Feasibility and Efficacy of a Well-Formulated Ketogenic Diet in delaying progression of Polycystic Kidney Disease in patients at risk for Rapid Progression

The Ohio State University

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Clinical Study Protocol

Study Title: "Feasibility and Efficacy of a Well-Formulated Ketogenic Diet in delaying progression of Polycystic Kidney Disease in patients at risk for Rapid Progression"

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SUMMARY AND BACKGROUND

ADPKD is the fourth most common cause of chronic kidney disease and accounts for 5% of patients with kidney failure [1]. It is the fourth most common cause for end stage renal disease (ESRD) which typically occurs in the sixth decade of life [17]. ADPKD affects more than 600,000 Americans and 12.5 million people worldwide [2].

ADPKD is characterized by the predisposition to cyst formation and enlargement. The progressive development and enlargement of cysts leads to fibrosis of the renal parenchyma and eventually ESRD [1]. The glomerular filtration rate (GFR) remains stable for many decades due to compensatory hyperfiltration. Therefore, it is imperative to utilize alternative prognostic biomarkers to assess progression to end stage renal disease (ESRD).

Due the genetic heterogeneity in ADPKD, there is significant phenotypic variability with regard to progression to ESRD. Therefore, it is critical to use biomarkers to assess patients' risk of progression to initiate early renal protective measures and therapeutics to delay progression. Factors that suggest rapid progression include hypertension onset ≤ age 35, urologic events such as hemorrhagic cysts or infection ≤ age 35, multiple family members with ESRD ≤ age 55, or PKD 1 mutations if genetics are known. Other objective measures of rapid progression include total kidney volume growth ≥ 5% per year (on 3 measurements done at least 6 months apart), GFR decline ≥ 2.5 ml/min/year over 5 years and Mayo Imaging Classification of Class 1C-1E. The Mayo Imaging Classification is used in patients with "typical" ADPKD in which individuals have bilateral, diffuse cystic disease. It is a validated calculator for measurement of height-adjusted total kidney volume (htTKV) and predicts trajectory of GFR and time to ESRD [1]. At present, Tolvaptan, a vasopressin V2 antagonist, is the only US Food and Drug Administration (FDA) approved drug for slowing progression of ADPKD in individuals at risk for rapid progression. However, Tolvaptan is associated with significant aquaretic effects and requires frequent lab monitoring for liver toxicity, making it challenging to prescribe.

Lifestyle interventions such as ketogenic diet have shown promise in animal models, yet long-term studies in humans are lacking. We propose that ketogenic diet will slow htTKV growth and thus slow disease progression. Furthermore, it may prove beneficial and reduce need for Tolvaptan, which is associated with significant side effects and financial burden or act as an alternative therapy for those who cannot or chose not to take Tolvaptan.

Background:

Autosomal dominant Polycystic Kidney Disease (ADPKD) is the most common genetic cause for ESRD. It is critical to assess individuals at high risk for disease progression in order to implement kidney protective measures early in disease course.

At present, caloric restriction is the only dietary intervention that has shown benefit with regard to disease progression in this unique population [2]. Animal models suggest metabolic reprogramming contributes to cystic epithelial proliferation and cyst growth [4]. The metabolic alteration in the cystic cell epithelia resembles that of cancer cells, known at the "Warburg Effect". Thus, glucose serves as the primary source of fuel in highly proliferative cystic cells, which consequently shift to aerobic glycolysis as opposed to oxidative phosphorylation through the tricarboxylic acid (TCA) cycle. In the former pathway, glucose is preferentially converted to lactate. The glycolytic products shift to anabolic pathways such as the Pentose Phosphate pathway that sustain cell proliferation and growth via reduction in AMP-activated protein kinase (AMPK) and upregulation of mammalian target of rapamycin (mTOR). This was demonstrated in *Pkd1* knockout mice. Furthermore, glucose deprivation led to reduced cystic cell proliferation [8]. Interestingly, 2-deoxyglucose (2DG), a molecule that inhibits glycolysis, restored normal levels of AMPK and acetyl-CoA carboxylase, reducing increase in total kidney volume [9]. Because of decreased glycolytic activity with administration of 2DG, activation of AMPK inhibits mTOR, which downstream inhibits cell proliferation and cyst growth [10].

Oxidative phosphorylation relies on fatty acid oxidation (FAO) for generation of ATP; impaired FAO is another hallmark in the abnormal metabolic pathways of ADPKD and leads to increased mitochondrial damage. In cystic cells, mitochondria no longer function as the energy sources, but supply the intermediates for anabolic pathways [10]. Additionally, glutamine is catabolized to α-ketoglutarate, which switches the TCA cycle in the opposite direction and produces acetyl-CoA for fatty acid biosynthesis. Mitochondrial function is thought to be mediated by the polycystins. Polycystin-1 (PC-1) and polycystin-2 (PC-2) are membrane proteins encoded by PKD1 and PKD2 genes. From a metabolic standpoint, PC-1 and PC-2 are present in the mitochondria-associated endoplasmic reticulum membranes. Abnormal polycystin proteins in the setting of ADPKD lead to reduced calcium-dependent oxidative phosphorylation and thus switching to the glycolytic pathway [7]. The abnormal pathways are depicted in Figure 1.

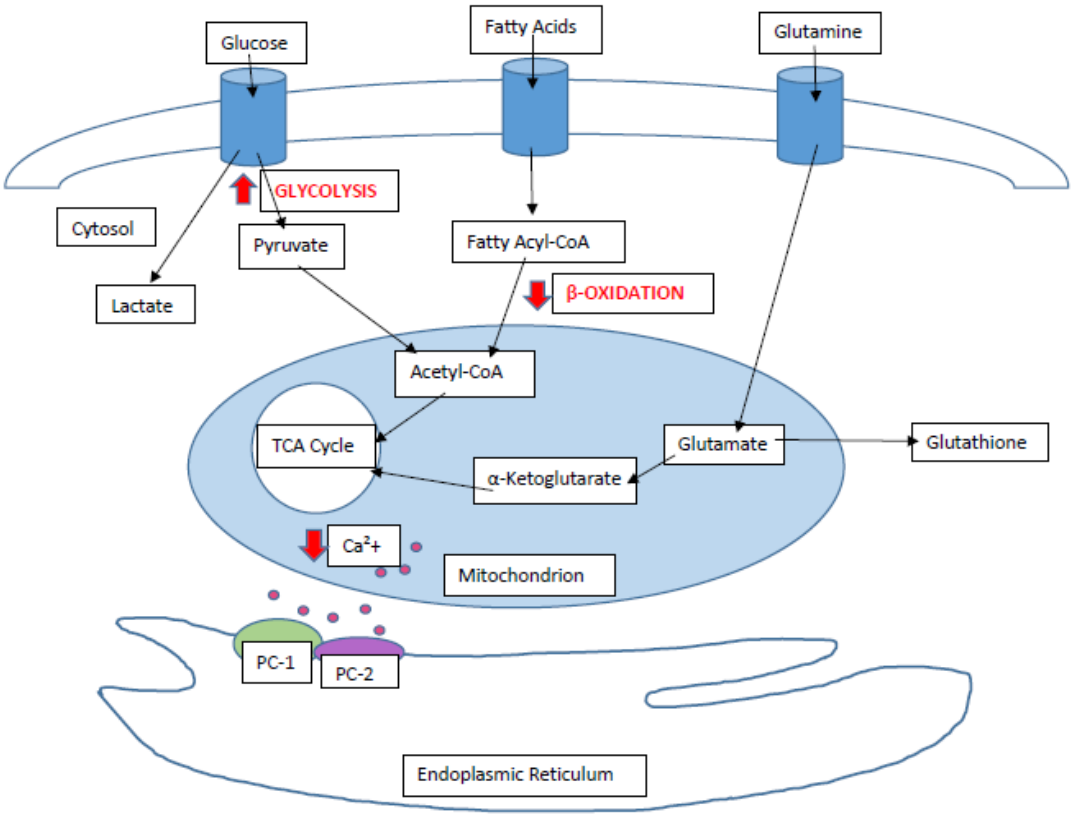

Figure 1

Animal models have shown slowed disease progression with diet interventions such as fasting and ketogenic diet, meant to diminish available glucose [7]. Metabolic pathways are under investigation as potential targets for both dietary and pharmacologic interventions. Ketosis improves the phenotype in mouse models of ADPKD, including reduction in fibrosis, reduced kidney and cyst size, and improvement in creatinine clearance. The proposed mechanism involves a switch from glucose metabolism to fat oxidation. The switch leads to activation of AMPK and induces peroxisome proliferator activated receptor α (PPARα) [6]. Activation of PPARα increases fatty acid utilization and oxidative phosphorylation [10]. Additionally, ketosis inhibits mTOR (a known modulator of cyst cell proliferation and fluid secretion). B-hydroxy-butyrate (BHB) elevation and ketogenic diet was shown to reduce cysts number and size in rat models [5]. This supports a theory that cystic cells are unable to utilize ketone bodies as a source of energy, and therefore use glucose as their main source of energy [12]. Ketosis inhibits STAT3 signaling to reduce cyst growth and fibrosis [16]. Figure 2 illustrates ketogenic diet effect.

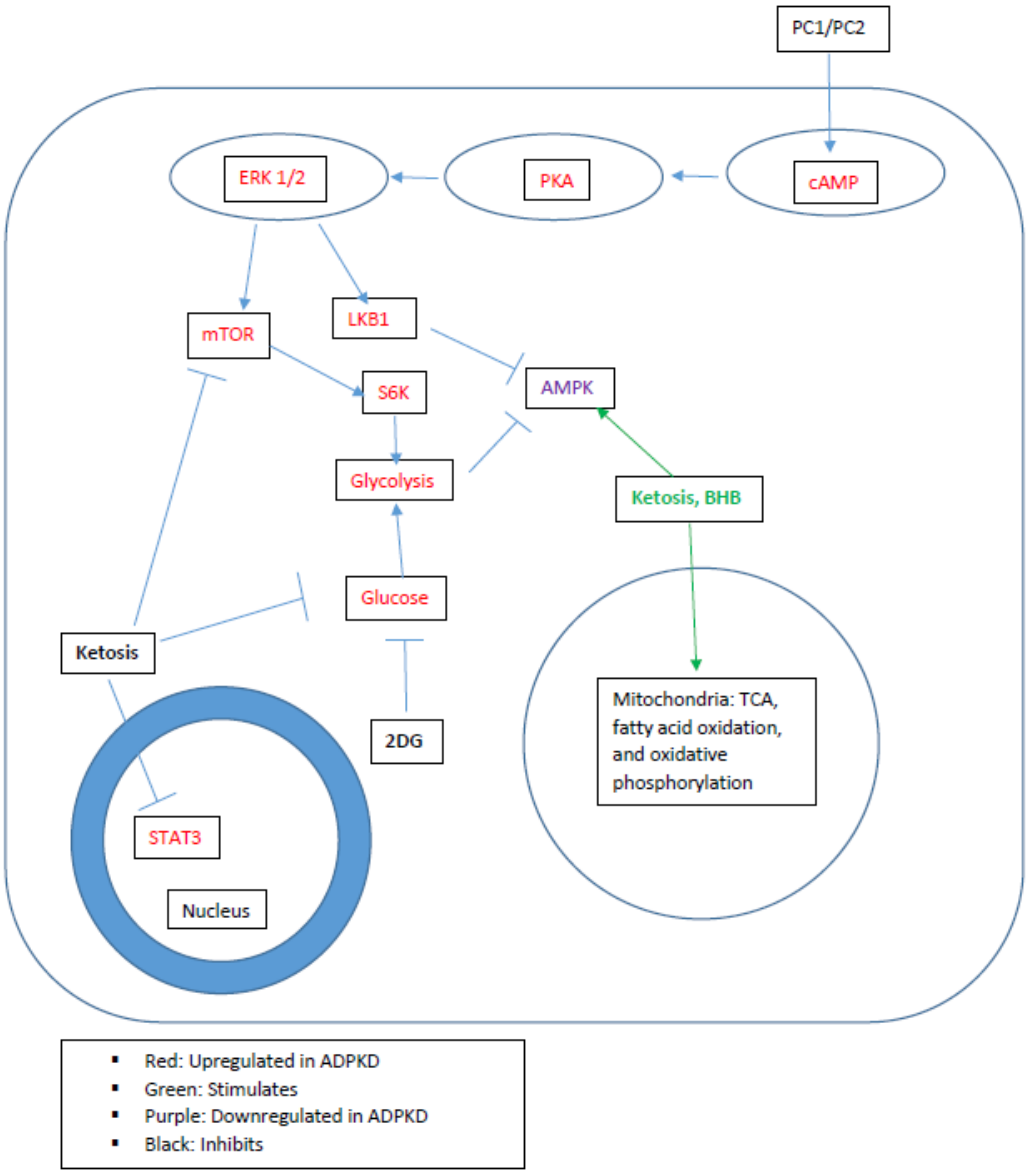

Figure 2

STUDY SYNOPSIS	
Title	Feasibility and Efficacy of a Well-Formulated Ketogenic Diet in delaying progression of Polycystic Kidney Disease in patients at risk for Rapid Progression
Study Design	Phase 1 open label single arm dietary intervention study
Study Center	The Ohio State University

Primary Objective	Renal MRI (non-contrast) measures of total kidney volume to assess disease progression, cyst growth/progression, and stiffness/fibrosis
Secondary Objective	Urine microalbumin, eGFR, body mass and composition
Number of Subjects	20 patients

Inclusion Criteria	1. Age 18-65 years old 2. Diagnosis of ADPKD by imaging and/or genetic testing 3. GFR ≥ 25 mg/dl 4. Patients at risk for rapid progression of ADPKD: Mayo Class 1C-1E along with any other high risk features including early decline in GFR, hypertension onset ≤ 35 years of age, urologic events ≤ 35 years of age, PKD 1 truncating mutation and PROPKD score of 4 or higher as determined by the treating physician 5. No changes of medications within the last three months. 6. Able to comply with dietary intervention 7. Ability to sign informed consent
Exclusion Criteria	1. Patients currently being treated with Tolvaptan 2. Diagnosed with diabetes. 3. Pregnancy 4. Contraindications to MRI 5. Ketogenic diet within the last three months 6. Severe kidney disease with GFR < 25 mg/dl 7. Unable to purchase food for the diet intervention 8. Gastrointestinal disorders that will interfere with diet intervention 9. Chronic alcohol or drug abuse

Target Population:

Patients with Class 1C-1E Mayo Clinic Imaging Class by MRI with assessment of high risk to progress to ESRD. This may include early onset hypertension ≤ 35 years of age, first urologic

event (hematuria or urinary tract infection) ≤ 35 years of age, and/or family history of rapid progression to ESRD by about age 55.

Study Duration:
12 months

OUTCOMES

Study Endpoints: Change in total kidney volume (TKV) from baseline to 1 year to assess disease progression. Change in albuminuria and GFR.

Primary Objective: The primary Objective will be to determine htTKV at baseline and 1 year after ketogenic diet intervention.

Secondary Objectives: The secondary endpoints will be measurement of GFR and 24-h urine microalbumin.

RESEARCH STRATEGY

This is a prospective study to determine ketogenic diet effect on htTKV, GFR, microalbuminuria. This is a single-center study of 20 patients with ADPKD and deemed high risk for progression to ESRD. This determined by combination of features of ADPKD and htTKV as assessed by prior computed tomography (CT) or MRI. Patients will be recruited from the Polycystic Kidney Disease (PKD) Clinic at Ohio State University Wexner Medical Center. Enrolled patients will have MRI for htTKV, urinary studies, blood tests at baseline, 6 months, and 52 weeks. Blood for GFR will be assessed three times over the course of the study including baseline, 6 months, and 1 year. Participants will follow ketogenic diet for 52 weeks. The Volek Lab Nutrition Team will manage the ketogenic diet.

METHODS

Description of Ketogenic Diet:
The goal of the KD will be to achieve a state of nutritional ketosis in each person as defined as blood BHB ≥0.5 mM. Typical BHB in a person consuming a well-formulated KD are between 0.5 and 4 mM. We will target BHB levels of >1 mM in participants in the KD, which are expected to be an order of magnitude higher than concentrations observed at baseline.

Baseline to Week 6: Participants will be provided a personalized ketogenic plan with up to 3 ready-to-eat "ketogenic-appropriate" meals per day and supplemental snacks in order to offset participant food costs, ensure nutrient quality, and facilitate adherence. In addition, natural low-carbohydrate/high-fat snacks, sources of fat, and electrolytes will be provided to meet caloric needs. This may include shelf stable items such as high-quality fats/salad dressings, salmon & sardine packets, jerky, cheese whisps, nuts and seeds. The quantity and content of these provided foods will vary from participant to participant based on caloric needs and personal preference.

Weeks 7-52: Participants will transition to a free-living ketogenic diet. They will be provided ongoing personalized support, coaching, and education by the Volek Lab Team to facilitate dietary compliance.

The ketogenic diet intervention will start after all baseline testing is complete. Each participant may require slightly different levels of coaching depending on their baseline knowledge and individual situation. Thus, the intensity of engagement may vary across participants. The ketogenic diet will follow general principles with the aim to achieve blood ketones >0.5 mM, which will require most participants to consume <50 g/day carbohydrate and ~1.5 g/kg reference weight protein. Fat will comprise the remaining calories with an emphasis on monounsaturated and saturated sources from whole foods. A wide range of foods will be incorporated including non-starchy vegetables, fruits (berries, olives, tomatoes, lemons/limes), meats (beef, chicken, pork, fish, lamb), nuts and seeds, oils (olive, canola, coconut), cheese, butter, cream, eggs, and fatty fish (salmon, sardines). Nutritional ketosis is associated with natriuresis that will lead to sodium and fluid loss if the extra sodium excreted is not compensated by individualized intakes. If untreated, the resultant hypovolemia can manifest in side effects and adrenal stress (often mis-characterized as 'keto-flu') that disrupts body fluid, electrolyte, and mineral status. Thus, slightly higher sodium and potassium intakes are required to offset the natriuresis, which will be achieved through the provision of broth/LMNT electrolyte packs and appropriate food sources and cooking methods.

MRI Imaging protocol for htTKV:

Patients will undergo investigational Magnetic Resonance Imaging for assessment of Total Kidney Volume at baseline and 1 year of study participation. MRI specific safety screening will be performed by a qualified imaging team member prior to the imaging session. Patients will be prepared for imaging by the imaging team member and imaging will be completed over the abdominal area, imaging will last no more than one hour. Studies will be carried out using FDA approved 0.55T, 1.5T, or 3T MRI units. Imaging sequences will include 2D T2-weighted Turbo Spin Echo and 3D T1-weighted fat-water separated sequences. Intravenous contrast agent will not be administered. Images of the abdomen and kidneys will be analyzed in order to calculate height-adjusted TKV according to established practices of the Mayo Imaging Classification [1].

The presence of pre- and post-treatment MRI and laboratory tests for comparing kidney size is crucial for evaluating the effectiveness of the treatment. However, there is a potential for inter and intra-observer bias in the measurement of kidney size. To address this issue, several steps and strategies can be implemented:

Standardized Protocols: Develop standardized protocols for measuring kidney size that all observers must adhere to. These protocols should include specific guidelines for image acquisition, positioning of patients, and measurement techniques.

Training and Calibration: We have ensured that that all observers receive comprehensive training on the measurement techniques and protocols, prior to the study. This training includes both theoretical and hands-on components. Additionally, periodic calibration sessions can be organized to minimize observer variability.

Quality Control Checks: We will regularly monitor the quality and consistency of measurements throughout the study. If significant variability is observed, additional training and calibration sessions should be conducted.

Interpretation Guidelines: We have developed clear guidelines for interpreting the MRI and lab results. This can help standardize the assessment of improvement in kidney size, reducing the risk of bias in interpretation.

Documentation and Audit Trails: Maintain detailed documentation of all measurements, protocols, and observer assignments. This information can be audited to ensure compliance and identify any potential sources of bias.

Table 1 | Table of Study Procedures.

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
MRI Imaging	X					X
Medical History Questionnaire	X	X	X	X	X	X
Vitals and Physical Exam	X	X	X	X	X	X
Blood Draw	X	X	X	X	X	X
Body Composition (DXA)	X	X				X

Study Procedures:

Serological Assessment and Health monitoring

Visit 1(Baseline):

- Record patient's medical history, including extrarenal manifestations
- Medications
- Pregnancy history if applicable
- Vitals
- Physical Exam
- Patients will undergo MRI non-contrast for evaluation of htTKV
- Blood will be drawn for baseline assessment of GFR, urine osmolality, and urine microalbumin to creatinine ratio
- Blood samples will be saved for metabolomics analysis
- Body composition will be assessed by dual-energy x-ray absorptiometry (DXA)
- Finger Stick BHB: Finger sticks will be taken daily in the morning, by the participant to determine ketone and glucose levels in a fasted state. Measurements will be reported daily in RedCap or Qualtrics.

CGM/CKM: Continuous Glucose Monitoring (CGM) and Continuous Ketone Monitoring (CKM) will be performed. This involves applying a sensor patch on the back of the arm to measure metabolites in interstitial fluid. The sensor can capture data for 2-weeks.

Visit 2 (6 Weeks):

- Same procedures in visit 1, except no MRI will be done at this visit.

Visit 3 (3 months):

- Same procedures in visit 1, except no MRI or DXA Scan will be done at this visit.

Visit 4 (6 months):

- Same procedures in visit 1, except no MRI will be done at this visit.

Visit 5 (9 months):
- Same procedures in visit 1, except no MRI or DXA Scan will be done at this visit.

Visit 6 (12 months): Same procedures in visit 1.
- Medications
  Vitals
- Physical Exam
- Patients will undergo MRI non-contrast for evaluation of htTKV
- Blood will be drawn for assessment of GFR, urine osmolality, and urine microalbumin to creatinine ratio
- Payment of $500

For testing at the PAES building in the Kinesiology Laboratory, subjects will arrive at the lab in the morning after an overnight fast. We will test for hydration, measure height and body mass, and then have participants complete surveys followed by resting blood pressure. A phlebotomist will obtain blood samples from a forearm vein in subjects after sitting for 10-min. Appropriate sample tubes will be used for EDTA, sodium heparin, or serum for different analytes. A serum tube will be sent to Quest Diagnostics to assess standard chemistry panel (liver enzymes, kidney function, lipids, etc.).

Serum and plasma will be immediately centrifuged and aliquoted into tubes and stored at -80°C until subsequent analyses. Samples will be thawed only once for analysis and assayed in duplicate for ketones, glucose, and insulin, which will be used to calculate an index of insulin resistance, and HbA1c. Extra serum and plasma will be archived for future analysis of biomarkers that may provide insight into novel associations with depression.

We will determine body composition from a single whole-body scan using dual-energy x-ray absorptiometry (iDXA, Lunar Corporation, Madison, WI). Total time for this visit will be approximately 1-2 hours.

DATA ANALYSIS AND STATISTICAL METHODS

The study will focus on percent change for TKV, albuminuria, and GFR pre and post the ketogenic dietary intervention. We will collect the following data for each patient including age, sex, race, baseline medications, BMI, albuminuria, GFR, and blood pressure. The univariate analysis will include the above variables and use analysis of variance to assess changes at baseline and at 12 months. Based on the univariate analysis, we will perform linear regression. Ketogenic diet administration will be forced into the model. Other variables will be chosen based on strength of the univariate analysis. Our outcome of interest will be percent change in TKV, albuminuria, and GFR. In regard to MR Imagining, we will utilize a statistical method to account for observer variability. Statistical techniques such as intra-class correlation coefficients (ICC) can quantify the level of agreement between different observers and help correct for any bias.

STUDY MONITOTRING

Diet Monitoring Protocol for Polycystic Kidney Disease (PKD) Study

The dietary guidelines for PKD closely align with CKD recommendations, emphasizing personalized sodium intake, starting at ~2300mg/day. In a well-formulated ketogenic diet, sodium levels may decrease due to reduced sodium-rich food consumption and the natriuretic effect of decreased carbohydrate intake. To address this, our dietary approach includes an additional 1-2 g/day of sodium, with adequate fluid intake to prevent hypovolemia and associated keto-flu symptoms. Recognizing individual variations, we aim to personalize sodium intakes within a general range of 1500-2300mg/day.

Monitoring Strategies:

Tailored Sodium Adjustment:

- Dietetics team to tailor sodium intake based on individual needs.
- Ongoing monitoring for signs of keto-flu, edema, rapid weight changes, and blood pressure variations.

Study Design Checkpoints:

- Incorporate consistent 24-hour dietary recalls and habitual dietary assessments.
- Regular check-ins with a registered dietitian for personalized support and adjustments.
- Education provided for recognizing high or low sodium responses.

Communication with Nutritionist:

- Participants to engage with a trained nutritionist for ongoing communication.
- Participants encouraged to share adherence to the dietary approach and address any questions or concerns.

Objective and Subjective Markers:

- Daily monitoring of blood ketones levels to assess ketosis.
- Address deviations individually with appropriate diet counseling and support.

Dietary Assessments:

- Submission of both acute (24-hour) and more habitual (30-day) diet assessments.
- Analysis of dietary data to ensure adherence and inform necessary adjustments.

Clinical Collaboration:

- Reinforce education on signs and symptoms associated with sodium balance.
- Work closely with clinicians to maintain consistent messaging and promptly address emerging issues.

This comprehensive monitoring protocol integrates various strategies to ensure effective tracking and management of the dietary approach for individuals with PKD. Regular communication,

objective markers, and collaboration with healthcare professionals contribute to a thorough assessment of diet adherence and individualized support throughout the study.

Monitoring Plan for Polycystic Kidney Disease (PKD) Ketogenic Diet Intervention Study

Objective:
The primary objective of this monitoring plan is to oversee and ensure the safety of study participants and the integrity of data collected during the Polycystic Kidney Disease (PKD) ketogenic diet intervention study. The primary marker of disease progression will be total kidney volume assessed via MRI.

Information to be Evaluated:

Patient Safety:

Adverse events related to the ketogenic diet, if any.
Monitoring of vital signs, kidney function, and electrolyte levels.
Compliance with dietary recommendations and any potential side effects.

Data Integrity:

Accuracy and consistency of MRI measurements of total kidney volume.
Proper documentation of patient data and dietary adherence.

Monitoring Personnel:

Principal Investigator (PI): The PI will have overall responsibility for the study and will lead the monitoring efforts.

Key Personnel : The study coordinator will assist in data collection, ensure compliance with the study protocol, and report any issues to the PI.

Timing of Monitoring:
Monitoring will be conducted at the following time points:

Baseline: Prior to enrollment, each participant's medical history, kidney function, and MRI scans will be assessed.

Regular Intervals: During the study, participants will be monitored regularly. This includes assessments of dietary adherence (weekly by Key Personnel), vital signs, anthropometrics, blood work and glomerular filtration rate (GFR) will be used for monitoring disease progression at the specified intermediate time points (Baseline, Week 6 and Month 3, Month 6, Month 9, Month 12), and kidney function (Baseline, Month 12 via MRI).

Adverse Event Reporting: Any adverse events related to the ketogenic diet or the study protocol will be monitored continuously and reported promptly.

Decisions and Actions:

Data Review Meetings: The study team will conduct regular data review meetings to assess the safety and progress of participants. This will involve a review of adverse events, dietary adherence, and MRI results.

Stopping Criteria: The study will include predefined stopping criteria to protect participant safety. If severe adverse events related to the ketogenic diet occur or if there is a significant worsening of kidney function, the study may be halted early.

Reporting: All monitoring findings, decisions, and actions will be documented in a monitoring report. Serious adverse events will be promptly reported to the Institutional Review Board (IRB) and relevant regulatory authorities, as required. Detailed Adverse enevt monitoring can be found below.

Participant Withdrawal: If participants wish to withdraw from the study, they will be allowed to do so at any time without prejudice.

Early Termination:
We anticipate that participants will follow the diet plan as closely as possible to ensure the study's success. In the event of a more than twofold increase in serum creatinine, reaching Stage II acute kidney injury, we may consider early termination from the study. However, we recognize that individual differences, especially in creatinine levels related to protein intake, may occur. Therefore, decisions about early termination will be made in consultation with a physician. Subsequent visits will involve careful consideration of the rate of increase in serum creatinine, allowing the physician to determine whether continued participation is safe and advisable for the participant's health. Our top priority is the well-being of each participant, and any decisions about early termination will be made with their best interests in mind.

Conclusion:
This monitoring plan for the PKD ketogenic diet intervention study is designed to ensure participant safety and maintain data integrity throughout the study. Regular assessments, reporting mechanisms, and decision-making criteria are in place to address unanticipated problems and make informed decisions regarding study continuation or termination.

Adverse Event Monitoring

An AE is defined as any untoward medical occurrence in an investigation participant following written informed consent that does not necessarily have a causal relationship with the study product. An AE can be any unfavorable or unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product. This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures (including laboratory test abnormalities).

Some side effects or GI symptoms could occur as an outcome of these dietary interventions; side effects listed in the beverage tolerability questions and reported will not be categorized as AEs but recorded as study outcomes. Side effects, outside of what is expected as a result of study product consumption, reported by participants and judged by the Investigators as medically relevant events and related to study product will be recorded as AEs.

Events should be considered AEs if they:

- Result in discontinuation from the study,
- Require treatment or any other therapeutic intervention,
- Require further diagnostic evaluation (excluding a repetition of the same procedure to confirm the abnormality),
- Are associated with clinical signs or symptoms judged by the Investigator to have a significant clinical impact.

Grading and Severity

The Investigator will evaluate all AEs with respect to their severity, and record the outcome and action taken on the AE study documents . AEs will be graded as:

Mild: Awareness of symptoms but easily tolerated

Moderate: Discomfort enough to interfere with but not prevent daily activity

Severe: Unable to perform usual activity

Relationship

The Investigator will also judge the likelihood that the AE was related to the study beverage and document this on the appropriate study documents as:

NOT RELATED	This category applies to those adverse experiences which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).
UNLIKELY	In general, this category can be considered applicable to those experiences that after careful medical consideration at the time they are evaluated, are judged to be, unlikely related to the study beverage.

POSSIBLY	This category applies to those adverse experiences for which, after careful medical consideration at the time they are evaluated, a connection with the study beverage administration appears possible but cannot be ruled out with certainty.
PROBABLY	This category applies to those adverse experiences that, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the study beverage.
DEFINITELY	This category applies to those adverse experiences which, the Investigator feels are incontrovertibly related to the study beverage.

Appropriate therapeutic action and follow-up measures will be performed by the Investigator in accordance with appropriate medical practice standard of care.

Serious Adverse Event Definition/Qualification

A SAE is defined as an AE that results in any of the following outcomes:

- Death (note that death is the outcome of a SAE and the cause of death should be listed as the AE),
- Life-threatening event,
- In-patient hospitalization or prolongation of existing hospitalization,
- A persistent or significant disability/incapacity,
- Congenital anomaly or birth defect,
- Any other important medical event that may not result in death, be life-threatening, or require hospitalization, may be considered a SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

In the event of a SAE, the participant may be dropped from the study if the Investigator deems it necessary.

Serious Adverse Event Reporting Instructions

If in the opinion of the Investigator the event meets the criteria of a SAE the following procedures will be followed:

- The Investigator will notify Institutional Review Board (IRB) of the SAE within the parameters and timeframe specified under the IRB Standard Operating Procedures (SOP). An initial report followed promptly by a complete report will be forwarded to the IRB, when applicable.
- If a participant is hospitalized or hospitalization is prolonged due to the SAE, the hospital discharge summary will be obtained if possible.

- If a death occurs and an autopsy is performed, a copy of the autopsy report will be obtained if possible.
- All efforts must be undertaken to obtain follow-up information promptly.

Recording of Adverse Events

All AEs (AE or SAE) will be recorded on the AE study documents. For participants who have an ongoing AE at their final study visit, follow-up information will be captured in the AE eCRF page which will be completed after 30 days.

Serious Adverse Event Follow-Up

For all ongoing SAEs occurring during the study, the Investigator must submit follow-up reports regarding the participant's subsequent course. All SAEs that are ongoing at the end of the study or upon discontinuation of the participant's participation must be followed until either:

- The event resolves, or
- The event/condition has stabilized (e.g., in the case of persistent impairment), or
- The event returns to baseline, if a baseline value is available, or
- The participant dies, or
- The event can be attributed to other than the study beverage, or to other than the study conduct.

Safety and Adverse Events via MRI

An adverse event is defined as illness, signs or symptoms that worsen over the course of the study. Abnormal lab tests, if deemed by the investigator to have resulted from ketogenic diet intervention, would be considered an adverse event. If the abnormal lab results lead to symptoms, further diagnostic tests, or withdrawal from the study, they are considered adverse events.

MRI at 0.55 Tesla, 1.5 Tesla, or 3 Tesla is considered a minimal risk procedure. There are no additional safety risks associated with the investigational MRI imaging. For patients receiving investigational 3 Tesla MRI (or lower field strength) imaging, the associated risks are the same as those that would normally be encountered in a standard of care MRI examination, and they are as follows:

Magnetic Resonance Imaging devices use three different types of electromagnetic fields that may pose potential risks to subjects undergoing MRI examinations. These are static magnetic fields, time varying magnetic field gradients, and radiofrequency (RF) electromagnetic fields.

1) Static magnetic fields exert forces on magnetic objects and induct electric currents in any conductive material, imposing two main risk categories:

a) Dislodgement or malfunction of medical implants or metallic foreign objects.

b) Current induction in tissue and subsequent alteration of physiologic function.

The FDA has classified MRI up to 8 Tesla as Non-Significant Risk.

In addition, subjects may be harmed by

- Loud noise from the MRI device.
- Some subjects may experience mild transient vertigo and/or metallic taste when they are being moved into the MRI system.
- Subjects may experience discomfort from the blood pressure cuff and finger clamp from the pulse-oximeter. Some subjects may experience feelings of being closed in if they are claustrophobic.
- Long-term effects of high field MRI are not known at this time.

Risk from static, time varying magnetic field gradients and radiofrequency fields are at or below the FDA limits and thus risks are identical to standard field strength MRI units.

All above listed known effects of exposure to static magnetic fields, switched magnetic field gradients and RadioFrequency are transient, i.e. vertigo, spontaneous visual light perceptions, metallic taste, peripheral nerve stimulation as well as RF heating will end once exposure to the fields is ended. Based on this, cumulative effects are not expected, however long-term effects are not known at this time.

To minimize risks from the static magnetic field as with standard MRI, the following subjects will be excluded from this study (see MRI Safety Screening Forms for further details):

- Subjects who have any type of bioimplant activated by mechanical, electronic, or magnetic means (e.g., cochlear implants, pacemakers, neurostimulators, biostimulators, electronic infusion pumps, etc.).
- Subjects who have any type of ferromagnetic bioimplant that could potentially be displaced or damaged.
- Subjects who have cerebral aneurysm clips.
- Subjects who may have shrapnel imbedded in their bodies (such as from war wounds), metal workers and machinists (potential for metallic fragments in or near the eyes), severe car accident victims.

Because effects of high field MRI are not known at this time also excluded are:

- Subjects who have permanent tattoo (e.g., eye liner) (may contain metallic coloring).

Also excluded are subjects incapable of giving informed written consent

- Subjects who cannot adhere to the experimental protocols for any reason, or have an inability to communicate with the researcher.
- Subjects who have limited mental ability to give informed consent, mentally retarded, altered mental status, mental disability, confusion, or psychiatric disorders.
- Prisoners

To minimize discomfort we will also exclude subjects who exhibit noticeable anxiety and/or claustrophobia or who exhibit severe vertigo when they are moved into the MRI system.

All subjects will be given ear protection to prevent risks from loud noise.

CONDUCT OF THE STUDY

1. **Ethics and Regulatory Considerations**

This study will be conducted according to Good Clinical Practice Guidelines, the Declaration of Helsinki (2004) and United State Code of Federal Regulation Title 21. Signed written informed consent for participation in the study will be obtained from all participants before protocol-specific procedures are carried out. Participants will be informed of their right to withdraw from the study at any time. Participants will be informed that their participation in the study is completely voluntary, personal information will be both coded with a study ID to preserve anonymity.

2. **Institutional Review Board**

The Investigator will ensure that an appropriately constituted IRB, in compliance with the requirements of 21 CFR 56, reviews and approves the clinical study. Before the study is started, the Investigator will forward copies of the protocol and consent form for this study to the IRB for review and approval. IRB approval must refer to the study by exact protocol title and number, identify the documents reviewed, and state the date of review. The IRB must be informed of all subsequent protocol amendments. No alterations, modifications to IRB-approved documents, including the protocol, protocol summary, consent form, recruitment materials and questionnaires will be allowed. The IRB must also be informed of all SAEs and of unexpected AEs as outlined in the IRB's SOPs or reporting guidelines.

3. **Informed Consent and Protected Health Information**

The study will be explained verbally as well as on the informed consent document. Each participant will be given ample opportunity to inquire about details of the study and to read and understand the consent form before signing it. It will be made clear that participants can withdraw from the study at any time.

Each participant's signed informed consent document must be kept on file by the Investigator. The participant should receive a copy of the informed consent document. A participant may not be admitted to the study unless informed consent of the participant (or his/her legally authorized representative) has been obtained.

4. **Participant Confidentiality**

The Investigator is responsible for ensuring that participants' anonymity will be maintained. For all the data collected over the course of the study for each participant (i.e., records, biological samples and questionnaires) a unique subject identifier (i.e., a code) will be assigned and used instead of the subject's name. The code for each participant which links the subject name with their identifier will only be available to research personnel. Electronic CRFs or other documents will identify participants by initials, number, or code, and not by name. The Investigator will keep a separate log showing codes, names, and addresses. Any records that contain the subject's name and identifier will either be stored in the Kinesiology file storage room in a file cabinet (locked) or protected on a computer via password protection on the individual digital file and password protection on the computer the file(s) are stored on. All other records that contain the subject identifier only will also be kept in either a file cabinet in our locked file storage room or on a password protected computer. Subject names will never be used in any presentation or publication resulting

from this study. The records will be maintained until the data are published and up to a maximum of ten years after the completion of the study. All records or biological data obtained after signing of the informed consent (including the screening visit, even for subjects that are not eligible for participation in the study) are treated with the same confidentiality safety measures as those subjects who qualify. Any information obtained during the prescreening for participants that were not eligible will be deleted

5. **Withdrawal of Participants from the Study**

Participants may be removed from the study for any of the following reasons:

- A participant requests discontinuation;
- The Investigator initiates removal for medical or compliance reasons;
- Occurrence of any AE or condition that could, in the Investigator's opinion, interfere with the evaluation of the effect of the study beverage or put the participant at undue risk.

It is understood by all concerned that an excessive rate of withdrawals can render the study uninterpretable, therefore, unnecessary withdrawal of participants should be avoided. Should a participant decide to withdraw, all efforts will be made to complete and report observations as thoroughly as possible. In the event that a participant is withdrawn from the study, the reason for the withdrawal will be documented in the eCRF.

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