

SPECIFIC AIMS:

Kidney stones (KS) are painful mineral deposits that affect 1 in 11 people in the United States. Lifestyle factors, genetics, and diet may contribute to the development of KS. The most common type of KS is composed of calcium oxalate (CaOx) crystals which form when urine becomes supersaturated with these constituents. Meals containing high amounts of oxalate can increase urinary oxalate excretion and promote crystal formation. Accumulation of crystals within the kidney can lead to stone formation. Unfortunately, treatment options to prevent stone formation/recurrence are limited, and this contributes to increased healthcare costs and diminished quality of life.

Several studies have reported that exposure of cultured renal cells to CaOx crystals results in inflammatory responses that include cytokine release and monocyte recruitment. Subsequently, crystals are degraded and dissolved by macrophages, which rely on reactive oxygen species (ROS) signaling and metabolism to mitigate inflammation. We previously reported that circulating monocytes from patients with CaOx KS have decreased cellular energetics and increased inflammation compared to healthy subjects. In my K01, we further determined that oxalate disrupts cellular energetics and redox status in a monocyte cell line and primary monocytes from healthy subjects. This was the first recorded evidence illustrating the impact of oxalate on monocyte metabolism. Our recent preliminary data demonstrates a single dietary oxalate load stimulates crystalluria in healthy subjects. We also observed that oxalate decreased monocyte cellular energetics in some healthy subjects similar to patients with KS and this may be mediated by mitochondrial complex I. In addition, rats fed a diet containing 5% hydroxy-L-proline (HLP; an oxalate precursor) develop increased urinary oxalate levels, renal crystals, and renal pro-inflammatory macrophages.

Building on these findings, the **long term goal** of this research is to understand how oxalate impacts monocyte and macrophage immunometabolism in CaOx KS disease. The **objective of this study** is to investigate the relationship between urinary crystals, macrophage activation, ROS, and pro-inflammatory signaling pathways following oxalate consumption. We will define the effects of oxalate enriched meals on crystalluria and monocyte and macrophage responses in human subjects and a rat animal model. The **central hypothesis** is oxalate disrupts mitochondrial complex I activity in monocytes and stimulates ROS generation, pro-inflammatory macrophage differentiation, and renal crystal deposition in CaOx KS disease (Fig. 1). To address this hypothesis, two specific aims will be tested:

Aim 1: Test the hypothesis that oxalate stimulates reverse electron transport (RET) through mitochondrial complex I in monocytes from healthy subjects.

- Healthy subjects will consume a previously optimized low oxalate or oxalate-enriched controlled diet over 14 days. A novel approach to quantify urinary nanocrystals will be evaluated using NanoSight technology.
- Monocyte cellular energetics and mitochondrial complex activity will be evaluated using a Seahorse XF Analyzer.
- Flow cytometry and molecular biology approaches will be used to evaluate monocyte and macrophage subtype characteristics by RNA sequencing, RET, mitochondrial properties, oxidative stress, synthesis of inflammatory cytokine/chemokine levels, and macrophage phagocytosis.

Aim 2: Test the hypothesis that oxalate mediated crystalluria induces ROS and disrupts macrophage immunometabolism in rat kidneys.

- Sprague-Dawley rats (male and female) will be fed either low (1%) or high (5%) HLP for 28 days. Urinary crystals will be confirmed using NanoSight technology and crystal deposition will be assessed via histology.
- Flow cytometry and molecular biology approaches will be used to assess mitochondrial properties, oxidative stress, RET, and inflammatory proteins in monocyte or macrophage populations from the circulation and kidney. Additionally, the cellular transcriptome of macrophages isolated from the kidney will be examined.

These aims complement the ongoing experiments in my K01 award. Successful completion of these aims will reveal mechanisms regarding the role of dietary oxalate on crystalluria and monocyte and macrophage responses during CaOx KS disease. The **impact of this research** may identify: 1) tools to assess stone risk and immune status, and 2) therapeutic targets to alter monocyte and macrophage immunometabolism in KS disease. The data generated from this proposal will advance our knowledge about the role of dietary oxalate and innate immunity in KS disease and serve as a key foundation for subsequent R01 funding.

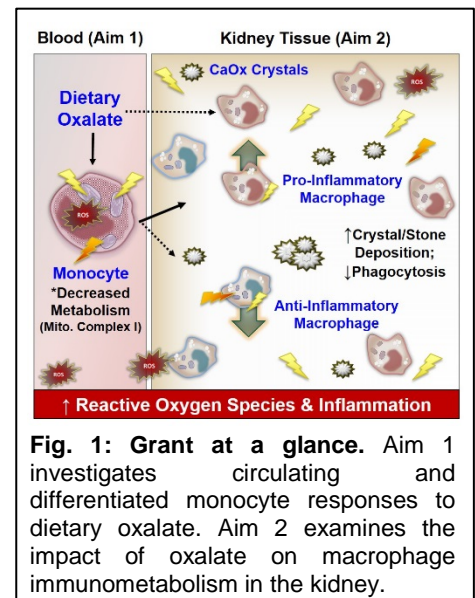


Fig. 1: Grant at a glance. Aim 1 investigates circulating and differentiated monocyte responses to dietary oxalate. Aim 2 examines the impact of oxalate on macrophage immunometabolism in the kidney.

RESEARCH STRATEGY—Significance:

KS Disease and Dietary Oxalate: The prevalence of KS formation is expected to rise and cause an extensive economic burden worldwide^{1,2}. KS are extremely painful and the etiology of KS disease is not fully characterized. Lifestyle factors, genetics, and diet contribute to KS disease^{1,3,4}. There are no treatments available to cure or prevent stone formation and recurrence. Oxalate is a small molecule found in plants and specific vegetables, nuts, and fruits⁵. It is absorbed in the intestine and excreted in the urine⁶. Meals containing high amounts of oxalate can increase urinary oxalate excretion^{3,7}, which is a risk factor for CaOx KS^{8,9}. It has been shown that a transient increase in urinary oxalate excretion develops 2–6 hours after dietary oxalate ingestion^{7,10,11}. During these periods, urine becomes super-saturated with CaOx leading to crystal formation and stone growth. Thus, there is a **critical need** to dissect mechanisms of how stones form in order to identify strategies to prevent KS.

Monocytes, Macrophages, and Immunometabolism: CaOx crystals increase monocyte chemoattractant protein (MCP-1) secretion in renal proximal tubular cells¹² in an effort to promote crystal clearance. Monocytes circulate in peripheral blood and can differentiate into pro- or anti-inflammatory macrophages within tissues. Pro- or anti-inflammatory macrophages regulate inflammation using glycolysis or oxidative phosphorylation, respectively¹³. Mitochondria play an important role in regulating inflammation within monocytes and macrophages¹³ using ROS signaling¹³⁻¹⁷; however, chronic over-production of mitochondrial ROS by the electron transport chain (ETC) can cause oxidative stress¹⁶. This can occur by RET which occurs when electrons flow backwards through complex I¹⁸. It has been shown that RET is important for macrophage metabolic reprogramming and activation¹⁹. Importantly, overproduction of ROS can lower the ability of monocytes and macrophages to resolve inflammation^{13,17} in chronic kidney disease (CKD) and acute kidney injury^{20,21}. CaOx crystals have been reported to induce inflammatory pathways in dendritic cells²², alter mitochondrial protein levels in human monocytes²³, and differentiate human monocytes into pro-inflammatory macrophages²⁴. We previously demonstrated that monocytes have decreased cellular energetics and increased inflammation in a cohort of CaOx stone formers compared to healthy subjects²⁵. We further determined that oxalate disrupts cellular energetics and redox status in primary monocytes from healthy subjects²⁶. A **large gap in our knowledge** is understanding whether oxalate from the diet impacts monocytes and macrophages and promotes crystal retention/growth during KS disease.

Oxalate Feeding Studies: Meals rich in oxalate that induce CaOx crystalluria could cause inflammation and alter monocyte responses. The experiments detailed in this proposal using humans and an animal model, will investigate the relationship between crystals, macrophages, ROS, and inflammation following oxalate consumption. Controlled optimized oxalate enriched meals will be used for the human studies^{7,27}. We and others have determined that HLP induces urinary oxalate and crystal formation in mice²⁸ and rats²⁹. HLP is an oxalate precursor and is found in western diets (e.g. gelatin and meat). Rats will be fed either 1% or 5% HLP to identify mechanisms that may be associated with stone disease that are limited with the use of humans. These models will help determine whether dietary oxalate mediated crystalluria alters monocyte and macrophage functions.

Scientific Premise: Prior studies have highlighted an important role of dietary oxalate in KS disease. However, a limited number of studies have focused on the importance of monocytes and macrophages in stone formation^{23,24,30,31}. There are no reports showing whether dietary oxalate influences the immune system in KS disease. Based on the literature and our preliminary data, understanding the significance of dietary oxalate on crystal formation and monocyte/macrophage function could bring valuable insight to the field. The **significance of this research** will 1) expand our scientific knowledge about the role of dietary oxalate on immunometabolism, 2) unravel the relationship between urinary crystals and monocytes/macrophages, and 3) provide a framework for identifying strategies to intervene in the pathogenesis of CaOx KS disease. The proposed studies will provide insight regarding ROS mediated alterations in monocyte and macrophage immunometabolism during crystal formation and could have prognostic value to assess stone risk. Ultimately, this could assist clinicians in patient care management (i.e. personalized dietary or prescription recommendations) and improve clinical outcomes.

Innovation: This will be the first study to evaluate the effects of dietary oxalate induced CaOx crystalluria on monocyte and macrophage immunometabolism in rats and humans. Novel approaches that will be used to address the objective of this proposal include: combining oxalate feeding studies, NanoSight Technology, Seahorse XF Analyzer, and RNA-sequencing. Longitudinal studies in healthy subjects on low and high oxalate diets will allow us to determine mechanisms linking crystalluria with monocyte responses. The NanoSight will identify urinary nanocrystals that are not visible with conventional microscopy and could be useful to predict stone risk. The impact of RET on monocyte immunometabolism has not been explored and will provide new insight not only to KS disease but other metabolic diseases where macrophages are altered. Additionally, cellular transcriptomics will identify pathways altered in monocytes in response to oxalate. Elucidating the significance of immunometabolism during KS disease could aid in identifying strategies to prevent KS formation.

Approach

Aim 1: Test the hypothesis that oxalate stimulates reverse electron transport (RET) through mitochondrial complex I in monocytes from healthy subjects.

Rationale: Mitochondria are a source and target for oxidative stress and the failure to maintain mitochondrial quality ultimately leads to cell death³²⁻³⁵. Many metabolic/systemic diseases are associated with increased systemic oxidative stress and mitochondrial dysfunction^{32,34,36-39}. We and others have used the Seahorse XF Analyzer to assess bioenergetic profiles in circulating immune cells isolated from healthy subjects and patient populations^{40,41}. We have previously determined that monocytes from patients with CaOx KS disease have decreased cellular energetics compared to healthy subjects²⁵. This could be due to a deficit in mitochondrial biogenesis, substrate availability, mtDNA damage, or respiratory protein damage^{32,42}. Such responses could provide evidence that the cell cannot satisfy the energy demand through mitochondrial respiration and could lead to oxidative stress through RET, mitochondrial damage, inflammation, and cell death^{18,43-49}. To identify potential stimuli responsible for the monocyte changes observed in patients, we tested whether oxalate could cause similar responses in monocytes from healthy subjects. We reported CaOx crystals (50 $\mu\text{g}/\text{ml}$) or sodium oxalate (0.1 mM) significantly decreased monocyte cellular energetics²⁶ (Data from K01; not shown). These data suggest dietary oxalate could reduce monocyte function in humans and is a scientific premise for this aim.

Preliminary Studies: Intake of high oxalate meals is associated with increased plasma and urinary oxalate levels and crystalluria^{3,7}. However, the effect of dietary oxalate on immune cells has not been elucidated. We have shown that a sodium oxalate load can increase urinary oxalate levels up to 6 hours in healthy subjects⁷. The amount of oxalate in the load was equivalent to that found in 100 grams of spinach, which is approximately a normal serving. Thus, we tested whether providing healthy subjects a 3 day low oxalate diet followed by a single spinach smoothie (700 mg oxalate load) would have any effect on crystalluria and monocytes.

Our preliminary data shows that a high oxalate load significantly increased urinary oxalate (both crystalline and soluble) levels (**Fig. 2A**). We observed nanocrystals (~180 nm) in post-oxalate urine using a NanoSight particle counter with a calcium-based fluorescence dye (**Fig. 2B**) and electron microscopy (**Fig. 2B inset**). It has been reported that nanocrystals cause greater injury to renal cells compared to larger crystals⁵⁰. Thus, the presence of nanocrystals within 5 hours could stimulate cellular responses to disrupt monocyte function. To investigate this further, we used the Seahorse XF Analyzer to evaluate monocyte cellular energetics and mitochondrial complex activity. We determined that the high oxalate load decreased monocyte cellular energetics compared to pre-oxalate samples in a small cohort of healthy subjects similar to results found in patients with CaOx KS (**Fig. 3A**). Interestingly, mitochondrial complex I activity was significantly decreased in this same cohort (**Fig. 3B**). These data suggest that mitochondrial complex I could play an important role in monocyte immunometabolism. To explore this further, we evaluated monocyte cellular transcriptomics using RNA sequencing. Ingenuity pathway analyses revealed alterations in key mitochondrial genes and cytokines in response to oxalate. As shown in **Fig. 4**, NADH:ubiquinone oxidoreductase core subunit V2 (NDUFV2), a mitochondrial complex I gene involved in electron transfer, was downregulated 5 hours following an oxalate load. However, succinate dehydrogenase assembly factor 2 (SDHAF2), a mitochondrial complex II assembly gene important for succinate activity was upregulated. Accumulation of succinate has been linked to increased RET¹⁸. Other key genes shown to be modified by oxalate were Interleukin-6 (IL-6) and Interleukin-10 (IL-10) (**Fig. 4**). Oxalate increased IL-6 expression (pro-inflammatory cytokine), which supports our prior findings demonstrating IL-6 is elevated in the plasma of KS patients²⁵. In contrast, IL-10, a cytokine important for activating anti-inflammatory macrophages was downregulated by oxalate. It has been reported that bone marrow-derived

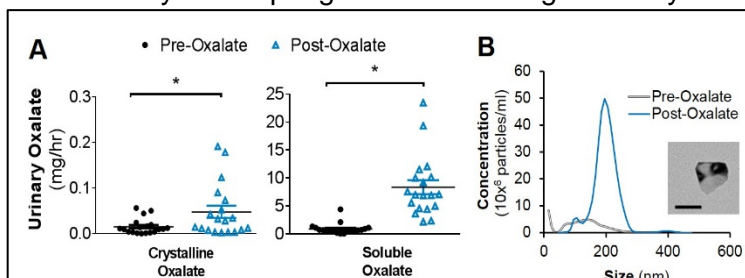


Fig. 2: A single oxalate load increases urinary oxalate levels and crystalluria in healthy subjects. (A) Urinary oxalate (crystalline and soluble) levels in healthy subjects following a dietary oxalate (spinach smoothie) load. * $p < 0.05$. **(B)** Size distribution of urinary crystals using the NanoSight NS300. Inset: Electron micrograph of post-oxalate urinary crystal. Scale bar, 200 nm.

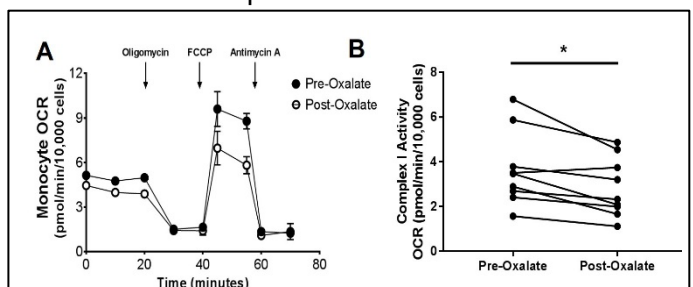


Fig. 3: A single oxalate load decreases monocyte cellular energetics and mitochondrial complex activity in healthy subjects. (A) Cellular energetics and **(B)** Mitochondrial Complex I activity in monocytes from healthy subjects following a dietary oxalate (spinach smoothie) load. $n = 5-6$ replicates/group. * $p < 0.05$.

macrophages from mice deficient in IL-10 have dysfunctional mitochondria⁵¹. These data suggests that oxalate may induce urinary crystals and disrupt monocyte mitochondrial function by RET and cytokine signaling.

Experimental Design and Methods: The experiments in this aim are designed to determine the impact of dietary oxalate over time on crystalluria and monocyte responses in healthy subjects.

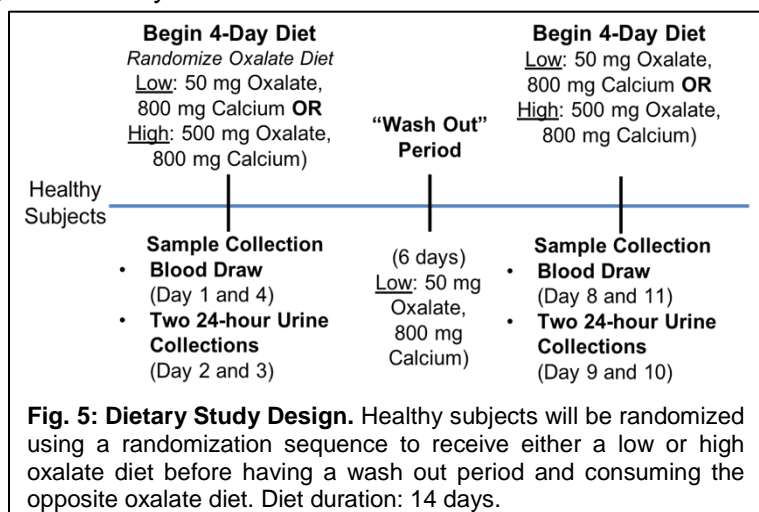
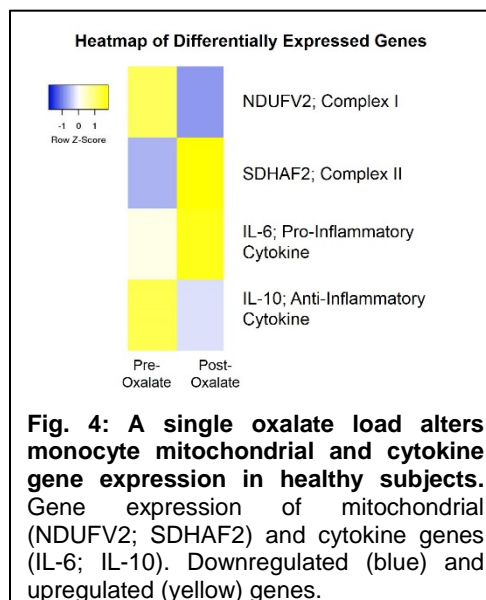
1.1. Participant enrollment: Healthy subjects (n=20; 10M; 10F) will be enrolled for these studies if they have a normal blood comprehensive metabolic panel, are non-tobacco users, non-pregnant, have a BMI between 20-30 kg/m², and without medical conditions (**Human Subjects Section**). A detailed clinical profile including family history of KS disease and serum/urinary values will be noted. Any changes in health status or any adverse events will be documented. Power analysis is described in the Sample Size and Statistical Analysis section.

1.2. Dietary Plan: Our laboratory has extensive experience with human controlled dietary studies^{7,27}. For these studies, participants will be randomly assigned to consume either a low or oxalate enriched diet for 4 days. Participants will then have a 6 day “wash out” period consisting of a controlled low oxalate diet before consuming the other oxalate diet for 4 days (**Fig. 5**). The washout period will be implemented to reduce any carry-over effects. These time points were selected based on our preliminary data and longitudinal studies illustrating a return to baseline in urinary oxalate and monocyte cellular energetics levels (data not shown). The intake of dietary oxalate and calcium will be controlled and will be targeted as follows: low oxalate diet (50 mg/2,500 kcal/day) or high oxalate diet (500 mg/2,500 kcal/day). Both diets will contain 800 mg/2,500 kcal/day of calcium. The oxalate contents outlined here are below and above the reported range of normal oxalate intake (~150 mg/2,500 kcal/day)⁵². Some of the meals will include: turkey, chicken, broccoli, or potatoes. Food preparation and distribution will be organized by experienced dietitians and personnel at the UAB Center for Clinical and Translational (CCTS) Bionutrition Unit. Food oxalate and calcium levels will be characterized by the Nutrition Data System for Research and will be confirmed using ion chromatography-mass spectrometry (IC-MS) to ensure unbiased results. Fluid intake will be controlled and recorded throughout the study. Each participant will serve as their own control.

1.3. Urinary Oxalate Levels and Nanocrystals:

Twenty-four hour urine samples will be collected and processed immediately to evaluate oxalate levels⁷ and crystal formation⁵³. Urinary oxalate levels will be measured using IC-MS^{7,28}. Urinary crystals will be isolated by a series of centrifugations and incubated with calcium specific dyes to accurately detect crystal size and count using the NanoSight NS300. Fourier-transform infrared spectroscopy (FTIR) will be used to detect whether CaOx or calcium phosphate crystals are present. Crystals will be further assessed using electron microscopy. Certified human urine quality controls and urine containing purchased CaOx crystals will be used as controls for these experiments.

1.4. Monocyte and Macrophage Analyses: Participants will have their blood drawn at the UAB CCTS Clinical Research Unit (CRU). Blood will be processed within 30 minutes. Plasma oxalate levels will be assessed using IC-MS. Monocytes will be isolated from blood to measure cellular energetics and mitochondrial complex activity using the Seahorse XF Analyzer^{40,41}. Flow cytometry will be performed to assess monocyte populations (e.g. CD14, CD11b/c) and intracellular cytokine signaling (i.e. IL-6, IL-1 β , and TNF- α). Proper gating and isotype controls will be included for flow cytometry analyses. Isolated monocytes will also be used to examine the effects of dietary oxalate on RET by measuring NADH activity, succinate, mitochondrial membrane potential and hydrogen peroxide production^{54,55}. Isolated monocytes will be treated with inhibitors (e.g. metformin) to validate the role of oxalate induced RET⁵⁶. In addition, monocyte transcriptomics will be performed and validated using quantitative real-time PCR (qRT-PCR). Lastly, monocyte differentiation and phagocytosis will be evaluated³¹.

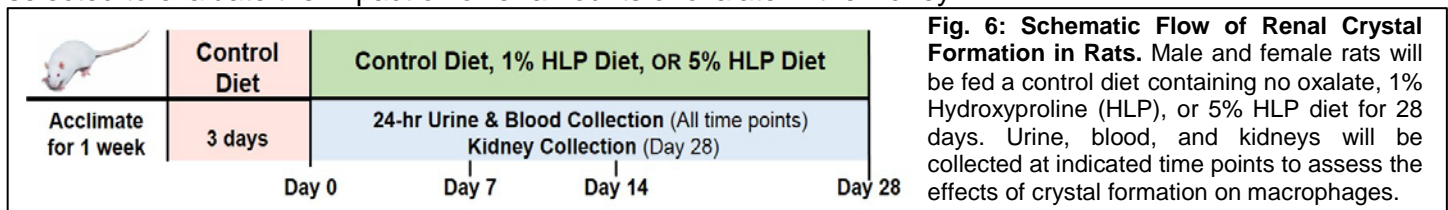


Expected Outcomes, Potential pitfalls and Alternative Approaches: We expect that oxalate enriched (high oxalate) meals will support our preliminary data and show that oxalate increases plasma and urinary oxalate levels and urinary nanocrystals. We anticipate increased crystalluria and plasma oxalate will correlate with decreased monocyte cellular energetics and mitochondrial complex I activity based on oxalate concentrations. It is also anticipated RET will occur through complex I and this will disrupt monocyte cellular energetics by increased ROS. If RET is not detected, we will investigate whether increased ROS from Complex I could be due to a reduced NADH pool which has been shown to affect mitochondrial metabolism⁵⁷. It is expected high oxalate mediated RET will reduce the ability of macrophages to phagocytose crystals. We anticipate experiments using RET inhibitors (e.g. metformin, rotenone) will reverse expected outcomes noted above. There may be a significant inter-individual variability that dampens differences and influences statistical analyses due to the sample size, which may complicate data interpretation. Such effects, however, should be limited by the tight dietary control that will be implemented. Another potential pitfall of the study will be diet adherence and inadequate 24-hour urine collections. Alternative approaches may involve expanding the sample size to account for biological variability and to use spouses or household members to limit the effect of environmental factors on measured outcomes. We have the expertise and tools required to complete this aim. We expect these studies will establish an appropriate model to test in patients with CaOx KS and help identify a potential diagnostic tool to predict stone risk.

Aim 2: Test the hypothesis that oxalate mediated crystalluria induces ROS and disrupts macrophage immunometabolism in rat kidneys.

Rationale: Several experimental and clinical studies have suggested oxidative stress and inflammation contribute to stone pathogenesis^{22,58-65}. It has been established that renal cells exposed to CaOx crystals or oxalate have increased oxidative stress and inflammatory responses including increases in the expression of MCP-1, Interleukin-2R (IL-2R β) receptor, and IL-6^{12,63,66,67}. Two laboratories previously reported that CaOx crystals increase IL-1 β secretion in dendritic cells²² and induce changes in mitochondrial proteins involved in metabolism in a human monocytic cell line²³. Taguchi et al. used colony stimulating factor-1 (CSF-1)-deficient mice to show anti-inflammatory macrophages play an important role in renal crystal formation during hyperoxaluria³⁰. A recent study established that CaOx crystals differentiates human monocytes into M1 macrophages²⁴. This likely occurs due to changes in metabolism and oxidative stress as indicated by our recent publication where we showed oxalate (soluble and insoluble forms) disrupts monocyte metabolism and redox status²⁶. Taken together, it appears that monocytes and macrophages may play an important role in stone pathogenesis. Understanding the interplay between inflammation, oxidative stress, and monocytes and macrophages is necessary to prevent KS growth and serves as a rationale for these studies.

Preliminary Studies: Our laboratory and others have previously determined that HLP feeding increases urinary oxalate excretion and crystal formation in mice²⁸. For the experiments outlined in this aim, six to eight week old Sprague Dawley rats (male and female) will be fed either a purified control diet containing no oxalate, a 1% HLP diet, or a 5% HLP diet for 28 days (**Fig. 6; Vertebrate Section**). The 5% concentration of HLP was selected as it has been shown to induce crystal formation in rats²⁹ and higher concentrations are toxic. The 1% HLP diet was selected to evaluate the impact of lower amounts of oxalate in the kidney.



To confirm the feasibility of this model, we evaluated urinary oxalate levels using IC-MS. As shown in **Fig. 7A**, rats fed 5% HLP had a significant increase in urinary oxalate levels compared to animals fed a control diet at Day 7 and 28. Furthermore, these same animals had increased renal crystal formation (white spots denoted by black arrows) at Day 28 (**Fig. 7B**). To determine whether this model could have any effect on innate immunity, we assessed live leukocyte populations in the kidney using flow cytometry. As shown in **Fig. 8**, rats fed 5% HLP had a significant increase in CD68⁺ leukocytes (monocyte and macrophage surface marker) within the kidney, suggesting increased inflammation. Myeloperoxidase (MPO) was used to not gate neutrophils. However, there was no difference in CD68⁺ cells in the circulation (data not shown). We further evaluated whether markers of inflammation and macrophage phenotypes would be expressed in the kidney using qRT-PCR. Both IL-6 and MCP-1 mRNA levels were significantly elevated in HLP fed rats (**Fig. 9A and B**). In addition, both iNOS (pro-inflammatory macrophage marker) and mannose receptor (anti-inflammatory macrophage marker) mRNA levels

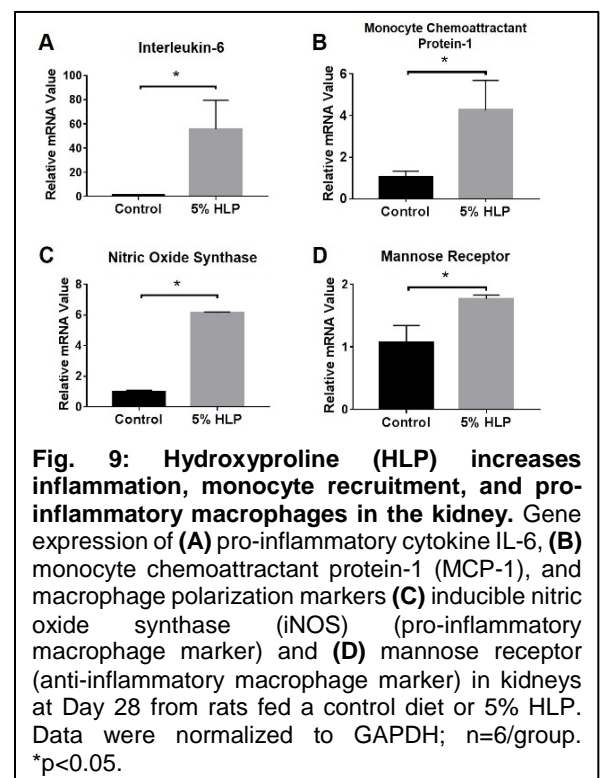
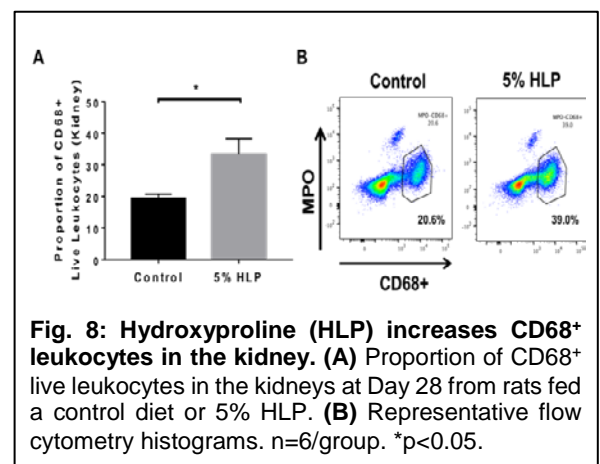
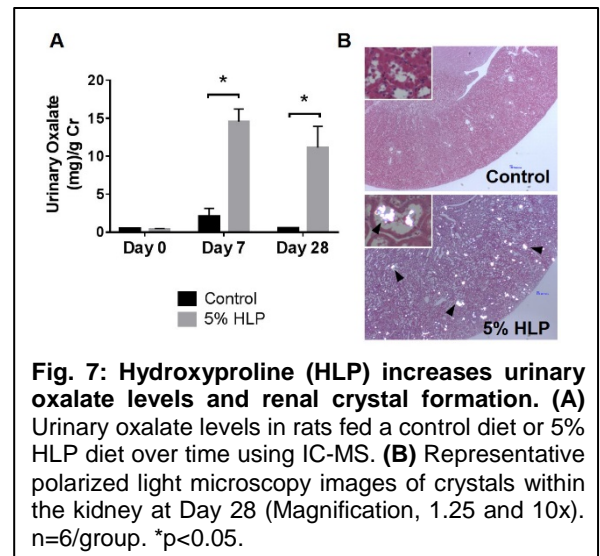
were significantly elevated (**Fig. 9C and D**). However, there was a significant increase in pro-inflammatory versus anti-inflammatory macrophages (6-fold, iNOS vs. 2-fold, mannose receptor). Our preliminary data demonstrates HLP feeding increases inflammation and monocyte recruitment and may stimulate pro-inflammatory renal macrophages differentiation. Thus, this model is ideal to investigate how oxalate impacts renal macrophages during crystal formation.

Experimental Design and Methods: The experiments in this aim are designed to investigate the effect of crystal formation on monocytes and macrophages within the circulation and kidney of age-matched male and female rats.

2.1. Dietary Plan and Crystal Formation: Animals will receive the control diet containing no oxalate for 3 days and then have their blood collected (i.e. retro-orbital) and be placed in metabolic cages to collect 24-hour urine samples. Next, animals will be randomly selected to be fed either a control diet, 1% HLP diet, or 5% HLP diet for 28 days. Food and water intake as well as body weight will be notated daily. Importantly, all diets are free of oxalate and will not influence oxalate results. In addition, diets will be prepared and stored frozen in -20°C for up to one year to minimize variability in diets provided to animals. All blood collection and nephrectomy surgeries will be performed at the UAB O'Brien Small Animal Microsurgical Core. Animals will be sacrificed at Day 28. Kidney morphology and crystal formation will be assessed using Hematoxylin and eosin (H&E) staining and quantified using a blinded and unbiased approach. Antibodies that stain for monocytes and macrophages (i.e. CD68, CD11b/c), pro- and anti-inflammatory proteins (i.e. IL-6, IL-10), and oxidative stress (i.e. nitrotyrosine) will be used for immunohistochemistry. Proper antibody controls will be included and histology scoring will be blinded to ensure unbiased results.

2.2. Urinary Analyses: Urinary oxalate will be assessed in 24-hour urine collections using IC-MS. Urinary crystal size and count will be examined using the NanoSight NS300, FTIR, and electron microscopy (Described in Aim 1.3). Inflammation (i.e. IL-10, IL-1 β , etc.) and oxidative stress (i.e. 8-hydroxydeoxyguanosine) will be measured in urine using immunoassays.

2.3. Characterization of Monocytes and Macrophages: Flow cytometry will be used to assess monocyte and macrophage populations in the circulation and kidney using surface specific antibodies (e.g. CD68, CD11b/c). In addition, intracellular cytokine and protein staining (i.e. IL-6, IL-1 β , iNOS, and TNF- α) will be performed following with GolgiPlug treatment to inhibit protein transport in the Golgi complex. Inflammatory responses will be validated using qRT-PCR to measure mRNA expression levels. Assessing both phenotypic markers and intracellular cytokines will provide insight regarding the populations and their immune responses. Additionally, cellular transcriptomics will be assessed in macrophages after cell sorting using flow cytometry to identify specific cellular pathways (i.e. mitochondrial metabolism) modified in response to 1 or 5% HLP. These data should provide insight about pathways inhibited or activated in monocytes by oxalate. Lastly, oxidative stress (i.e. CellRox and MitoSox Red dyes) and mitochondrial membrane



potential (JC-1 dye) will be evaluated in macrophages from the kidney using flow cytometry. Complex I inhibitors (i.e. Rotenone, Metformin) and antioxidants (i.e. MitoQ) will be used to confirm ROS production.

Expected Outcomes, Potential Pitfalls and Alternative Approaches: It is expected that 5% HLP will increase urinary oxalate levels, crystal formation, and pro-inflammatory macrophages in rat kidneys to a greater extent than 1% HLP. It is also anticipated oxidative stress and inflammation will be elevated in the circulation and kidney. It is expected there will be changes in cellular pathways such as oxalate and mitochondrial metabolism in macrophages which will result in increased RET, oxidative stress, and pro-inflammatory macrophage differentiation primarily in rats fed 5% HLP. If this does not occur, we will evaluate these endpoints at an earlier time point (Day 7) since oxidative stress could impact macrophages (e.g. signaling and differentiation) prior to crystal formation/deposition. Based on our past experience with animal models and feeding studies²⁸, we don't anticipate any difficulties completing the proposed studies. Alternative strategies would be to use either glyoxylate or sodium oxalate injections to induce crystal formation in rats. Daily glyoxylate injections (60 mg/kg; 5x/week) have been shown to generate intra-tubular crystal deposition in mice^{68,69} and rat models⁷⁰. In addition, a single injection of sodium oxalate solution (70 mg/kg) causes hyperoxaluria and CaOx crystals within tubules of rats^{71,72}. Future approaches will examine the effect of HLP on macrophages and crystal formation over time.

Sample Size and Statistical Analysis: Power calculations were performed using nQuery Advisor + nTerim 3.0, and assume a two-sided statistical test and a type I error rate of 0.05. For **Aim 1**, a sample size of 20 healthy subjects will be needed. We obtained estimates of the standard deviation (SD) for two key bioenergetic parameters from preliminary data on healthy subjects: maximal respiration of 2.33 pmol/min/10,000 cells and reserve capacity of 1.95 pmol/min/10,000 cells. With a sample size of 20 healthy subjects, and also assuming a paired t-test and prior assumptions, we will have 80% power to detect changes of at least 1.54 pmol/min/10,000 cells in maximal respiration and 1.29 pmol/min/10,000 cells in reserve capacity. For **Aim 2**, 48 rats will be used; 8 rats will be used per group to encompass biological variability. With a sample size of 8 rats per group, and also assuming a two-group t-test and the initial assumptions for all power calculations, we will have 80% power to detect effect sizes of 1.5 and greater as statistically significant between two groups. For **All Aims**, descriptive results will be presented as mean \pm SD. All statistical tests will be two-sided. Differences will be considered significant at $p < 0.05$. Statistical analysis will be performed using SAS software (version 9.4 or later). For **Aim 1**, the primary method of analysis will be mixed models repeated measures analyses, as there will be two diets (low oxalate, high oxalate) and two time points per diet. An appropriate structure for the covariance matrix will be selected for these models using the final data. Correlations between study parameters will be assessed using Pearson correlation analysis. Outcome variables that deviate greatly from a normal distribution will be log-10 transformed or will be analyzed using nonparametric techniques. For **Aim 2**, the primary group comparisons will be performed at Day 28. Differences between groups will be tested using analysis of covariance, with gender being controlled for; post hoc analyses will be performed using the Tukey-Kramer test. The paired t-test will be used for exploratory comparisons of oxalate levels between selected time points. **RNA-Seq analysis:** STAR will be used to align raw RNA-Seq fastq reads to the reference genome⁷³. Following alignment, HTSeq-count will count the number of reads mapping to each gene⁷⁴. Normalization and differential expression will be applied to count files using DESeq2⁷⁵. **Systems Biology analysis:** Data containing gene identifiers and corresponding expression values will be uploaded into Ingenuity Pathway Analysis to generate networks. Each identifier will be mapped with its corresponding object. A fold change cutoff of ± 2 and $p < 0.05$ will be set to identify Network Eligible molecules whose expression are significantly differentially regulated. These molecules will be overlaid onto a global molecular network based on Ingenuity's Knowledge Base. Next, networks of Network Eligible Molecules will be algorithmically generated based on connectivity. Functional Analysis will identify biological functions and/or diseases most significant to the entire data set. Molecules from the dataset that meet the fold change cutoff of ± 2 and $p < 0.05$, will be considered for analysis using Right-tailed Fisher's exact test.

Potential Hazards: All high-risk hazards (e.g. blood, urine), materials, procedures, or situations will be minimal and handled according to UAB Occupational Health and Safety guidelines.

D. Timeline and Future Directions: Aims 1 and 2 will be completed in Year 1-2. Data generated will result in at least 2 high-impact publications illustrating the significance of dietary oxalate, crystalluria and monocytes in KS disease. **Future studies for my R01** proposal consist of testing whether assessing crystalluria can predict stone risk and/or the quality of innate immunity in patients. Additionally, monocyte cellular energetics and complex I activity will be assessed in patients on controlled diets. In addition, several potential therapies (e.g. MitoQ, a mitochondrial targeted antioxidant) to preserve monocytes and/or ameliorate inflammation during stone formation will be examined in HLP fed rats and oxalate treated cell culture models. Lastly, crystal proteomics will be evaluated to determine whether immune cells interact with urinary crystals.

SUMMARY STATEMENT

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(Privileged Communication)

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Review Group: DDK-D
 Kidney, Urologic and Hematologic Diseases D Subcommittee

Meeting Date: 06/19/2019	RFA/PA: PAR18-103
Council: OCT 2019	PCC: KTR UTR
Requested Start: 09/01/2019	

Project Title: Dietary Oxalate and Innate Immunity in Kidney Stone Disease

SRG Action: Impact Score:20
Next Steps: Visit https://grants.nih.gov/grants/next_steps.htm
Human Subjects: 30-Human subjects involved - Certified, no SRG concerns
Animal Subjects: 30-Vertebrate animals involved - no SRG concerns noted
Gender: 1A-Both genders, scientifically acceptable
Minority: 1A-Minorities and non-minorities, scientifically acceptable
Age: 3U-No children included, scientifically unacceptable

Project Year	Direct Costs Requested	Estimated Total Cost
1	75,000	108,186
2	75,000	108,186
<u>TOTAL</u>	<u>150,000</u>	<u>216,372</u>

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

ADMINISTRATIVE NOTE

INCLUSION OF CHILDREN PLAN UNACCEPTABLE

SCIENTIFIC REVIEW OFFICER'S NOTES

RESUME AND SUMMARY OF DISCUSSION: This application was submitted in response to program announcement PAR18-103 entitled "Limited Competition: Small Grant Program for NIDDK K01/K08/K23 Recipients (R03 Clinical Trial Optional)". The Principal Investigator proposes to "investigate the relationship between urinary crystals, macrophage activation, reactive oxygen species, and pro-inflammatory signaling pathways following oxalate consumption." These innovative studies are an expansion of the applicant's K award. The Principal Investigator has published extensively in the important area of kidney stones and is well qualified to conduct the outlined experiments. The outstanding collaborators and a superb environment increase the likelihood of successful completion of the research plan within the 2-year period of support requested. The experimental approach is straightforward, feasible, and likely to provide data for future R-level applications. Minor weaknesses noted do not detract from the high merit of the application that, overall, is rated outstanding.

DESCRIPTION (provided by applicant): Kidney stone (KS) disease is becoming more prevalent in the United States and is associated with systemic diseases and lifestyle factors such as diet. A major risk factor for stone formation is an elevation in urinary oxalate, which can be derived endogenously or from the diet. Dietary oxalate intake may induce supersaturation of calcium oxalate (CaOx) which may generate crystals of this stone forming salt in urine and perhaps the nephron. CaOx crystals that interact with renal epithelium activate innate immunity by releasing cytokines and chemokines to stimulate monocyte recruitment. We previously reported that patients with CaOx kidney stone (CaOx KS) disease have altered monocyte cellular energetics and increased inflammation. In addition, we have shown oxalate directly disrupts cellular energetics and redox homeostasis in monocytes from healthy subjects. The long-term goal of this research is to understand how oxalate impacts monocyte and macrophage immunometabolism in KS disease. The objective of the current study is to investigate the relationship between urinary crystals, macrophage activation, reactive oxygen species (ROS), and pro-inflammatory signaling pathways following oxalate consumption. The central hypothesis is oxalate disrupts mitochondrial complex I activity in monocytes and stimulates ROS generation, pro-inflammatory macrophage differentiation, and renal crystal deposition in CaOx KS disease. Aim 1 will test the hypothesis that oxalate stimulates reverse electron transport (RET) through mitochondrial complex I in monocytes from healthy subjects. Aim 2 will test the hypothesis that oxalate mediated crystalluria induces ROS and disrupts macrophage immunometabolism in rat kidneys. No studies to date have investigated the connection between dietary oxalate, crystalluria, and immune responses in CaOx KS disease. A novel approach to assess urinary nanocrystals and their influence on monocytes and macrophages will be investigated. The impact of this research will help us understand how monocytes and macrophages respond to crystals, and may identify potential approaches to assess stone risk, reduce stone formation, and stone recurrence. The data generated from this proposal will advance our knowledge about the role of dietary oxalate on immune cells in KS disease and serve as a key foundation for subsequent R01 funding.

PUBLIC HEALTH RELEVANCE: Diets containing high amounts of oxalate are associated with the development of calcium oxalate (CaOx) kidney stone disease. The goal of this proposal is to understand the role of dietary oxalate on the immune system during kidney stone pathogenesis. This study will produce valuable data displaying the impact of dietary oxalate on innate immunity and may help guide effective interventions for CaOx KS disease.

CRITIQUES: The written critiques of individual reviewers are provided in essentially unedited form below. These critiques were prepared prior to the meeting and may not have been revised afterwards. The "RESUME AND SUMMARY OF DISCUSSION" above summarizes the final opinions of the committee.

CRITIQUE 1

Significance: 1

Investigator(s): 2

Innovation: 1

Approach: 2

Environment: 1

Overall Impact: Calcium oxalate stones are quite common, and their prevalence appears to be increasing worldwide. Pathogenesis of these stones is not well understood. Animal model data indicate inflammatory aspect to stone formation which is not supported by the existing clinical data and their interpretation. Dr. Mitchell and associates have shown that stone formers have decreased monocyte mitochondrial functions and that oxalate disrupts cellular energetics and redox homeostasis in primary monocytes and human monocyte derived cell line. The applicant would like to continue this line of research, feeding high oxalate diet to non-stone forming humans and inducing hyperoxaluria in a rat model. This is an excellent approach. The environment at the UAB Department of Urology is outstanding for this type of research. Mentors are of high caliber. These studies will provide more data and publications for Dr. Mitchell to apply for her independent R01.

1. Significance:

Strengths

- Idiopathic calcium oxalate (CaOx) nephrolithiasis is a common urological disorder with high rate of recurrence. Pathogenesis is still not well understood. Results of animal model studies suggest an involvement of oxidative stress and inflammation. Exposure of the renal epithelial cells to oxalate and CaOx crystals results in production of reactive oxygen species and various markers of inflammation such as cytokines and chemokines. But clinical data are limited. There are two sources of oxalate, diet and endogenously produced. PI is proposing to investigate dietary oxalate induced changes in the innate immunity in humans and a rat model of hyperoxaluria, with emphasis on mitochondrial functions in monocytes/macrophages. Preliminary data show that oxalate/ CaOx crystal exposure causes degradation in mitochondrial function of the monocytes and influences monocytes to macrophages differentiation.
- This is a continuation of the K award and will help the PI to generate additional data and publications in support of a future R01 application.
- Project is unique and different from mentor's main area of research.

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- Dr. Mitchell is an Assistant Professor in the Department of Urology of School of Medicine at The University of Alabama at Birmingham.
- Focus of her research has so far been mitochondrial involvement in development of oxidative stress. She has published 20 articles including a well written review on dietary oxalate and formation of kidney stones, published in *AJP (Renal)*.
- She is mentored by two of the foremost experts on oxalate and kidney stones.

Weaknesses

- Productivity as indicated by first authorship, with review and editorial aside, is limited.

3. Innovation:

Strengths

- Even though suggestions have been made in the past about involvement of inflammatory processes in the formation of kidney stones, systematic examination of immunological aspect of the disease has not been tried. Thus, it is a new direction in stone research.

Weaknesses

- None noted.

4. Approach:

Strengths

- Dr. Mitchell is proposing to investigate the effect of oxalate consumption on monocytes through two aims. Aim 1 proposes to test the hypothesis that oxalate affects monocyte function through reverse electron transport (RET) of mitochondrial complex I. In this aim she plans to recruit normal healthy individuals, put them on oxalate diet, and determine monocyte cellular energetics and mitochondrial complex I activity. This is an important step in understanding the role of dietary oxalate. The plan is well laid out and PI and co-investigators, and mentors have expertise to help carry out the studies.
- 2nd aim is similar to the first aim but hyperoxaluria will be induced in an animal model with access to what happens inside the kidneys at a given time. They will be analyzing urine, serum and kidneys.
- Using both human and animal specimens is a big plus.

Weaknesses

- Animal model studies have shown involvement of NADPH oxidase in the development of inflammation in kidneys in response to an exposure to oxalate and CaOx crystals. NADPH oxidase plays an important role in monocyte activities also. This reviewer understands that PI has only two years and can only analyze just so much. But it is important to at least mention the possibility of other sources of reactive oxygen species.

5. Environment:

Strengths

- Outstanding environment with some of the foremost authorities on the subject available for consultation.
- All the necessary equipment's are available.

Weaknesses

- None noted.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Acceptable

Inclusion Plans:

- Sex/Gender: Distribution justified scientifically
- Race/Ethnicity: Distribution justified scientifically
- Inclusion/Exclusion Based on Age: Distribution justified scientifically

Vertebrate Animals:

YES, all criteria addressed

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommended as requested

CRITIQUE 2

Significance: 2

Investigator(s): 2

Innovation: 1

Approach: 2

Environment: 1

Overall Impact: This is an R03 application from Dr. Mitchell who is an Assistant Professor of Urology at UAB. The PI has established a new research program at UAB in part through her K01. The PI has a history of productivity with particular expertise in mitochondria bioenergetics. Her K01 proposal applied these approaches to investigate the role of monocyte mitochondria bioenergetics in kidney stone disease. Importantly, the preliminary data indicate that even in healthy individuals, oxalate loads change energetics. The PI proposes to further investigate these effects using oxalate-diet changes in healthy humans (Aim 1) as well as oxalate loading of rats (Aim 2). The over-arching hypothesis is that innate immunity and immune response may be an indicator of metabolic disruption and an indicator of KS-disease or perhaps damage. Through this R03, the PI continues to build her scientific network as she investigates alternative targets to lessen kidney stone burden.

1. Significance:

Strengths

- Oxalate dis-metabolism and urinary supersaturation are known to contribute to kidney stones, but there is growing evidence that at least infection (increased cytokines, monocytes & macrophages) contribute as well, especially manifesting as Randall's plaque. However, therapeutically current clinical approaches have focused on KS removal, overt oxalate control or urinary Ca control
- The PI and her collaborators have found that mitochondrial energetics is altered in kidney stone (KS) formers resulting in her K01 (monocyte mitochondria in KS disease). The R03 proposal will take a deeper dive into innate immunity (monocyte and macrophage mitochondria) in KS with oxalate challenges in humans (Aim 1) as well as in rat kidneys (Aim 2)
- The PI and her growing team thus propose a distinct set of regulatory points to (a) better understand the pathobiology (immune changes) in KS patients and models and (b) develop a new paradigm to approach KS treatment or prevention
- PI can show nanocrystal (Fig 2) and gene expression changes (Fig 4) between pre- and post-oxalate load. These results provide new and discrete targets to follow in KS patients

Weaknesses

- None noted

2. Investigator(s)

Strengths

- PhD (UAMS; Lee Ann MacMillan-Crow, PhD) studying mitochondrial health: 5 papers, 2 as 1st author

- Post-PhD (2011; UAB) with Dr. Victor Darley-Usmar (bioenergetics and autophagy): 11 papers, 4 as 1st author
- 2014 – Instructor in Urology working with Drs. Holmes and Assimos: 4 papers, 1 as senior author
- 2016- Assistant Prof Urology, UAB
- 2016: K01 DK106284 (Monocyte Mitochondrial Dysfunction and Kidney Stone Disease)
- PI is making significant strides to team-building by establishing collaborations and to seek out expertise to help answer questions

Weaknesses

- None noted

3. Innovation

Strengths

- PI continues to incorporate additional investigators in her team bringing new approaches to KS research
- Monitoring monocytes (Aim 1), macrophages (Aim 2) or certain cytokines could be an important tool for monitoring who is at clinical risk of KS and potentially determining if diet or other therapies are successful prior to waiting for KS recurrence
- Nanocrystal (Fig 2) and gene expression changes (Fig 4) between pre- and post-oxalate load as new and discrete targets in KS. Data seems to indicate that specific mitochondria enzymes may change

Weaknesses

- (minor) perhaps the PI could consider examining mitochondrial DNA / RNA changes (separate from chromosomal DNA) – expression or protein synthesis control. As the PI continues to develop this line of investigation, especially as additional ethnic / racial groups are added, mitochondrial DNA may play more of a role, particularly as a predictive tool as it is passed through maternal inheritance

4. Approach

Strengths

- Two-pronged approach to use humans (Aim 1) and a rat model (Aim 2) to evaluate innate immunity and mitochondrial function in response to oxalate. While it may seem that the aims are replicates, experimental variables may be controlled differently in each. Additionally, the PI will be able to make more explicit statements about sameness or differences between humans and a rodent model.
- Mitochondrial function, nanocrystals and gene expression will be followed in both aims using established techniques.
- Succinct study for the time frame and resources

Weaknesses

- Monocytes in rats would be followed as well to mirror Aim 1 in humans. It is clear why there is additional work performed on rats (more easily controlled and accessed), but it will be important to establish as many parallels or differences as possible between the models
- (minor for R03 but worth considering for R01):
 - For R01, probably want some data for various KS-patient populations

- While it is clear why the PI chooses to have both a patient aim as well as an animal model aim, there is a concern that for this particular pathology rodents with hydroxyproline feeding may not appropriately or at least fully represent the spectrum of human KS causes (e.g., hypercalciuria, Ca-metabolism defects, etc.). The PI might consider at least attempting to broaden the rodent treatments or performing experiments on rodent-strains or mutants which have issues other than oxalate metabolism

5. Environment

Strengths

- Excellent environment at UAB in both Urology and Nephrology.
- Training environment and general scientific environment is very strong and very supportive. The PI is flourishing and growing her team and network

Weaknesses

- None noted

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

- appropriate and minimal risk. Diet change for normal / healthy humans

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Acceptable

Inclusion of Women, Minorities and Children:

- Sex/Gender: Distribution justified scientifically
- Race/Ethnicity: Distribution not justified scientifically
- Inclusion/Exclusion of Children under 18: Excluding ages <18; justified scientifically appropriate w/ UAB-IRB approval

Vertebrate Animals:

YES, all four points addressed

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3

Significance: 2

Investigator(s): 1

Innovation: 3

Approach: 2

Environment: 1

Overall Impact: Stone disease is a debilitating, common condition on the rise and calcium oxalate is the most common constituent. This is the first submission for this well-written 2-year R03 application

from Tanecia Mitchell, PhD, an Assistant Professor at University of Alabama Birmingham since 2016 who studies the effects of hyperoxaluria and calcium oxalate crystals. She received her K01 in Fall 2016 and has been productive in leadership and academically (6 of 20 publications since 2016). Her working environment is not surpassed for this area of study, with mentorship, clinical acumen, and translational/basic science resources, as well as the 1) Center for Clinical and Translational Science Bionutrition Unit, 2) the UAB-UCSD O'Brien Core Center for AKI, 3) Center for Free Radical Biology, and 4) Bio-Analytical Redox Biology Core. She works in conjunction with Dr. Dean Assimos, who is her chairman, clinical collaborator, and mentor, given his long track record of NIH funding and contributions to the field of oxalate stone disease. She presents strong preliminary data on oxalate-induced monocyte changes in mitochondrial function, oxidative stress and inflammation generated from the first 2.5 years of her K award demonstrating feasibility of her studies. Her central hypothesis is that oxalate disrupts mitochondrial complex I activity in monocytes and stimulates ROS generation, pro-inflammatory macrophage differentiation, and renal crystal deposition in calcium oxalate kidney stone disease. Dr. Mitchell shows her versatility in her two aims wherein she will perform dietary oxalate loading studies on normal humans and normal rats. Biological samples will be queried for monocyte cellular energetics (reverse electron transport through mitochondrial complex I) and ROS/macrophage immunometabolism, respectively. There is a large repertoire of assays.

Many strengths include her crisp grantsmanship, her impressive track record, the outstanding research environment and the well-planned proposal. Minor weaknesses included specific aspects of the human subject's exclusion criteria and protocol, slight concerns of mechanism rationale, somewhat over-ambitious for 2 years and that all experiments are observational (without interventional basic science experiments). The proposal is very safe – enthusiasm is quite high.

1. Significance:

Strengths

- Stone disease is a debilitating, common condition on the rise and calcium oxalate is the most common constituent. It is an important problem.
- The pathobiology is incompletely understood, and this proposal addresses this.
- She is developing a niche field in oxalate induced mitochondrial and oxidative stress.

Weaknesses

- The statement..." There are no treatments available to cure or prevent stone formation and recurrence." Is partially true (no cures) and mainly false (there is prevention for stone formation and recurrence).
- The aims address only mechanisms and pathological endpoints. There are no planned interventions. The clinical trial is not earthshattering and is safe. No immediate outcome that would lead to change in clinical practice.

2. Investigator(s):

Strengths

- Tanecia Mitchell, PhD, an Assistant Professor at University of Alabama Birmingham since 2016. PhD from Univ Arkansas Medical Sciences. Post doc at UAB in Mitochondrial Biology. She received her K01 in Fall 2016 and has been productive in leadership and academically.
- 9 travel awards
- On Board Oxalosis Society
- 20 publications: 6 since 2016, 8 first author, and 4 senior authors
- High profile journals (*AMJ Phys*, *Redox Biol*)
- Bioenergetic Health Index (BHI) – new concept – biomarker of bioenergetic health in individuals. 2014

- Dr. Dean Assimos, who is her chairman, clinical collaborator, and mentor, given his long track record of NIH funding since 2002 and contributions to the field of oxalate stone disease. He will provide human subjects.

Weaknesses

- None noted.

3. Innovation:

Strengths

- Studying monocytes and macrophages in oxalate stone disease is not new but a close look at mitochondrial and oxidative stress may find new observations
- No true innovations but new applications of prior experimental techniques
- Dietary human clinical trial is not novel but will have assays not previously explored.

Weaknesses

- NanoSight has been used for oxalate stone studies previously
- No therapeutics easily in sight.

4. Approach:

Strengths

- Well-written and planned proposal without a single typographical error. Excellent grantsmanship. Well planned preliminary data, study design, statistics, pitfalls, alternative strategies
- May be slightly over-ambitious for 2 years

Weaknesses

- Minor weaknesses:
- It is unclear what is a biologically important high oxalate concentration for *in vitro* studies.
- It is unclear what is a biologically important low monocyte OCR or complex I activity. At what level is cell death a significant concern?
- The human subjects exclusion criteria and protocol – should check for acute illness
- How soon after the oxalate diet will the blood samples be drawn? How was this time rationale derived?
- Slight concerns of mechanism rationale for Aim 1. If all healthy humans eat intermittent boluses of high oxalate foods and if, as she proposes, this leads to mitochondrial and oxidative stress in monocytes and macrophages, then why doesn't everyone (all healthy humans) make stones? How does this pathobiological mechanism separate healthy from stone-formers?
- All experiments are observational (without interventional basic science experiments). The proposal is very safe. Would have liked to see some therapeutic experiments.

5. Environment:

Strengths

- Mentorship, clinical acumen, and translational/basic science resources
 - Center for Clinical and Translational Science Bio Nutrition Unit, 2) the UAB-UCSD O'Brien Core Center for AKI, 3) Center for Free Radical Biology, and 4) Bio-Analytical Redox Biology Core.
- Beautiful environment

Weaknesses

- None noted.

Study Timeline:

Strengths

- Study timeline good but could have more details
- Feasible to recruit only n=20 with relatively simple

Weaknesses

- Unclear if will be incentivized to aid retention

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Acceptable

Inclusion Plans:

- Sex/Gender: Distribution justified scientifically
- Race/Ethnicity: Distribution justified scientifically
- Inclusion/Exclusion Based on Age: Distribution not justified scientifically
- The PI states stone disease is rare in children - but it is not that rare. It may be difficult to recruit children however. Not stated.

Vertebrate Animals:

YES, all criteria addressed

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS: ACCEPTABLE

INCLUSION OF WOMEN PLAN: ACCEPTABLE

INCLUSION OF MINORITIES PLAN: ACCEPTABLE

INCLUSION OF CHILDREN PLAN: UNACCEPTABLE

As noted in Critique 3, justification for exclusion of children is unsatisfactory.

VERTEBRATE ANIMALS: ACCEPTABLE

SCIENTIFIC REVIEW OFFICER'S NOTES:

The resource sharing plan is adequate.

The authentication plan for key biologicals and/or chemical resources is adequate.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Recommended direct cost levels are estimated and are subject to further adjustment based on the Institute's standard budget calculation practices.

Footnotes for 1 R03 DK123542-01; PI Name: Mitchell, Tanecia R

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html>. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

Kidney, Urologic and Hematologic Diseases D Subcommittee National Institute of Diabetes and Digestive and Kidney Diseases Initial Review Group NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

DDK-D
06/19/2019 - 06/20/2019

Notice of NIH Policy to All Applicants: Meeting rosters are provided for information purposes only. Applicant investigators and institutional officials must not communicate directly with study section members about an application before or after the review. Failure to observe this policy will create a serious breach of integrity in the peer review process, and may lead to actions outlined in NOT-OD-14-073 at <https://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-073.html> and NOT-OD-15-106 at <https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-106.html>, including removal of the application from immediate review.

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