that drives hypertrophic podocyte stress resulting in progressive loss of podocytes from glomeruli(4). We have also shown that podocyte loss itself drives progressive glomerulosclerosis and kidney failure (5). By 3-months post transplant kidney allograft glomeruli have increased their glomerular volume by 20-25% and experience a 6-fold increase in rate of podocyte detachment, ranging from a 2-fold increase in stable allografts to a 10-20 fold increase in transplant glomerulopathy (6). In simulation studies these factors can quantitatively account for the observed shorter-than expected allograft half-life at about 15 years and the powerful donor age effect on allograft survival (7). Compatible with this concept glomerulosclerosis is a significant contributor to late allograft failure as demonstrated by several reports (8, 9).

In the setting of the kidney allograft there are therefore at least two factors driving glomerular volume increase potentially leading to hypertrophic podocyte stress and glomerulosclerosis. (i) The compensatory kidney hypertrophy that occurs in the transition from the 2 kidney to the 1 kidney state as the single allograft picks up twice the normal work load. (ii) Post-transplant weight gain that is associated with additional glomerular volume increase driven by growth factors, nutrients and other pathways. (iii) Development of overt diabetes will further amplify growth signaling and glomerular enlargement. **We hypothesize that the combination of these factors drives the observed accelerated podocyte detachment rate leading to progressive glomerulosclerosis and shorter-than-expected allograft half-life.** If this hypothesis is correct then slowing the rate and amount of glomerular volume increase should reduce the degree of hypertrophic stress and rate of podocyte detachment leading to preservation of glomerular structure and function. This hypothesis has been directly validated in a nephrectomy model in rats where even quite modest caloric intake reduction (CIR) is protective of development of glomerular enlargement, podocyte hypertrophic stress, proteinuria, glomerulosclerosis and kidney failure. **As a first step towards implementing these concepts in humans we will perform a small pilot study designed to test the feasibility of conducting such a study as well as to test the hypothesis that modest CIR can reduce the amount and rate of glomerular growth that accompanies kidney transplantation.**

**Aim 1:** Test the hypothesis that modest CIR (~500 kcal/day) reduces the glomerular growth rate associated with kidney transplantation. For this pilot study, we will randomize 15 overweight or obese patients (BMI: >25 kg/$m^2$) patients to dietician guided modest CIR or standard of care. All patients will undergo standard protocol biopsy at time 0 and 3 months post-transplantation. We will compare the difference in glomerular volume, number of Ki67 positive (actively dividing) glomerular cells, DAPI positive (all glomerular cell nuclei) and TLE4 positive (podocyte nuclei) intraglomerular cells from at least 8 glomeruli/patient. In those patients in whom the CIR protocol reduced post-transplant weight gain we expect to see lower glomerular growth rate as demonstrated by reduced glomerular volume increase and a lower number of Ki67 positive intraglomerular cells. As podocytes don't divide we also expect to see a lower proportion of podocytes (TLE4 positive/total DAPI positive nuclei) reflecting the increased growth rate of non-podocyte cells in the glomerulus.

**Aim 2:** Test the hypothesis that modest CIR reduces podocyte hypertrophic stress as assessed by a reduction in the urinary podocyte detachment rate. For this aim we will collect urine samples at the time of 3-month protocol biopsies and compare the urinary podocin to creatinine ratio as well as markers of podocyte "hypertrophic stress" including the urine podocin to nephrin ratio across both groups(10). We expect a lower urinary podocyte detachment rate as well as lower urine podocin to nephrin ratio in the intervention arm.

These studies will provide insight into the feasibility of conducting larger clinical studies using CIR in the early post-transplant period. Further, they may provide insights into the mechanistic role of caloric restriction on post-transplant glomerular growth, as well as their impact on reducing post-transplant weight gain, post-transplant diabetes mellitus and hypertension, all of which alone or in combination can affect allograft and patient survival. With the average cost of dialysis at 100,000 USD/year, even a 1-year improvement in allograft longevity of the approximately 15,000 kidney transplants performed every year translates into cost savings of approximately 1.5 billion dollars.

## BACKGROUND INFORMATION

Weight gain after kidney transplantation is a common clinical problem. Approximately 50-90% of kidney transplant recipients gain weight after transplantation (1). The increase in weight is likely the result of a change in diet, lifestyle and improved sense of well-being especially as the dietary restrictions of CKD/ESRD are lifted (11). Depending on underlying baseline demographics, weight gain in the first year after transplantation can vary from 2.7 to 11.8 kilograms. In one U.S.-based study the proportion of obese transplant recipients increased from 34% at baseline to 50% at 12 months (1). Along with the adverse effects of an elevated pretransplant body mass index (BMI) on long term renal transplant outcomes (12), increased weight gain post-transplant has been associated with all cause and death censored allograft loss (3, 13). Further, post-transplant weight gain has been associated with development of post-transplant diabetes mellitus as well as hypertension (13).

The cause of reduced allograft survival (death censored) among obese kidney transplant recipients is thought to be due to the increased recipient and donor kidney size mismatch (a surrogate for nephron mass) among overweight and obese transplant recipients (14). Previous nephrectomy studies performed by Brenner et al. have popularized the concept of progressive glomerulosclerosis in remnant kidneys due to hyperfiltration injury as a mechanism. This was postulated to be the cause of reduced kidney survival in both native kidneys as well as allografts (15, 16). Progressive glomerulosclerosis is now thought to occur as a result of podocyte depletion in the glomeruli, regardless of the etiology of upstream podocyte injury (5, 17, 18). Almost all glomerular diseases have increased podocyte detachment in parallel to increasing glomerulosclerosis and progression to End Stage Kidney Disease (ESKD) (17).

The reduction in podocyte density can be the result of podocyte loss (absolute density reduction) from any injurious process, an increase in **glomerular volume** (resulting in a “relative” podocyte density reduction) or some combination of both. Experimental nephrectomy models have demonstrated an increase in glomerular volume as a part of the compensatory hypertrophic response in the remnant kidney. In such a setting, the increase in glomerular growth is accompanied by an increased in the total number of cells in the glomerulus. Because podocytes are post-mitotic cells, the increase in the number of cells in the growing glomerulus is due to an increase in non-podocyte glomerular cells(19). As the glomerulus increases in size, the podocytes have to cover the increase in filtration surface area. While podocytes can hypertrophy to cover this increase in filtration surface area, a mismatch between the amount of glomerular volume increase and the podocytes ability to hypertrophy can result in the podocytes experiencing significant “circumferential” and “hoop” stresses that can cause them to detach and be shed in the urinary space (20, 21). Thus, limiting the amount of glomerular growth (hypertrophy) could, reduce the mismatch between glomerular volume, available podocytes (and their capacity to hypertrophy) thereby improving long term renal outcomes (by reducing the burden of progressive glomerulosclerosis).

Dietary protein and CIR has been previously shown to reduce rate of progression of CKD(22). Previous nephrectomy studies have also demonstrated that the increase in glomerular volume and total glomerular cell counts in the stressed glomerulus is dependent on caloric intake, a phenomenon mediated by the mTORc1 pathway(4). In a study of aging Fischer 344 rats, caloric restriction not only limited the increase in glomerular volume accompanying nephrectomy, but also the total number of non-podocyte glomerular cells(19). We have recently shown that caloric restriction prevents focal segmental glomerular lesions in rat models of glomerular hypertrophy. Further there was a reduction in the number of dividing non-podocyte cells as (determined by Ki67 nuclear staining see preliminary unpublished data) as well as lower G2M cell cycle scores on an unbiased transcriptomic analysis of isolated glomeruli. These observations and experiments would suggest that caloric restriction could have a significant effect in ameliorating hypertrophic stresses on glomerular growth and thereby reduce burden of glomerulosclerosis.

Among kidney allografts, serial surveillance biopsy studies of **stable** allografts have demonstrated that along with chronic microcirculatory injury from antibody mediated rejection, *denovo* progressive glomerular

disease is likely to play an important role in long term allograft outcomes (8). The etiology of this progressive glomerulosclerosis in allografts is unclear but is likely to be a combination of immune and non-immune events. Our lab has shown that by 3 months post kidney transplantation, the glomerular volume has increased by approximately 25%, accompanied by a **proportional 25% reduction in podocyte density** (as a function of volume). Thus, the hypertrophic glomerular volume increase of transplantation and the accompanying relative reduction in podocyte density can itself poise the allograft for glomerulosclerosis (threshold for glomerular destabilization is 30% reduction in podocyte density)(5, 6). Since caloric intake plays a critical role in glomerular growth and because weight gain is very common after transplantation, **we hypothesize that modest caloric intake reduction (CIR) early after kidney transplantation can reduce the glomerular growth associated with kidney transplantation and may improve long term allograft survival, by reducing glomerular hypertrophy mediated progressive glomerulosclerosis.**

With the average death censored allograft half-life of kidney transplants at 15 years (7), assessing the impact of such interventions may need prolonged follow up. Fortunately, we have at our disposal excellent biochemical (1-year serum creatinine, proteinuria) as well as histological surrogates (post-transplant glomerular volume) (23, 24), that may offer indirect evidence of the efficacy of an intervention on long term allograft outcomes.

The academic environment at the University of Michigan, expertise in glomerular disease, nutrition and obesity, weight management program, a large volume kidney transplant center that has a robust kidney allograft surveillance biopsy program (time 0, 3 months, 6 months and 12 months post-transplant) make it ideal to carry out the intervention trial proposed in this application.

**INNOVATION**

While the relationship between CIR and post-transplant glomerular growth has been defined in experimental models, to date no human experiments have been carried out. To assess the impact of any dietary/weight loss intervention on human kidney disease would require serial biopsies of the native kidneys. A procedure that is not only painful, but also technically challenging and has a high complication rate especially among overweight and obese individuals. The superficial placement of renal allografts and ongoing active surveillance biopsy program can offer unparalleled access to renal tissue for further study. Defining the role between nutrition and glomerular growth in humans will not only have implications on kidney transplantation but may also allow us to better understand on how the obesity epidemic may be contributing to the increasing burden of CKD and ESRD. Further, it will provide insights in to understanding the infrastructure needed to successfully carry out any study that involves dietary interventions in kidney transplant recipients asd well as patients with chronic kidney disease.

**PRELIMINARY DATA**: Glomerular hypertrophy following nephrectomy in rat models of progressive glomerulosclerosis is accompanied by an increase in cell division among non-podocyte glomerular cells. Preliminary rat studies performed on Wild Type (WT) and Transgenic (TG) rats (podocin promoter-AA-4E-BP1) rat models in which the podocytes capacity to hypertrophy in response to glomerular growth is impaired thereby leading to progressive glomerulosclerosis and ESKD within 1 year (or 12 weeks if they undergo nephrectomy). Rats underwent a nephrectomy (1K: one kidney state) or sham nephrectomy (2K: two kidney state) while on an Ad Lib Diet (ALD). Data (table below) obtained from whole renal cortical tissue demonstrates actively dividing intraglomerular and cells from other compartments but not podocytes (as assessed by Ki67 staining and transcriptomic analysis). Further, isolated glomerular Affymetrix transcriptome obtained 3 weeks after nephrectomy (focal segmental glomerulosclerotic lesions were

present) were used to obtain a G2M signature value derived from the "Hallmark_G2M" gene list downloaded from the GSEA/MSigDB molecular signatures database at the Broad Institute(25). It was found that genes associated with cell cycle pathways were significantly increased after nephrectomy in both WT and TG animals corresponding with glomerular growth.

| Ki67 positive nuclei per field in different kidney compartments | | | | | |
|---|---|---|---|---|---|
| | Intraglomerular | Podocyte | PEC | Periglomerular/Interstitial | Tubular |
| TG.1K. ALD | 10.3±1.7** | 0.01±0.1 | 2.8±0.7** | 8.2±2.2** | 4.0±1.1** |
| TG. 2K.ALD | 6.4±0.9** | 0.1±0.1 | 0.9±0.4 | 3.2±0.5 | 2.0±0.4 |
| WT.1K.ALD | 4.5+1.0** | 0.0+0.0 | 0.7+0.4 | 3.2+0.8 | 2.5+0.9 |
| WT.2K.ALD | 6.7+1.0* | 0.0+0.0 | 0.6+0.3 | 2.6+0.2 | 2.8+1.1 |

**PEC- Parietal Epithelial Cell. The TG.1K.ALD group developed glomerulosclerosis. Other groups did not.**

These findings are compatible with the concept that glomerular growth as represented by increased cell cycle scores and increased cell division among non-podocyte glomerular cells was associated with development of progressive glomerulosclerosis (first focal and then global glomerulosclerosis) in rat models. If the hypothesis of impact of glomerular growth on long term allograft outcomes was correct, then modest caloric restriction should have an impact on post-transplant glomerular growth.

## PREVIOUSLY PUBLISHED STUDIES SUPPORTING FEASIBILITY

By the applicant team:

1. Yang et al. JASN 2015. Demonstrates that allograft recipients have a 6-fold increased rate of podocyte detachment and that post-transplantation kidney allografts have an increased glomerular volume and reduced podocyte density, and podocyte number per glomerulus decreases in association with development of transplant glomerulopathy(6).
2. Naik et al. JCI Insight 2016. Demonstrates that the podocyte depletion hypothesis can account for the unexpectedly short allograft half-life and the donor-age effect on allograft outcomes(7).
3. Nishizono et al. JASN, manuscript accepted for publication. Demonstrates in a rat model that CIR reduces glomerular enlargement, podocyte stress, podocyte reduction rate and glomerulerulosclerosis

By other investigators:

- Previous dietary intervention trials in clinical transplantation demonstrate feasibility in reducing weight gain post-transplant. A previous study compared patients who received weekly dietary advice to identical historical controls. At baseline both groups had identical weights. By 4 months post-transplant the intervention group had gained on average 2 kilograms compared to 7 kilograms in the historical control. By the end of the first year, the intervention arm had gained 6 kilograms compared to the 12 kilograms in individuals that received standard post-transplant dietary advise(11).

## STUDY DESIGN AND METHODOLOGY

Prospective randomized single center clinical trial with un-blinded endpoint evaluation. The study is a pilot interventional trial comparing the impact of modest caloric intake reduction on glomerular growth and podocyte parameters after kidney transplantation. This pilot study plans to enroll 30 patients at the University of Michigan. We will follow patients for 12 months after their transplant.

### Study Endpoints

**Primary Endpoint for Aim 1:** Assess differences in change of glomerular volume from immediate post perfusion biopsies to those obtained at 3 months between both arms. Further we will also investigate the

differences in number of Ki67 positive, DAPI positive (all glomerular cells), TLE4 positive (all podocytes) intraglomerular cells from at least 8 glomeruli/patient.

**Primary Endpoint for Aim 2:** Assess differences in urinary podocyte detachment rate (measured as urine podocin mRNA to creatinine ratio) as well as markers of podocyte "hypertrophic stress" such as urine podocin to nephrin ratio.

**Secondary Endpoints:** Monitor at months 3 and 12, differences in glomerular filtration rate (GFR) using both creatinine (modified diet in renal disease) as well as serum cystatin C based equations (to account for the possibility that caloric restriction may also affect lean muscle mass and thus creatinine levels), urine protein to creatinine ratio (UPCr), differences in weight as well as glycosylated hemoglobin (HbA1c).

**Study Eligibility**

Patients presenting for living or deceased kidney transplantation at the University of Michigan Hospital.

**Inclusion Criteria:**

- Recipients of Living and Deceased donor kidney transplants where a post perfusion biopsy is obtained
- BMI ≥25 kg/$m^2$ at the time of randomization
- Age range: 18-70
- Immediate graft function.
- Standard triple immunosuppression (Tacrolimus, Mycophenolate Mofetil, Prednisone) with or without induction.
- No experimental drugs.
- Have smartphone or active internet connection at home.

**Exlusion Critieria :**

- Patients who do not meet eligibility/inclusion criteria or who do not provide consent.
- Patients on dual antiplatelet agents or who are on oral anticogaulation
- Patients who did not receive a post-prefusion biopsy
- Early post-op wound complications
- Patients who have had Bariatric Surgery (concerns for malnourishment)
- Patients who will be taking Angiotensin Converting Enzyme Inhibitors (ACEi) or Angiotensin Receptor Blockers (ARBs) within the first 3 months' post-transplant

**Study Setting, Patient Enrollment and Randomization**

***Study Setting*:** Single center study at the University of Michigan Hospital where approximately 200 kidney transplants are performed every year

***Patient Enrollment***: Patients who meet the inclusion criteria and provide written informed consent after adequate explanation of the risks and benefits will be randomized in a 1:1 fashion to the modest CIR or the standard of care arm. Study participation will end 12 months after transplantation.

**Study procedures, Interventions,tests follow up, concurrent therapies and study end**

***Study Procedures*:** Patients who consent, will be randomized to modest CIR or the standard of care arm. The standard of care arm patients will obtain dietary guidance per current University of Michigan Transplant Center policy, where a one time face-to-face visit is provided in the first month after kidney transplant and then as requested by the patient or referred by physician.

**Dietary Intervention:** The intervention group will be seen by the kidney disease dietician at all clinical visits for the first 3 months (weekly for month 1, every other week for months 2 and 3). During months 2 and 3, in the weeks that the patients are not in clinic, they will receive telephone calls from the dietician. Thus the intervention involves weekly assessment for the first 3 months (in person or over the telephone). After 3 months, the patient will not receive any further active dietary intervention from the dieticians unless the patient wishes to continue using the dieticians advice per clinic protocol. Patients will be given adequate explanation of their caloric goals and dietary constituents such as the proportion of carbohydrates, fats and proteins they should consume. A daily log of all dietary intake will be recorded using the MyFitnessPal (https://www.myfitnesspal.com) application or website (if no smartphone is available). The dietician will review weekly diet log, goals and reinforce dietary recommendations. If the patient desires, additional sessions with the dietician will be provided to help patient reach their dietary goals. All patients in the intervention arm will be actively encouraged to increase physical activity as tolerated with the intent of being able to perfrom 150 minutes of cardiovascular exercise per week.

Previous studies have suggested providing 30 Kcal/Kg early after transplantation to account for the high catabolic rate and weight loss immediately after transplantation, followed by 25 Kcal/Kg for the long term dietary maintenance (26).However, these studies likely provided a higher caloric intake than may be necessary as the majority of the weight loss early after transplantation is thought to be due “fluid-weight” loss from solute and post acute tubular necrosis related diuresis. For the intervention arm, the estimated daily caloric intake (assuming a sedentary lifestyle), necessary to maintain steady body weight will be calculated using the Mifflin St-Jeor equation (27). For the first 7 days, we aim to provide the recommended calories to account for the higher catabolic rate early after transplantation. By the end of the first week, a net reduction of 500 kcal/day will be done allow an estimated weight loss of about 1lbs/week. If the goal of 1 lbs/week is not achieved we will increase the net caloric deficit by another 250 kcal/day to achieve the goal.

For this study, the goal of the dietary intervention is reduction of overall caloric intake. However, we intend to maintain an adequate proportion of carbohydrates, proteins and fats as has been recommended previously for the purpose of kidney transplant patients (26). On average fat will provide <30% of all caloric intake, carbohydrates will provide 50% of total calories and protein at 1.3-2.0 g/kg in the first 7 days (to account for catabolic effects of surgery), followed by 0.8-1.0 g/kg for the remainder of the intervention.

For patients randomized to standard of care arm, the dietician will meet and provide instructions per the current University of Michigan Transplant Center protocol.

***Tests, Procedures and Follow up*:**

**Measurement of Glomerular Volume**

Only patients with post perfusion biospies are consented. Per transplant center protocol, all patients will also get a 3 month allograft biopsy. Biopsies from post perfusion and 3 months will be used to obtain glomerular volume. Glomerular volume measurements will be made on biopsies with at least 8 glomeruli. With assumption that glomeruli are spherical, the radius (R) will be calculated as $R = r \times 4/\pi$ as described by Weibel (28). Glomerular tuft volume will then be given by $4/3\pi R^3$. Globally, sclerotic glomerular will be excluded from the analysis. 3 month protocol biopsies will also be stained with monoclonal antibodies against TLE4, DAPI and Ki67 to identify podocytes, all nucleated cells in the glomerulus and actively dividing cells, respectively. All counting of cells will be done manually by either Dr. Abhijit S. Naik (PI) or Dr. Roger C. Wiggins (Co-I).

**Urine processing:** Left over urine (ideally, at least 50 cc) will be collected at the time of 3 month biopsies. The Urine will be centrifuged at 4°C for 15 minutes at 4000 rpm (3200×*g*). Two 2-ml aliquots of the supernatant will be removed and stored at −20°C for protein, creatinine, and other measurements. The urine pellet will be treated as mentioned previously(6).The pellet will then be suspended in RLT/*β*-mercaptoethanol buffer and then frozen at −80°C for assay.

**Urine RNA Preparation and Quantitative RT-PCR Assay and Interpretation**: The total urine pellet RNA will be isolated using the protocol of the RNeasy. Quantitation of the absolute podocin mRNA abundance will be performed using RT-PCR. Probe for human NPHS2 (podocin) will be used. All data will come from 2-*μ*l samples measured in duplicate. Standard curves will be constructed for each assay using serially diluted cDNA standards. Assays will be accepted only if the *r*2 is >0.97 for standard curves. Human podocin cDNAs of known sequence and concentration will be used as standards for each assay so that the data can be calculated on a molar basis for each probe.

**Urine data analysis (Urine Podocin mRNA to Creatinine ratio)**: All assays will be performed against cDNA standards. Data will we first be expressed per milliliter of urine, and then expressed per gram of urine creatinine. All measurements will be carried out at the Wiggins laboratory.

During each clinic visit patients vital signs including weights and a physical examination will be performed per the University of Michigan Transplant Center protocol.

Lab frequency will be twice weekly for 3 months; weekly months 3-6; biweekly month 6-12 and monthly months 12-24 per UMHS transplant protocol or as desired by treating physician. Serum Creatinine and proteinuria measurements will be performed at the University of Michigan Hospital and Health Systems.

**Serum Cystatin C levels** will be added on to standard of care labs at months 3 and 12 after kidney transplantation and will be performed at the UMHS.

**Serum Prealbumin levels** will be added on to standard of care labs at months 1 and 3 after kidney transplantation to assess for malnutrition.

Patients will have the option of having an additional sample collected (6ml of blood) at their 3-month biopsies for future studies. The samples will be stored (-80) at Dr. Roger Wiggins laboratory for up to 5 years after collection and then be destroyed. If the patient decides to drop out of the study we would destroy their samples. The samples will be identified by an identification number only and will be kept confidential.

All pertinent demographic variables including age, gender, race, weight, height, body surface area etc. Donor information on age, gender, weight, race will also be recorded. Type of induction therapy, maintenance therapy at time of discharge and any changes over the first year will be recorded with reason for change. At all prespecified time points recipient vital signs including weight, development of post transplant diabetes,initiation of hypoglycemic agents, rejection will be recorded.

**Concurrent Therapies**

***Immunosuppression*:** Induction agents will be used according to center protocol. Experimental agents will not be used. Maintenance immunosuppressive therapies will be according to center protocol (Thymoglobulin, Mycophenolate Mofetil and Prednisone). Use of mammalian target of rapamycin inhibitor (mTORi) such as Sirolimus or Everolimus will be discouraged until 3 months after kidney transplantation, unless there is a compelling clinical reason.

***Others***: No Angiotensin Converting Enzyme Inhibitors (ACEi) or Angiotensin Receptor Blockers (ARBs) are allowed in patients in the study until after 3 months' post-transplant at which time the timing of initiation will be deferred to the attending physician taking care of the patient.

Hypertension, dyslipidemia and other medical conditions will be treated per the UMHS transplant center's cardiovascular disease prevention and treatment protocols and determined by individual transplant physicians and surgeons caring for the given patients.

**End of Study**

***Completion of study***: The study will end 12 months after transplantation. Patients will continue to receive care for their medical conditions related or not to the study as per the UMHS transplant center.

***Study Withdrawal*:** Patients or their physicians can withdraw the patient from the study at any time.

**Data collection and Storage:** The proposed study intends to use a web-distributed data entry clinical research data managemnt on the Research Electronic Data Capture (RedCap) platform.

**Statistical considerations and analytical plan:** Previous animal studies have shown that approximately 5 animals in each arm (dietary restriction vs. ad lib) has been sufficient. However, given lack of any previous data in humans and higher expected variability, we plan on recruiting 15 patients in each arm in this pilot trial. Further trials will use data obtained from this trial to determine the number of patients necessary to adequately power future studies. The unpaired "t" test will be used to compare differences in glomerular: volume, DAPI positive nuclei, Ki67 positive nuclei, TLE4 positive nuclei. Differences in means of proportions will be measured z test . A linear regression model will be utilized to quantify the relationship between the continuous variables. Best-fit curve estimation, including quadratic and cubic models, will be utilized, if necessary, to demonstrate nonlinear associations between two continuous variables.

**Safety Monitoring:**

Although we do not anticipate any adverse events due to caloric restriction, all adverse events including serious adverse events that could be related or possibly related will be reported. Reducing caloric intake may cause hypoglycemia in some individuals.

**Potential Pitfalls:** Patient may not follow instructions on diet leading to higher than desired caloric intake causing the study endpoints to be affected. However, this may offer further insight into study design feasibility and allow us to make appropriate changes for future studies.

As glomerular hypertrophy can also be driven by donor and recipient body size mismatch we have tried to minimize that possibility by using the average BMI groups for both donors and recipients (BMI:25-30 $kg/m^2$). Other interventions that can affect glomerular hypertrophy including the use of ACEi/ARBs, mTORi which will be avoided in the first 3 months post transplant. Theoretically , the use of antiproliferative drugs used in transplantation may limit cell growth and thus we may not see the full benefit of reducing hypertrophy by dietary interventions. However, since the control and the intervention arms are on the same immunosuppressive agents, we believe that the effect of caloric restriciton on glomerualr growth would still be possible to observe.

**Anticipated Result:** The percent change in glomerular volume from baseline to 3 months, number of total glomerular cells (DAPI positive cells), Ki67 positive cells as well as the ratio of TLE4 positive cells to total number of DAPI positive glomerular cells (TLE4/DAPI ratio), podocyte detachment rate, podocin/nephrin ratio and proteinuria will all be lower in the intervention arm.

**Protection of Human Subjects:**

**Risk to the patient:**. Since this is a dietary intervention study there is no more than minimal risk to the patient. Reducing caloric intake may cause hypoglycemia in some individuals, however we do not expect this to be a significant clinical problem. For Aim 1, we will utilize renal biopsy tissue that is already being collected as part of standard of care treatment and no additional tissue or cores are being collected for the purpose of this study. Aim 2, involves collection of urine specimen that is already being collected as part of standard of care. We will use only left over urine samples for our analysis. This includes baseline clinical and laboratory information as well as outcome variables. Patients will not undergo repeat venous puncture or biopsies for the purpose of the study outside of UM protocol. The data will be analyzed will be coded to reduce the risk of breach of confidentiality. .

**Responsible Conduct of Research**

I have successfully completed the online module “Program for the Education and Evaluation of Responsible Research and Scholarship (PEERRS)” at the University of Michigan. The modules covered in this educational activity were:

- Foundations of Good Research Practice
- Research Administration
- Conflict of Interest
- Human Subjects – Biochemical & Health Sciences
- Human Subjects – Social & Behavioral Sciences
- Authorship, Publication and Peer Review
- Animal Subjects

| PROCEDURE | STUDY ENROLLMENT | 1 MONTH | 2 MONTH | 2 MONTH | 3 MONTH | 3 MONTH | 12 MONTH |
|---|---|---|---|---|---|---|---|
| Weeks | 0 | 1-4 | 5 & 7 | 6 & 8 | 9 & 11 | 10 & 12 | 52 |
| Inform Consent | X | | | | | | |
| Inclusion/Exclusion Criteria | X | | | | | | |
| Dietary Intervention | | X | | X | | X | |
| Dietary Phone Call | | | X | | X | | |
| Biopsy post-perfusion & Month 3 | *X | | | | *X→ | | |
| Urine (50 cc 3 mth) | | | | | *X→ | | |
| Serum Cystatin C | | | | | *X→ | | *X |
| Prealbumin | | *X | | | *X→ | | |
| Blood sample (for future use) | | | | | *X | | |
| Immunosuppression Review | | X | | X | | X | |
| Vital Signs | | X | | X | | X | |

*Biopsy- extra slide only at Post-Perfusion & Month 3 (SOC)

*Serum Cystatin C will be added on to SOC labs at 3 and 12 months

* Prealbumin will be added on to SOC labs at 1 and 3 months

*Extra 6ml green top tube for future use at time of 3 month biopsy

*Urine 50 cc only at Month 3

**References:**


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