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TITLE: Dehydration as a Cause of Chronic Kidney Disease: Role of Fructokinase

PRINCIPAL INVESTIGATOR: Richard J Johnson MD

CONTRACTING ORGANIZATION: University of Colorado, Denver
Aurora, CO 80045

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14. ABSTRACTOur studies evaluate how recurrent dehydration can cause chronic kidney disease, an important question for the military and public. We hypothesize, based on preliminary data, that dehydration associated renal injury results from hyperosmolarity induced activation of renal aldose reductase-fructokinase. We have made good progress and are on target for completing all studies. Aim 1 test the role of selective renal knockout of fructokinase in dehydration induced kidney disease, and we have now generated the floxed fructokinase mouse that is critical for conducting these studies. Aim 2 investigates the role of vasopressin receptors and uric acid, and we have completed studies of the Vasopressin 2 receptor and demonstrated vasopressin 2 receptors are responsible for some of dehydration induced kidney damage. Aim 3 tests the role of rehydration with fructose solutions with or without blocking of vasopressin receptors. These studies were delayed due to administrative issues but are ready to be completed and are still within our proposed milestones. In summary, we are documenting how recurrent dehydration causes chronic kidney disease, documenting a role for vasopressin and aldose reductase.					
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1. INTRODUCTION:

Our studies are aimed at identifying how recurrent dehydration may lead to chronic kidney disease. This is important for the military as well as for the general population. In preliminary studies we developed the first model of chronic kidney disease from recurrent dehydration and found evidence that the renal injury is mediated by hyperosmolarity that activates an enzyme pathway in the kidney (aldose reductase-fructokinase) that leads to tubular injury. Here we will proceed with three aims to further identify the role of this pathway. Aim 1 will evaluate the role of selective knockout of fructokinase in the renal tubule on dehydration induced kidney disease. Aim 2 will investigate the role of downstream mediators, including the role of vasopressin receptors and uric acid in driving dehydration induced kidney injury. Aim 3 will evaluate the role of rehydration with fructose solutions with or without blocking of vasopressin receptors. These studies should provide major insights into how recurrent dehydration may cause chronic kidney disease

2. **KEYWORDS:** *Dehydration, Chronic kidney disease, vasopressin, uric acid, fructose*

3. ACCOMPLISHMENTS:

What were the major goals of the project? According to our revised and accepted SOW, our primary goals consisted of three aims:

Aim 1 involved generating an inducible tissue specific knockout mice for fructokinase. This required generating a mouse whose fructokinase gene is floxed (the floxed fructokinase mouse) and then selectively knocking fructokinase from the renal tubule. The milestone for the first year was to generate the KHK floxed mouse, for which we accomplished (see below). The milestone for year two is to breed the fructokinase floxed mouse and to cross it with a Cre mouse targeting the tubule. We have already initiated on the work to achieve the second year milestone.

Aim 2 involves testing the role of vasopressin and uric acid in our mouse model of recurrent dehydration induced chronic kidney disease. Here there are 4 sets of experiments—studies using V1a and V1b receptor knockout mice, studies using wild type mice administered allopurinol to lower uric acid, and a study using desmopressin that only stimulates vasopressin type 2 receptors. Here we are also on target, as we have completed the desmopressin experiment and our preliminary results show that stimulation of vasopressin 2 receptors is accelerating kidney damage. We also performed the allopurinol study but will have to repeat it as in our original study the allopurinol was not effective at lowering the uric acid at the dose of allopurinol we used. In terms of milestones, we had planned to finish the V1 receptor knockout mice in the first year, but we

have completed 60 percent of the work by obtaining the mice and breeding them in preparation for our study. For the V2 receptor studies, we are ahead of our milestone as we completed the experiment in the first year, and it was originally slotted for year two. The allopurinol study was performed in year one, but we will repeat it in year two. The other milestones are all for subsequent years. Hence, overall we are on target to complete our work.

Aim 3 tests the effect of rehydration in our model of chronic kidney disease, by comparing the administration of fructose versus water and the impact of blocking vasopressin in this process. There have been unforeseen administrative challenges in executing the subcontract to Instituto Nacional de Cardiología Ignacio Chavez. Many of these challenges were related to multiple contract document translations and finding mutually agreeable terms. We believe the latest iteration of the contract will be mutually agreed upon and executed in the upcoming weeks. The studies to be conducted in Mexico will be able to be performed in the second year. Indeed, our collaborator, L. Gabriela Sanchez-Lozada, has in the meantime performed related studies on a different grant that has confirmed our basic premise, that rehydration with sugary beverages worsens dehydration associated renal damage and can be blocked by conivaptan (a vasopressin antagonist). Thus, we are hopeful that this next year we will be able to complete much of this aim. Interestingly, according to our timeline, we had planned the renal hemodynamic studies on year 1, with completion of the analyses in years two and three. We are still on track for completion of these milestones on time, with the hemodynamic studies now included in year 2.

What was accomplished under these goals?

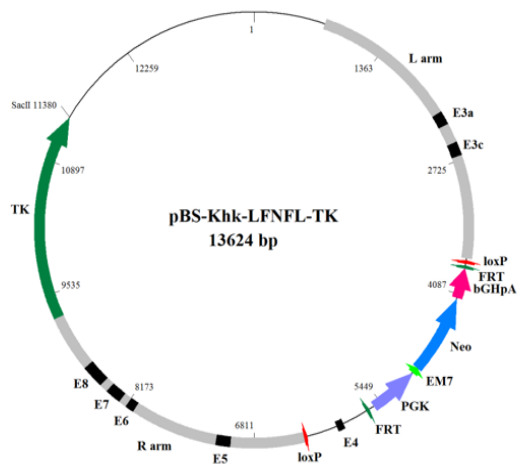
1) Major activities.

The primary activities included a) Generation of the Floxed Fructokinase knockout mouse; b) Obtaining and Breeding of the Vasopressin 1a and 1b receptor knockout mouse; c) Demonstration that stimulation of vasopressin 2 receptors with desmopressin is worsening dehydration-associated kidney disease. These studies are discussed in detail below.

2) Specific objectives;

a. The goal of Aim 1 for the first year was the development and breeding of a loxP mouse that would allow us to test the role of rena-specific fructokinase in mediating dehydration associated chronic kidney disease. A key part of this aim is the development of a loxP fructokinase mouse which was the focus (and milestone) of our first year effort.. A large number of steps was required to generate this mouse. First, we designed a construct using a p85 vector that will conditionally knockout exon 4 of the fructokinase C isoform due to the presence of loxP sites flanking the exon (see Figure). The construct was used to transfect mouse ES cells (EC7.1 ES cells,

mouse 129 background). Clones carrying the construct were selected using neomycin and karyotyped, with a good cytogenetic profile. The neomycin cassette was removed and the new clones were rekaryotyped, also with a good cytogenetic (91% diploidy) profile. A specific clone (B1-E7-7) was then injected into mouse blastocysts (B6 background) and injected in B6 mice, with the first chimeric male born in June 2015 (see photo). We are currently performing germline breeding with rekaryotyping of the F1 pups. We expect to have a loxP-KHK C mouse colony by the end of the year. This will allow us to cross the mouse with the TetOn LC-1 a Pax8-rTA Cre transgenic mouse. This will allow us to determine the role of fructokinase in the kidney in our model of heat-dehydration induced renal

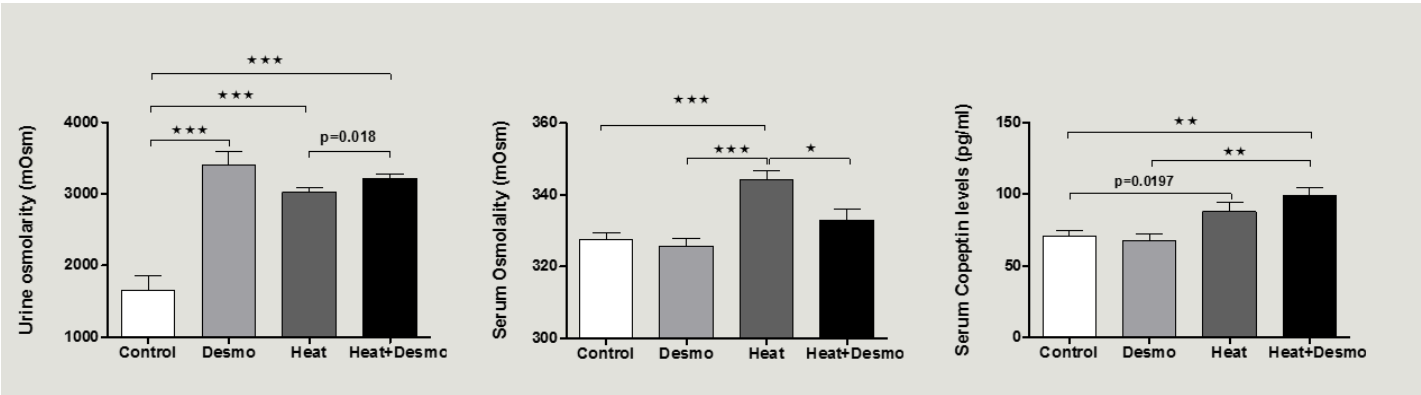


We have generated a construct pBS vector that will conditionally knockout exon 4 of both the A and C isoform of KHK. The system includes a Neo positive marker and a thymidine kinase negative marker



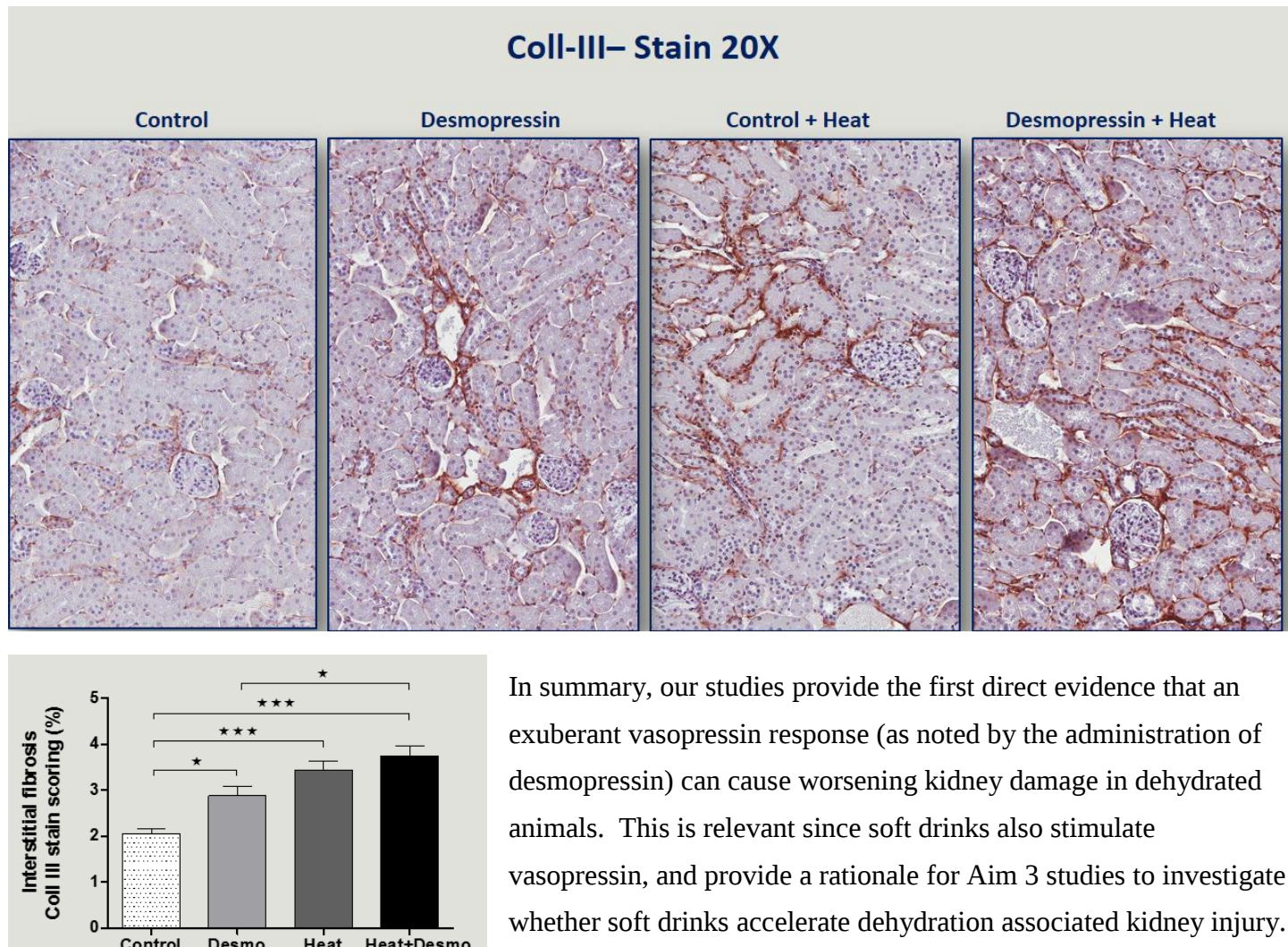
injury (aim 1) but will also be invaluable for future studies on the role of fructokinase in other organs (liver, brain, adipose, islets).

b). The goal of Aim 2 was to evaluate the role of vasopressin receptor 1A, vasopressin receptor 1B, vasopressin 2 receptor, and uric acid in the model of heat-dehydration induced kidney disease. We have completed the study using vasopressin 2 receptor stimulation with desmopressin. In this experiment we induced heat/dehydration and studied mice administered vehicle or mice administered desmopressin for 5 weeks. We also had two additional controls groups (normal mice with no heat/dehydration, and control mice administered desmopressin). The primary findings are shown in the next set of figures. First, control mice administered



desmopressin showed the expected rise in urine osmolality, with normal or slightly suppressed serum osmolality, and no change in serum copeptin levels (copeptin measures a vasopressin precursor).

Heat/dehydration also causes a rise in serum and urine osmolality, but the addition of vasopressin worsened the urine osmolality and improved the serum osmolality, which is expected since it should lead to greater water retention. Importantly, the administration of desmopressin resulted in worsening of the renal disease, as shown by more severe renal fibrosis (collagen III staining) and as quantified by computer imaging software (Aperio).



Another part of Aim 2 was the procurement and breeding of the vasopressin receptor knockout mice. Jackson Laboratories provided the embryos for both vasopressin receptor knockout mice (V1a receptor KO ; B6;12P2-/Avpr1a^{tm1Dgen}/J and the V1b receptor KO; B6;129X1-/Avpr1b^{tm1Wsy}/J), and then we had to breed them for our studies. As it turns out, breeding the vasopressin 1b mouse was slightly difficult, but we in fact had success breeding both the vasopressin 1A and vasopressin 1B knockout mice. By summer 2015 we were ready to perform the studies, but we had our renewal for the ACCURO at that time. Although we had local IACUC approval to perform these studies as part of a larger IACUC protocol, ACCURO preferred a separate

IACUC protocol just for the DOD studies. As such, we submitted our local IACUC protocol in late July, received approval from our IACUC in early September, and are currently waiting for the ACCURO approval so we can perform the studies with these mice, and we have recently been given the go ahead to proceed with these studies.

We also performed the allopurinol study earlier this year in which we treated mice with our heat dehydration protocol with or without allopurinol in order to test the hypothesis that uric acid may be playing a role in the kidney damage. Unfortunately, the study failed as the uric acid was not significantly lowered by the dose of allopurinol (150 mg/kg/d) that we used. Our dose had been calculated based on the dose that is effective in rats, but it seems higher doses are needed to lower uric acid in the mouse. We will have to perform pilot studies to identify the safe dose before we attempt this study again.

3) Significant results/key outcomes

Our primary successes include 1) the development of a Lox-P fructokinase mouse; 2) Completion of the DDAVP study. The former discovery now allows us to perform site specific knockout of fructokinase in the proximal tubule and hence we are well on our way to completing aim 1. The latter study is important as it suggests that vasopressin 2 receptors may be the key receptors involved in driving kidney disease from dehydration. Additional analyses are ongoing, but our results are already generating some new insights into the mechanisms by which dehydration and heat cause kidney disease.

4) Other achievements.

The development of the Floxed fructokinase mouse will have major implications for future studies that extend well beyond the DOD grant. First, it will allow us to explore the role of CNS fructokinase in sugar craving, and of islet cell fructokinase in the development of diabetes. The liver fructokinase can also be targeted and may be responsible for the fatty liver and insulin resistance. Of course, targeting the kidney might provide the insight of how dehydration causes kidney injury via the fructokinase pathway.

5) Challenges/ stated goals not met.

We also had some challenges this year, primarily related to administrative issues. First, because our animal model involves dehydration and heat exposure, it took longer than usual to get IACUC approval. We should note that the animals tolerate the procedure well, and we have no mortality. Nevertheless, the concern for intermittent dehydration is such that the reviews took longer than expected, for both the regular IACUC at our institution and for the ACCURO approval. Second, there were also some challenges with our subcontract with Mexico. The original RFA by the DOD encouraged international collaboration, and our collaborator (L G

Sanchez-Lozada) is an expert renal physiologist who has collaborated with us previously on numerous studies and on several NIH-funded applications. However, the challenge was that her institute was not registered with the DOD and required a long process for registration (including several English-Spanish translations), and her IACUC also required ACCURO approval. There was also an adjustment to the Statement of Work that related to some minor changes in protocol that required modifying the protocol and waiting for approval. There have been unforeseen administrative challenges in executing the subcontract to Instituto Nacional de Cardiología Ignacio Chavez. Many of these challenges were related to multiple contract document translations and finding mutually agreeable terms. We believe the latest iteration of the contract will be mutually agreed upon and executed in the upcoming weeks. The good news is that these challenges were temporary and did not significantly slow the overall time line, and we feel confident we can complete the studies in Aim 3 (that largely involve this subcontract) in the next two years.

What opportunities for training and professional development has the project provided?

The grant was not meant to provide training or professional development, and so at one level there is nothing to report. However, the studies are opening up information on the role of dehydration in kidney disease. Indeed, the principal investigator was invited to speak on dehydration and chronic kidney disease at a meeting in Costa Rica in November 2015 on the epidemic of chronic kidney disease that is occurring among sugarcane workers in Central America. He was also invited to give a state of the art talk on this topic at the American Society of Nephrology meeting in San Diego in November 2015 and also at the Presidential symposium of the Experimental Biology meetings in April 2016 in San Diego. In addition, Carlos Roncal, who is supported by the DOD grant, was invited to speak on dehydration and kidney disease at a National Dialysis Conference in Miami in January 2016. He also has been mentoring individuals in our laboratory on techniques involved for inducing dehydration and methods for evaluating this from studies of the urine and blood. These individuals are funded from other grants.

How were the results disseminated to communities of interest?

Our studies on dehydration and kidney disease are currently being supported by grants from the DOD as well as from Danone Research Foundation and La Isla Foundation. The DOD studies are completely distinct. However, Dr Johnson has had the opportunity to lecture on this research, which includes studies supported by the DOD grant, to a variety of groups—including the American Society of Nephrology (Nov 2015), the Japanese Society of Nephrology (June 2015), the Experimental Biology Meetings (April 2016), and the Central American coalition on Mesoamerican Nephropathy (November 2015). These lectures provide an avenue for disseminating the work to the scientific community.

What do you plan to do during the next reporting period to accomplish the goals?

We are poised to complete Aim 2 in the next year. We have the vasopressin 1a and 1b knockout mice and they are actively breeding, and hence we should be able to complete these studies and analysis. We also plan to perform the allopurinol studies (again) and to finish the analysis of the DDAVP studies. The studies using the floxed mouse (Aim1) to target the kidney are now also underway. Specifically, we will need to breed more of the floxed mouse and then cross it with a Cre mouse targeting renal tubular cells. We hope to have this mouse bred during the next year. Finally, our goal is to complete the experiments (not complete analysis) for Aim3 in the second year, which will keep us on target with our projected milestones.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our studies document for the first time the importance of chronic recurrent dehydration as a mechanism for causing chronic kidney disease. This has led to huge interest from the academic nephrology societies around the world, especially as it is becoming apparent that there are epidemics of chronic kidney disease emerging in Central America, India and Bangladesh, Sri Lanka, and elsewhere. As such, there is great interest in our research, especially with the realization that this disease may increase with global warming and worldwide water shortages. Our DOD studies are elucidating the role of the vasopressin and fructokinase systems, and also the importance of the rehydration fluid. This year we have made the floxed fructokinase knockout, bred the vasopressin receptor knockout mice, and initiated studies on the role of vasopressin and uric acid. We have already shown that it is the vasopressin receptor 2 that is responsible for much of the renal injury induced by dehydration. As the analyses are ongoing, we will have a better assessment of the specific impact of the DOD studies. However, with the increasing evidence that recurrent dehydration may be causing epidemics of chronic kidney disease worldwide, the importance of our work remains extremely high.

What was the impact on other disciplines?

Our work is identifying global warming as a factor driving kidney disease, and as such is causing some concern as the first epidemic disease induced by global warming. This has generated interest in many other disciplines (general medicine) as well as by the lay public (NPR, BBC). The DOD grant is exploring the mechanisms, and as the results are generated, will likely have an impact on our understanding of the cause of disease and the importance and risks associated with dehydration. We believe our reports will identify chronic kidney disease as the first major human disease due to global warming.

What was the impact on technology transfer?

Our work is heightening interest into the correct ways for rehydration when in the dehydrated state. Indeed, our work is generating concern that the current use of WHO sugar rehydration packages for dehydration may have injurious effects on kidney health (based on preliminary studies performed by Dr Sanchez-Lozada in preparation for specific aim 3 studies of the DOD proposal). While this data is not generating new intellectual property, it is generating interest in current approaches to the treatment of dehydration.

What was the impact on society beyond science and technology?

Our studies could lead to a reevaluation of sports drinks and rehydration packages for the hydration of individuals who are exposed to heat and dehydration.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

No significant changes were made to the approach other than a minor revision of the Statement of Work plan as it related to Aim 3. The proposed change to the SOW was submitted and approved by the DOD in the summer of 2015.

Actual or anticipated problems or delays and actions or plans to resolve them

We had several delays in our first year. While our animal care protocol was approved by both our local IACUC and ACCURO, the renewal was delayed slightly as we decided to incorporate the DOD proposal as a single IACUC protocol and not to have it be a component of an existing IACUC protocol that included other funded studies. This required submitting a new IACUC protocol and then having it approved by ACCURO. This resulted in a delay of performing animal studies of two months—however, we continued to analyze tissues from our prior studies during this time so the actual delay in work was minimal.

The second delay related to obtaining an agreement between my institution (University of Colorado) and our collaborator's institution (Cardiologia University in Mexico City) as it related to the subcontract. There were three major problems—the first was to the time needed to have official translations made of the subcontract and negotiations between the institutions—the second was the requirement of Cardiologia to have a first payment advance prior to any invoice—and the third was the agreement on the wording of the subcontract as it related to the institution and trust funds that administer the research monies at Cardiologia. The good news is that resolution of the issues has occurred and our collaborator should be able to perform the key experiments for Aim 3 during this next year.

Changes that had a significant impact on expenditures

The payment to Mexico was delayed due to the administrative issues raised above. However, our collaborator should be able to complete the experiments of Aim 3 in the next year, with the final year to analyze and report the findings of those experiments, thereby staying in line with our milestones.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals.

None

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS:

Publications, conference papers, and presentations

Nothing to report (yet)

Books and book chapters

Nothing to report

Other publications, conference papers, and presentations.

1. Roncal-Jimenez C, Garcia-Trabanino R, Barregard L, Lanaspa MA, Wesseling C, Harra T, Aragon A, Grases F, Jarquin E, Gonzalez MA, Weiss I, Glaser J, Sanchez-Lozada LG, Johnson RJ. Heat Stress Nephropathy from Exercised-Induced Uric Acid Crystalluria: a Perspective on Mesoamerican Nephropathy? *Am J Kid Dis* (in press)
2. Garcia-Trabanino R, Jarquin E, Wesseling C, Johnson RJ, Gonzalez MA, Weiss I, Glaser J, Vindell JJ, Stockfelt L, Roncal C, Harra T, Barregard L. Heat stress, dehydration and kidney function in sugarcane cutters in El Salvador—a cross-shift study of workers at risk of Mesoamerican Nephropathy. *Environ Res* (in press)
3. Roncal-Jimenez C, Lanaspa MA, Jensen T, Sanchez-Lozada LG, Johnson RJ. Mechanisms by Which Dehydration May Lead to Chronic Kidney Disease. *Ann Nutr Metab*. 2015;66 Suppl 3:10-3
4. Roncal-Jimenez C, Garcia-Trabanino R, Wesseling C, Johnson RJ. Mesoamerican Nephropathy or Global Warming Nephropathy. Blood purification (in press)
5. Johnson RJ, Garcia-Trabanino. On the Etiology of Mesoamerican Nephropathy. CENCAM conference,

Website(s) or other Internet site(s)

We do not have a website that details our results of the DOD study

Technologies or techniques

We have not introduced any new technologies or techniques.

Inventions, patent applications, and/or licenses

Nothing to Report

Other Products

Our research is generating great interest in the role of dehydration and global warming in chronic kidney disease. However, we do not have any major findings from the DOD grant yet that will lead to major changes in current practices. We hope that our studies in the next two years will provide such information.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name:	<i>Richard J Johnson</i>
Project Role:	<i>Principal Investigator</i>
Researcher Identifier (e.g. ORCID ID):	2 months
Nearest person month worked:	
Contribution to Project:	<i>Dr Johnson has overseen the design, performance and analysis of the studies</i>
Funding Support:	<i>DOD funding</i>

Name:	<i>Zhilin Song</i>
Project Role:	<i>Coinvestigator</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month	6 months

worked:	
Contribution to Project:	<i>Dr Song is performing studies to identify the effects of dehydration on the vasopressin axis in the hypothalamus.</i>
Funding Support:	<i>DOD funding</i>

Name:	<i>Carlos Roncal</i>
Project Role:	<i>Co-Investigator</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6 months
Contribution to Project:	<i>Mr. Roncal is performing all of the experiments in Aim 2 and overseeing their analyses.</i>
Funding Support:	<i>DOD funding</i>

Name:	<i>Laura G Sanchez-Lozada</i>
Project Role:	<i>Co-Investigator</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1 month
Contribution to Project:	<i>Dr Sanchez Lozada oversaw the administrative aspects of executing the subcontract.</i>
Funding Support:	<i>DOD funding</i>

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

For Dr. Richard Johnson, M.D., the following project has ended since the previous submission:

1R01DK080764-01 (Johnson, Garin)

09/01/11-08/31/14

2.4 months

NIH/NIDDK \$150,000
CD80 Expression on Podocytes and the Pathogenesis of Minimal Change Disease

Goal: The goal is to study the role of CD80 in Minimal Change Disease.

There have been no changes in active support for Carlos Roncal, Zhilin Song or LG Sanchez Lozada.

What other organizations were involved as partners?

We have a collaboration supported by the DOD with Gaby Sanchez-Lozada at the Cardiology University as part of our DOD proposal.

Organization Name: Instituto Nacional de Cardiología Ignacio Chavez

Location of Organization: Mexico City, Mexico

Partner's contribution to the project: Will be responsible for completion of aim 3.

In-kind support None

Facilities None

Collaboration None (other than our DOD collaboration with Dr Sanchez-Lozada)

Personnel exchanges None

Other. None

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Not applicable

9. APPENDICES:

We are including a paper in press in Blood Purification that summarizes the theme of our research. It was not funded by DOD but directly relates to what we are studying.

Mesoamerican Nephropathy or Global Warming Nephropathy?

Carlos A. Roncal-Jimenez¹, Ramon García-Trabanino², Catharina Wesseling³, and Richard J Johnson¹.

¹Division of Renal Diseases and Hypertension, University of Colorado Denver, Anschutz Medical Campus, Aurora CO, USA; ²Scientific board, Department of Investigation, Hospital Nacional Rosales, San Salvador, El Salvador; ³Unit of Occupational Medicine, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

Word count 1150

Running Title: CKD from Global Warming

Abstract

Background. An epidemic of chronic kidney disease of unknown cause has emerged along the Pacific Coast of Central America. The disease primarily affects men working manually outdoors, and the major group affected is sugarcane workers. The disease presents with an asymptomatic rise in serum creatinine that progresses to end stage renal disease over several years. Renal biopsies show chronic tubulointerstitial disease. While the cause remains unknown, recent studies suggest that it is driven by recurrent dehydration in the hot climate. Potential mechanisms include the development of hyperosmolarity with the activation of the aldose reductase-fructokinase pathway in the proximal tubule leading to local injury and inflammation, and the possibility that renal injury may be the consequence of repeated uricosuria and urate crystal formation as a consequence of both increased generation and urinary concentration, similar to a chronic tumor lysis syndrome. The epidemic is postulated to be increasing due to the effects of global warming.

Summary. An epidemic of chronic kidney disease has taken more than 20,000 lives in Central America. The cause is unknown, but appears to be due to recurrent dehydration. Potential mechanisms for injury is renal damage as a consequence of recurrent hyperosmolarity and/or injury to the tubules from repeated episodes of uricosuria.

Key Messages. The epidemic of chronic kidney disease in Mesoamerica may be due to chronic recurrent dehydration as a consequence of global warming and working conditions. This entity may be one of the first major disease attributed to the climate change and the greenhouse effect.

Key Words: Chronic Kidney Disease, Mesoamerican Nephropathy, Sugarcane, Uric acid, Global Warming

An epidemic of chronic kidney disease of unknown etiology is occurring in Central America, primarily along the Pacific Coasts. First described in 2002,[1] it likely has been present since the early 1970s, but has increased markedly over the last two decades.[2] To date there have been nearly 20,000 deaths, in part because most of the victims do not have access to dialysis.[3]

Clinically, the disease presents primarily in men working manually in the sugarcane fields, but it has also been observed in other groups that work manually outdoors, such as construction workers, subsistence farmers, etc. Most of the subjects are asymptomatic until they start developing signs of uremia.[4] However, there is evidence that the disease has been slowly progressive, beginning with an asymptomatic rise in serum creatinine, in association with low grade or absent proteinuria with occasional red cells and white cells present in the urine sediment. However, some subjects complain of “sandy urine” that causes dysuria, although uninfected when tested by urine culture.[5] Blood pressure may be slightly high, but not at levels (>160 mm Hg) commonly associated with renal progression. Most strikingly, the disease does not appear to be due to any of the common causes of end stage renal disease, such as diabetes, hypertension, glomerulonephritis, or polycystic kidney disease. Thus, the disease has been called “Mesoamerican Nephropathy”, and is thought to be a new form of kidney disease.[4,6]

There has been much effort to identify the cause of the disease. Renal biopsies, when performed, show a chronic tubulointerstitial disease with focal inflammation, and with signs of glomerular ischemia and secondary glomerulosclerosis.[7] The recognition that this is primarily a chronic tubulointerstitial disease has raised the possibility that toxic metals might be involved in the etiology, such as lead, cadmium, arsenic — however, to date the preliminary studies performed do not support these as a cause. Silica released from the burnt sugarcane remains a possibility, but it does not explain why the disease is also observed in other groups. Likewise, while some studies have focused on agrichemicals and pesticides, no specific pesticide has been identified, and furthermore it does not explain the presence of this disease in other groups. Likewise, infections such as leptospirosis and Hanta virus have been considered, but clinical manifestations of these diseases are largely lacking.

The Role of Dehydration as a Cause of Mesoamerican Nephropathy

One common characteristic to all groups developing this disease, however, is recurrent dehydration occurring during heavy work in the hot environment.[5,8-10] Indeed, some studies have shown that the disease is more common in sugarcane fields that are at lower altitude, compared to those at higher altitude, where the temperature is less hot. Workers are starting early in the morning before the day gets too hot, but studies have shown that the heat indexes cross into the unsafe zone, defined by the Occupational Safety and Health Administration, well before 10:00 in the morning, while workers continue cutting cane for several more hours on a typical day.[8] As such many of the workers develop signs of dehydration during the day (fainting, light headed, jittery), heat strokes are often confused with fever, dysuria is frequent, and urine analyses show signs of urinary concentration (elevated specific gravity, elevated urine osmolality) at the end of the workday.[5,8-10] This happens cyclically, on a routinely daily basis.

An animal model of dehydration associated CKD. Historically, dehydration (loss of water) and volume depletion (salt loss) are thought to result in “pre-renal” kidney disease, in which no frank injury occurs unless blood pressure and renal blood flow drop to levels that cause acute tubular necrosis. However, we recently developed an animal model of recurrent dehydration associated with heat exposure in mice. As a remarkable finding, these mice developed impaired renal function and tubulointerstitial fibrosis after 5 weeks.[11] Interestingly enough, the timing of hydration was key; indeed, if hydration was provided immediately after each cycle of dehydration the renal injury was prevented, whereas if the same total amount of hydration was provided until the end of the day, the renal injury ensued. This study thus suggests that adequate hydration needs to be provided to the sugarcane workers while they are in the fields, not afterwards.

The mechanism by which the kidney injury was developing was also explored. The mice lost salt and water by sweating in their feet, so they tended to become hyperosmolar during the day, and this activated an enzyme system (aldose reductase-sorbitol dehydrogenase-fructokinase) in the proximal tubules. This enzyme system converts the glucose being reabsorbed in the proximal tubule to fructose, which is then metabolized by fructokinase to generate oxidants and uric acid that causes local tubular injury. Interestingly, when mice lacking fructokinase were recurrently dehydrated, they were protected from kidney damage.[11]

New insights into Kidney Injury

Recently our group identified a potentially more important mechanism to account for the development of Mesoamerican Nephropathy. Specifically, exercise under heat stress is known to cause subclinical rhabdomyolysis that is associated with the release of nucleotides and a rise in serum uric acid.[12,13] Indeed, hyperuricemia is very common among the sugarcane workers, and serum uric acid increases during the work day.[9] In turn, during the day, dehydration leads to urinary concentration and acidification, thereby resulting in high urinary uric acid concentrations that can exceed solubility. Urate crystals are common in the urinary sediment of sugarcane workers (Figure 1). Indeed, we found that many of the sugarcane workers develop urine uric acid levels greater than 100 mg/dl per day, which is similar to that observed in subjects suffering from acute kidney injury following chemotherapy (tumor lysis syndrome).[14] Thus, we have proposed that the Mesoamerican Nephropathy may be caused by repeated episodes of hyperuricosuria and urate crystal formation that occurs through hard work on hot days when hydration is limited or delayed.

Why the Epidemic?

The last century has seen a progressive rise in temperatures, with an acceleration in the last 20 years. While the absolute increase in temperature is relatively subtle (0.8 degrees Celsius), this has led to a dramatic increase in exceptionally hot days. Indeed, studies show that 75 percent of extremely hot days (exceeding the 99th percentile) are currently attributable to global warming.[15] As an example, in the summer of 2015 there was a major heat wave in Karachi with over 40,000 people suffering from heat stroke, and another heat wave in Iran where the ambient temperature crossed 160 degrees Fahrenheit.[16,17] For those subjects working daily in the sugarcane fields extremely hot days are common,[9] and the intensity of the work adds up to further increase body core temperature.

It is interesting that epidemics of chronic kidney disease are now being reported in multiple countries, primarily in the tropics, including India, Bangladesh, Sri Lanka, Egypt, Mexico and Central America. A common theme is that the CKD is occurring in subjects working manually outside, directly exposed to sunshine,

frequently in areas where there is significant water shortage or scarce access to potable water. In conclusion, we propose that the Mesoamerican Nephropathy is essentially a consequence of Global Warming, and may be one of the first major diseases described as a consequence of extensive fossil fuel use and the greenhouse effect.

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Figure 1 Urine sediment from a sugarcane worker in Nicaragua. The urine uric acid Crystals are box-like and negatively birefringent.

