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# Chronic kidney disease of unknown etiology in Nicaragua: investigating the role of environmental and occupational exposures

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# CHRONIC KIDNEY DISEASE OF UNKNOWN ETIOLOGY IN NICARAGUA: INVESTIGATING THE ROLE OF ENVIRONMENTAL AND OCCUPATIONAL EXPOSURES

by

**REBECCA L. LAWS**

B.S., Duke University, 2006
M.P.H., Boston University, 2011

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Approved by

First Reader

Michael D. McClean, Sc.D.
Associate Professor of Environmental Health

Second Reader

Madeleine K. Scammell, D.Sc.
Assistant Professor of Environmental Health

Third Reader

Daniel R. Brooks, D.Sc.
Associate Professor of Epidemiology

Outside Reader

David J. Friedman, M.D.
Assistant Professor of Medicine
Beth Israel Deaconess Medical Center
Harvard Medical School

“The idea that some lives matter less is the root of all that is wrong with the world.”

– Paul Farmer

Dedicated to Donald Cortez

1972 — 2013

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Rebecca L. Laws

July 2015

**CHRONIC KIDNEY DISEASE OF UNKNOWN ETIOLOGY IN NICARAGUA: INVESTIGATING THE ROLE OF ENVIRONMENTAL AND OCCUPATIONAL EXPOSURES**

**REBECCA L. LAWS**

Boston University School of Public Health, 2015

Major Professor: Michael D. McClean, Sc.D., Associate Professor of Environmental Health

ABSTRACT

**Background:** In Central America, an epidemic of chronic kidney disease (CKD) primarily affects younger, male agricultural workers. Hypothesized causal agents include heat stress, agrichemicals, and heavy metals, among others, but the etiology remains unknown. Our aims were to assess job-specific changes in kidney function and injury during the sugarcane harvest season, characterize hydration practices and metals exposure, and determine whether these agents are associated with kidney function and injury. The overall goal was to address this public health emergency to inform intervention and prevention strategies.

**Methods:** We recruited 284 sugarcane workers, representing seven job tasks, and 51 miners, all from northwestern Nicaragua. We sampled sugarcane workers at two time points (before and near the end of the harvest season) and miners at one time point. As a marker of kidney function, we measured serum creatinine to estimate glomerular filtration rate (eGFR). As markers of kidney injury, we measured urinary neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), N-acetyl-D-glucosaminidase (NAG), albumin, and creatinine. We measured blood lead and urinary

cadmium, uranium, and total arsenic in a subset of sugarcane workers and miners.

**Results:** Kidney function (eGFR) declined during the harvest season in seed cutters, irrigators, and cane cutters compared to other jobs, and kidney injury biomarkers (NGAL, IL-18) increased during the harvest, most notably among cane cutters. Electrolyte solution consumption appeared protective in cane cutters, a job with high heat exposure and heavy manual exertion. Though metal biomarkers were not elevated, there was some evidence that combined exposure to multiple metals was associated with decreased kidney function and increased injury. Albuminuria was rare.

**Conclusions:** These findings provide evidence that occupational exposures are involved in the etiology of CKD. Heat stress and volume depletion may play a role, most likely in combination with one or more other agents, possibly environmental exposure to low-level metals. Our results do not support the hypothesis that agrichemicals are causal, but future studies that quantify exposure to specific agents are needed. The limited albuminuria and presence of tubular injury markers supports a tubulointerstitial disease that could occur with repeated subclinical injury leading to clinically apparent CKD over time.

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## CHAPTER ONE. INTRODUCTION

Over the past two decades, a drastic increase in the prevalence of chronic kidney disease (CKD) has been identified in some Central American regions (Brooks et al. 2012; Ramirez-Rubio et al. 2013b; Weiner et al. 2013; Wesseling et al. 2013a; Wesseling et al. 2014). The etiology of this CKD epidemic is unknown, and is not explained by common risk factors such as diabetes or hypertension (Cuadra et al. 2006; O'Donnell et al. 2011; Ramirez-Rubio et al. 2013a; Torres et al. 2010). Due to its unique characteristics, the disease is referred to as chronic kidney disease of unknown etiology (CKDu), chronic kidney disease of non-traditional etiology (CKDnt), or Mesoamerican Nephropathy (MeN).

Treatment options for end-stage renal disease are limited in this resource-poor region, so a CKD diagnosis is usually terminal (Correa-Rotter et al. 2014; Weiner et al. 2013). Though exact data are lacking, some have estimated upwards of 20,000 deaths due to MeN (Ramirez-Rubio et al. 2013b). The Pan American Health Organization (PAHO) has recognized this epidemic as a serious public health problem that requires immediate and coordinated action (Ordunez et al. 2014a; Ordunez et al. 2014b).

### *Overview of Chronic Kidney Disease (CKD)*

Chronic kidney disease (CKD) is defined as "abnormalities of kidney structure or function, present for greater than three months, with implications for health" (KDIGO 2013). Glomerular filtration rate (GFR) is considered the best overall indicator of kidney function, as it becomes reduced after widespread structural damage (KDIGO 2013). GFR

can be measured directly, but is typically estimated based on serum creatinine, or alternatively, serum cystatin C, as a function of age, sex, and race. Normal GFR in young adult men and women is approximately 125 mL/min/1.73 $m^2$ (KDIGO 2013). CKD, on the other hand, is characterized by a progressive loss of kidney function over time, and the severity is staged (1–5) according to estimated GFR (eGFR) (Table 1.1). An eGFR <60 mL/min/1.73 $m^2$ indicates a moderate decrease in kidney function and is the cutoff value for CKD stage 3. Stage 5 (eGFR <15 mL/min/1.73 $m^2$) requires renal replacement therapy in the form of dialysis or kidney transplant. Of note, eGFR >60 mL/min/1.73 $m^2$ only constitutes CKD if some form of kidney damage is present.

**Table 1.1.** Stages of Chronic Kidney Disease

| Stage | Description | Estimated GFR (mL/min/1.73 $m^2$) | Action |
|---|---|---|---|
| 1 | Kidney damage with normal or increased GFR | >90 | • Diagnosis<br>• Treatment of comorbidities<br>• Slow progression |
| 2 | Kidney damage with mildly decreased GFR | 60–89 | • Estimate progression |
| 3a | Mildly to moderately decreased GFR | 45–59 | • Evaluate and treat complications |
| 3b | Moderately to severely decreased GFR | 30–44 | • Treat complications |
| 4 | Severely decreased GFR | 15–29 | • Preparation for kidney replacement therapy |
| 5 | Kidney failure | <15 | • Replacement |

Source: adapted from KDIGO 2013, NKF 2002

Though primarily based on eGFR, cause of disease and proteinuria are also considered in CKD staging. Cause is generally assigned based on whether there is underlying systemic disease that affects the kidney (*e.g.* diabetes) or primary kidney

disease, as well as location of abnormality within the kidney (*i.e.* glomerular, tubulointerstitial, vascular) (KDIGO 2013). Proteinuria is the term used to indicate increased levels of protein in the urine, and may suggest problems with the glomerulus (*e.g.* increased permeability) or tubules (*e.g.* incomplete reabsorption). In most kidney diseases, but not all, albumin is the predominant protein found in the urine, and is associated with disease progression. For these reasons, albumin-to-creatinine ratio (ACR) is also incorporated into CKD staging with three categories: A1 (<30 mg/g), A2 (30–300 mg/g), and A3 (>300 mg/g) (KDIGO 2013). Albuminuria is a common characteristic of glomerular diseases and is often increased before GFR decreases (KDIGO 2013). Tubulointerstitial diseases, on the other hand, are typically characterized by presence of low-molecular weight proteinuria (*e.g.* $\beta_2$-microglobulin), without substantially increased albuminuria (KDIGO 2013). Regardless of whether the primary location of injury is glomerular, tubular, or vascular, chronic progression will eventually result in a common manifestation of histological and functional alterations, leading to generalized fibrosis and glomerulosclerosis, and will continue even in the absence of the triggering insult (Lopez-Novoa et al. 2011).

Acute kidney injury (AKI) is an abrupt (within hours to days) reduction in kidney function, and is defined by a doubling of serum creatinine or reduction in urine volume output (Van Biesen et al. 2006). Once thought to be a completely reversible syndrome, there is now evidence that AKI may result in permanent kidney damage, increasing the risk for chronic kidney disease and end-stage renal disease (Bedford et al. 2012; Coca et al. 2012; Venkatachalam et al. 2010). Although less documented, an emerging hypothesis

is that repeated subclinical kidney injury may also increase one's risk for CKD.

Within the past decade, several novel biomarkers of kidney injury have been identified. Since serum creatinine is an insensitive and late marker of disease (Slocum et al. 2012), these biomarkers of kidney injury may be useful tools for earlier diagnosis of clinically-relevant disease, as well as for localization of injury to a specific site in the kidney (Bonventre et al. 2010; Charlton et al. 2014; Vaidya et al. 2008). Furthermore, these markers may be useful for monitoring disease progression and severity, and may help to identify individuals at greatest risk for developing CKD (Bolignano et al. 2009; Chawla et al. 2014; Devarajan 2008; Vaidya et al. 2008).

*CKD Worldwide*

Chronic kidney disease is a global public health problem that creates a substantial burden on health systems (Levey et al. 2007a; Levey et al. 2007b; Nugent et al. 2011). The prevalence is increasing worldwide, largely driven by the widespread increase in diabetes and hypertension, the two most common risk factors for CKD (El Nahas and Bello 2005; Grams et al. 2013). In the United States, CKD prevalence has steadily increased from 10% in 1988–1994 to 13% in 1999–2004 (Coresh et al. 2007). Though the early and least severe stages account for most CKD cases, the age- and sex-adjusted incidence of end-stage renal disease (ESRD) has also increased, by 42% between 1991 and 2001 (Coresh et al. 2007). In the US and Europe, CKD is common only in older populations (age >60) and affects men and women equally. For example, in the United States, the prevalence of CKD is 21% among those age ≥60 years but is less than 2%

among those age <60 years (O'Donnell et al. 2011). The disease typically affects the glomeruli of the kidney and is characterized by significant proteinuria.

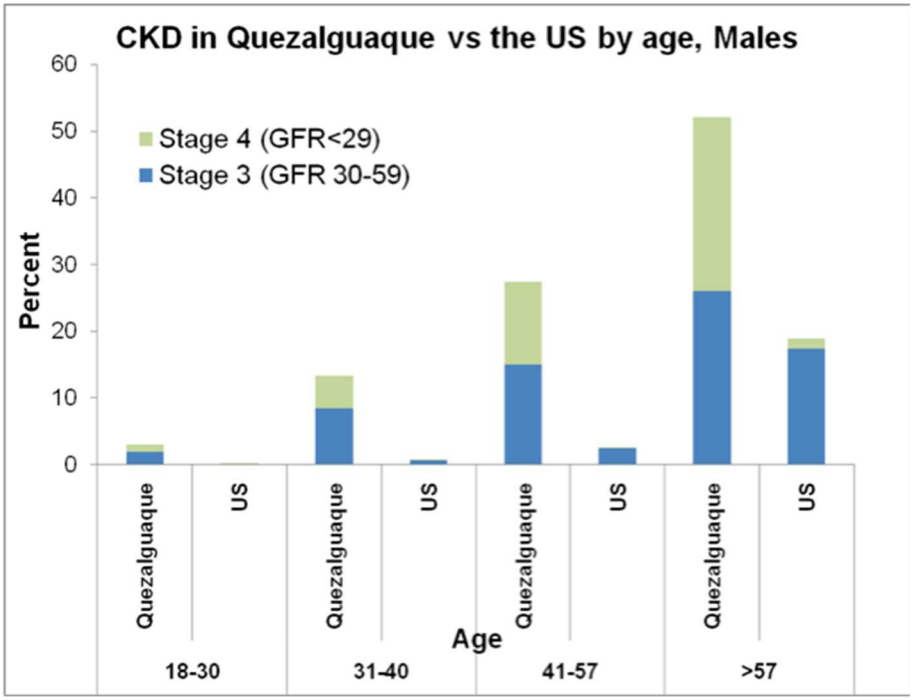
In addition to diabetes and hypertension, other common risk factors include family history of CKD, autoimmune diseases, and systemic infections (Vassalotti et al. 2007). Environmental and occupational exposures to nephrotoxic agents (*e.g.* heavy metals, solvents, aristolochic acid) usually account for a small percentage of CKD, particularly when compared to diabetes and hypertension, and tend to damage the tubules rather than the glomeruli (Carvalho et al. 2003; Sabolic 2006; Soderland et al. 2010). Tubulointerstitial disease typically presents with low proteinuria and is far less common than glomerular disease.

*Epidemiology of CKD in Central America*

The pattern of CKD in Central America contrasts with what we see in more industrialized countries. There is little evidence of diabetes or hypertension, and high-grade proteinuria is uncommon, pointing to a tubulointerstitial rather than glomerular disease process (O'Donnell et al. 2011; Orantes et al. 2011; Peraza et al. 2012; Raines et al. 2014; Ramirez-Rubio et al. 2013a; Sanoff et al. 2010; Torres et al. 2010). Supporting this empirical evidence, biopsies indicate interstitial fibrosis and tubular atrophy, with secondary glomerular damage (Lopez-Marin et al. 2014; Wijkstrom et al. 2013). The disease disproportionately affects men, often at ratios greater than 3:1 (Cuadra et al. 2006; O'Donnell et al. 2011; Orantes et al. 2011; Peraza et al. 2012; Torres et al. 2010). Furthermore, these otherwise healthy men are developing disease at relatively young

ages, often in the third to fifth decade of life. In Quezalguaque, Nicaragua, for example, the prevalence of CKD stages 3 and 4 in men aged 31–40 is nearly 15 times that of similarly aged men in the United States (Figure 1.1) (O'Donnell et al. 2011).

**Figure 1.1.** Comparison of CKD Prevalence in Quezalguaque and the U.S. by Age, Males




Source: O'Donnell et al. 2011

This unusual CKD was first observed in the 1970s, though it is primarily within the past decade that the epidemic has been described in formal reports or peer-reviewed journals (Cerdas 2005; Cuadra et al. 2006; Domínguez et al. 2003; Garcia-Trabanino et al. 2005). Throughout Central America, CKD incidence data are lacking, but mortality data indicate that El Salvador has the highest overall age-adjusted mortality rate due to kidney disease in the world, at approximately 52 deaths per 100,000 people, and that CKD is the second leading cause of mortality among men of working age (MOH 2010). Nicaragua and Honduras are also among the top ten countries with the highest mortality

due to kidney disease in the world (WHO 2008). These data provide evidence that the CKD epidemic concentrates primarily in the Pacific coastal lowlands, extending from southern Mexico to Panama. For example, the provinces of Chinandega and León on the Pacific coast of Nicaragua have CKD mortality rates of 73 and 77 per 100,000, respectively, compared to 19.6 per 100,000 for the country as a whole (MINSA 2011). Reports also suggest an increasing trend over time in these affected areas (Cuadra et al. 2006).

Over the past 5–10 years, a number of prevalence studies have been conducted in the region. All measured serum creatinine to estimate GFR, and some included nested case-control studies to evaluate potential CKD risk factors. Results from affected communities in Nicaragua and El Salvador estimate the prevalence of reduced kidney function (as defined by eGFR <60 mL/min/1.73m$^2$) between 9 and 18% in the general population and between 14 and 26% in men (Lebov et al. 2015; O'Donnell et al. 2011; Orantes et al. 2011; Peraza et al. 2012; Raines et al. 2014; Sanoff et al. 2010; Torres et al. 2010). These studies have also consistently demonstrated that agricultural workers are a high-risk population, particularly those with a history of working in the sugarcane, cotton, corn, rice, and banana industries (Orantes et al. 2014; Peraza et al. 2012; Raines et al. 2014; Sanoff et al. 2010). Increased risk has also been observed in communities of miners, subsistence farmers, and fishermen, but notably, not in coffee-growing communities (Laux et al. 2012; Peraza et al. 2012; Sanoff et al. 2010; Torres et al. 2010). Finally, it appears that persons living at a lower altitude are at greater risk of CKD than those living at higher elevations (Laux et al. 2012; Peraza et al. 2012; Torres et al. 2010).

Table 1.2 provides an overview of some of these features common to Mesoamerican Nephropathy.

**Table 1.2** Characteristics of Mesoamerican Nephropathy

| **Features Common to MeN** |
| --- |
| Males more commonly affected than females |
| Individuals affected at younger ages than expected |
| Common risk factor is agricultural work (current or former) |
| Not associated with diabetes or hypertension |
| Little evidence of proteinuria |
| Pathology indicates varying degrees of glomerulosclerosis with tubular atrophy and interstitial fibrosis |

*Potential Causes of Mesoamerican Nephropathy*

Many possible etiologies of Mesoamerican Nephropathy have been posited, but currently the cause(s) remains unknown. Given the demographics of the disease, environmental or occupational etiologies are suspected. Hypotheses include heat stress, agrichemicals, heavy metals, nephrotoxic medications, systemic infections (*e.g.* leptospirosis), fructose intake, and genetic factors (Correa-Rotter et al. 2014; Cuadra et al. 2006; Roncal Jimenez et al. 2014; Weiner et al. 2013; Wesseling et al. 2014). Several of these are reviewed below, and a more in-depth evaluation of each potential etiologic agent is available in Weiner *et al.* 2013, Correa-Rotter *et al.* 2014, and Wesseling *et al.* 2014.

Heat stress, described as repeated exposure to heat and strenuous labor with associated repeated volume depletion and dehydration, has garnered particular consideration (Brooks et al. 2012; Johnson and Sanchez-Lozada 2013; Peraza et al. 2012; Torres et al. 2010; Wesseling et al. 2013a). The epidemiologic pattern of MeN is consistent with this hypothesis:

- mortality is highest in lowland areas with high temperatures;
- occupational groups that are most affected perform heavy manual labor in hot environments; and
- communities in cooler, high altitude areas have lower CKD prevalence than similar communities in hotter, low altitude areas.

Renal tubules are particularly vulnerable to reduced perfusion from dehydration, potentially leading to AKI. However, heat stress is not a known cause of CKD and there could be other explanations for the patterns described above.

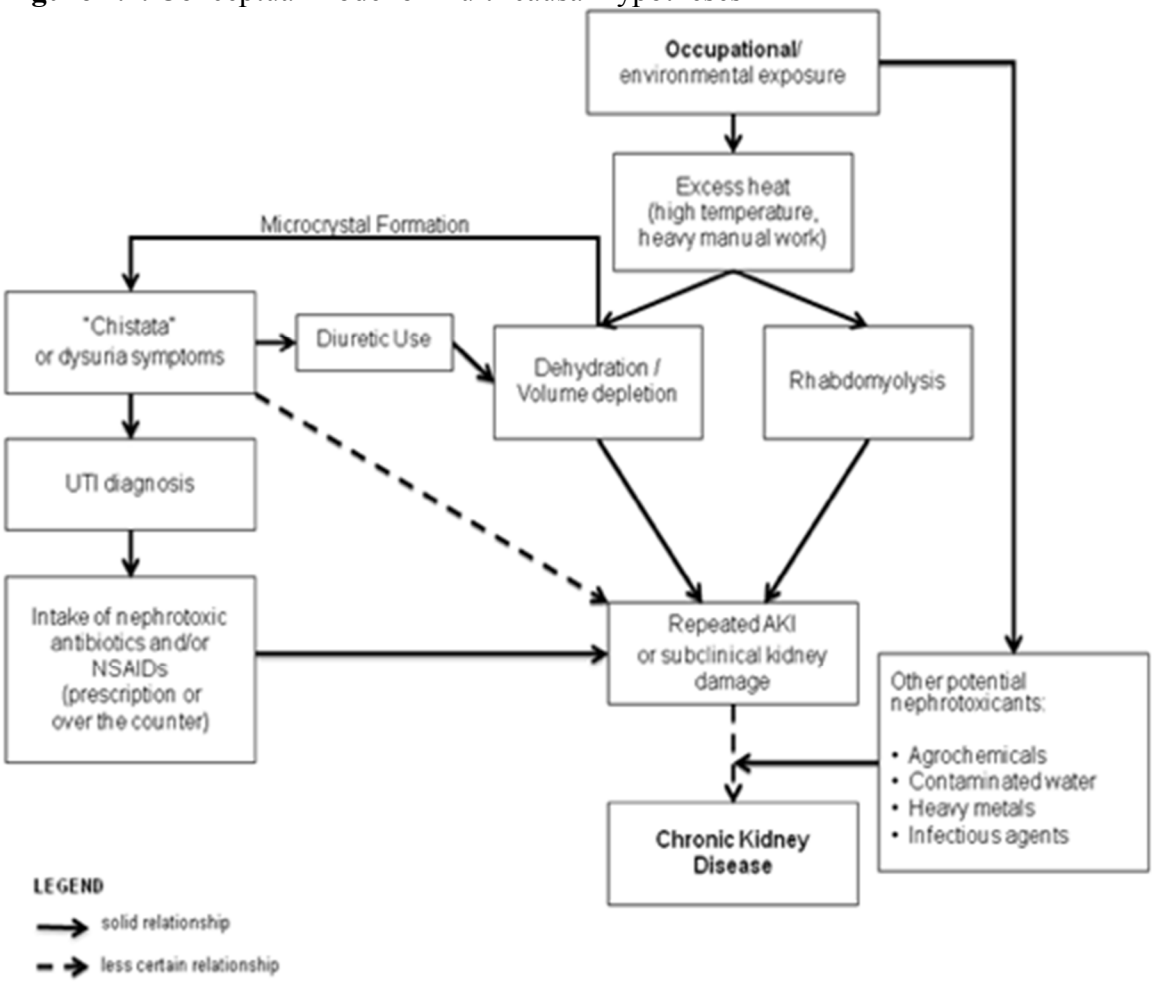
Agrichemicals are also an important hypothesis, as they are used extensively in the region and widely believed among community members to be the cause of the epidemic. Several studies have reported an association between agrichemicals and CKD (Orantes et al. 2011; Orantes et al. 2014; Raines et al. 2014). One major limitation, however, is the basis of these findings on self-reported agrichemical use. Additionally, there is evidence of increased CKD risk in workers in non-agricultural industries, presumably without occupational exposure to agrichemicals (Torres et al. 2010). This hypothesis remains controversial and requires further investigation. In fact, due the perceived link to CKD, El Salvador's National Assembly voted in 2013 to ban 53 agrichemicals, including 2,4-D, paraquat, DDT, and glyphosate, among others (Chavkin 2013).

Heavy metals such as lead and cadmium are well-known nephrotoxicants, most directly affecting the proximal tubules (Gonick 2008; Sabath and Robles-Osorio 2012; Soderland et al. 2010; Vicente-Vicente et al. 2010). Furthermore, these metals may be

elevated throughout the Pacific region of Central America due to a series of active volcanoes (Lopez et al. 2012). Arsenic is of particular concern, as reports have identified arsenic contaminated well water in several communities in western Nicaragua (Bundschuh et al. 2012; Lopez et al. 2012; McClintock et al. 2012). Cadmium, lead, and uranium are also of concern and have been identified in high concentrations in soils of other volcanic areas (Bubach et al. 2014; Mora 2003; Queirolo et al. 2000). To date, however, there has been no comprehensive assessment of metals in this population.

Not all hypothesized etiologic agents are known causes of CKD; therefore, it is possible that some unknown biological pathway is at play. Additionally, the causal mechanism is most likely multifactorial (Brooks et al. 2012; Ramirez-Rubio et al. 2013a; Wesseling et al. 2013a). For example, it is plausible that an environmental exposure occurring in the community (*e.g.* arsenic in drinking water) could be modified by a separate occupational factor (*e.g.* heat stress), resulting in disease that would not otherwise occur as the result of either risk factor alone. Ramirez-Rubio (2013a) proposed a conceptual model describing how multiple risk factors may be involved in the etiology of MeN, using heat stress as an example (Figure 1.2). Most postulated scenarios involve a mechanism of multiple episodes of subclinical AKI, leading to residual damage post-recovery, which eventually progresses to CKD even in the absence of overt AKI. Adding to this already complex multi-causal web, these workers live in extreme poverty and are a vulnerable population, such that social factors (*e.g.* lack of health care, poor diet) may be predisposing individuals to disease (Hossain et al. 2009; Rossi-Rocha et al. 2007).

**Figure 1.2.** Conceptual model of multi-causal hypotheses




Source: Ramirez-Rubio 2013a

*Research Objectives*

Currently, the Mesoamerican Nephropathy is severely understudied. In fact, considering the magnitude of this public health crisis, investigations to date have been quite limited, primarily consisting of cross-sectional surveys of serum creatinine/eGFR and self-reported exposure information (O'Donnell et al. 2011; Orantes et al. 2011; Peraza et al. 2012; Sanoff et al. 2010; Torres et al. 2010). Analyses have primarily focused on between-industry comparisons, but not within-industry (*i.e.* job-level)

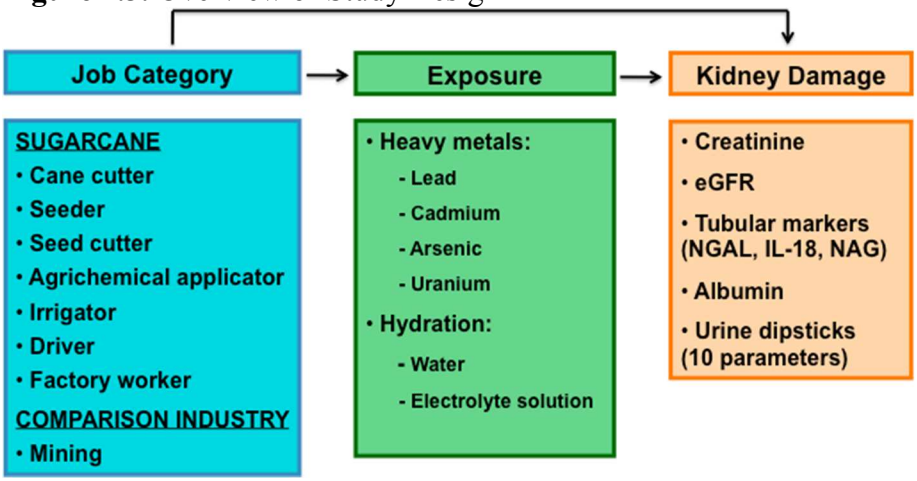
comparisons. A consortium of researchers investigating MeN, the Consortium for the Epidemic of Nephropathy in Central America and Mexico (CENCAM), highlighted the need for repeated measures studies, an improved approach to exposure assessment, and use of novel biomarkers of tubular injury to better characterize the type of renal disease (Wesseling et al. 2014). Critically, it is important to identify the environmental and/or occupational factors that play a role so that exposure reduction strategizes can be designed accordingly.

This research sought to address these key data gaps and to optimize the design of future research in the region. We utilized repeated measures to examine changes in biomarkers of kidney function and injury among sugarcane workers during one harvest season and evaluate differences by job category in a population at risk for Mesoamerican Nephropathy. We also quantified exposure to heavy metals, providing the first comprehensive data addressing this important hypothesis. To address these aims, we recruited a cohort of 284 sugarcane workers from the largest sugarcane company in western Nicaragua. We collected blood and urine samples before the 2010–2011 harvest and again at late-harvest (approximately five months later). At both time points, we obtained information about work practices and occupational history; at late-harvest, we also asked workers about typical hydration practices during the harvest. Finally, we recruited a population of miners from the same area for inclusion in the assessment of heavy metals; these workers were sampled at one time point, corresponding to the late-harvest investigation.

We assessed exposure in different but complementary ways. First, because the

causal agent is unknown, job task served as a surrogate for exposure. Workers were engaged in seven distinct job tasks (cane cutter, seeder, seed cutter, agrichemical applicator, irrigator, driver, and factory worker). All sugarcane workers live in the same region under similar socioeconomic conditions, but the various job tasks are different in terms of potential exposure to hypothesized causal agents (*i.e.* heat stress, agrichemicals) (McClean et al. 2010). A finding that biomarkers of kidney function and injury are not different by job category would suggest that the primary etiologic agent is environmental and not occupational, whereas a finding that kidney damage is higher in certain job tasks would provide information about which occupational factors may be important. Second, we used biomarkers to quantify exposure to nephrotoxic heavy metals. Total arsenic, cadmium, and uranium were analyzed in urine, while lead was analyzed in whole blood. Lastly, we used self-report to assess typical hydration practices during the harvest. Sugarcane workers were asked about the quantity of water and electrolyte solution packets (100 mL, distributed by the company) consumed during a typical workday. Outcome measures were biomarkers of kidney function and injury and included serum creatinine (used to estimate GFR), as well as urinary albumin and three novel biomarkers of kidney injury: neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), and N-acetyl-D-glucosaminidase (NAG). The study design is outlined in Figure 1.3.

**Figure 1.3.** Overview of Study Design




Our primary aim in *Chapter 2* was to evaluate changes in kidney function among sugarcane workers during the six-month harvest season and assess job-specific differences in these changes. Kidney function was measured using estimated glomerular filtration rate (eGFR) from serum creatinine; we also evaluated proteinuria as an indicator of location of injury within the kidney. Secondary aims were to explore associations between self-reported hydration and kidney function, both overall and within individual job categories, and evaluate the effect of employment duration on kidney function.

*Chapter 3* employed a similar study design as *Chapter 2*, but evaluated novel biomarkers of kidney injury to assess the potential for tubular injury in these workers. We evaluated job-specific changes in NGAL, IL-18, and NAG, and explored relationships between water and electrolyte solution consumption and these biomarkers. Additionally, we assessed relationships between changes in biomarkers of kidney injury and eGFR, to determine if subclinical injury may be associated with chronic decline in kidney function.

The primary aim of *Chapter 4* was to examine potential associations between nephrotoxic metals (*i.e.* arsenic, cadmium, lead, uranium) and biomarkers of kidney

function and injury among sugarcane workers and miners in northwestern Nicaragua. We also aimed to characterize exposure to these metals and compare exposure levels to those of the United States adult population using the National Health and Nutrition Examination Survey (NHANES). Finally, we evaluated differences in metal biomarkers by job category, providing important information about exposure pathways.

*Chapter 5* summarizes the main conclusions from *Chapters 2–4* and puts these findings into a broader context regarding Mesoamerican Nephropathy. Methodological limitations, public health implications, and directions for future research are discussed.

## CHAPTER TWO. CHANGES IN KIDNEY FUNCTION AMONG NICARAGUAN SUGARCANE WORKERS

Rebecca L. Laws[1], Daniel R. Brooks[2], Juan José Amador[2], Daniel E. Weiner[3], James S. Kaufman[4], Oriana Ramírez-Rubio[2,5], Alejandro Riefkohl[2], Madeleine K. Scammell[1], Damaris López-Pilarte[2], José Marcel Sánchez[2], Chirag R. Parikh[6,7], Michael D. McClean[1]

[1]Department of Environmental Health, Boston University School of Public Health, Boston, MA, USA
[2]Department of Epidemiology, Boston University School of Public Health, Boston, MA, USA
[3]Division of Nephrology, Department of Medicine, Tufts Medical Center and Tufts University School of Medicine, Boston, MA, USA
[4]Research Service, VA New York Harbor Healthcare System and Department of Medicine, New York, NY, USA
[5]Preventive Medicine and Public Health Department, Universidad Autónoma de Madrid, Madrid, Spain
[6]Section of Nephrology, Department of Medicine, Yale University and VA Medical Center, New Haven, CT, USA
[7]Program of Applied Translational Research, Department of Medicine, Yale University, New Haven, CT, USA

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## ABSTRACT

**Background:** There is an epidemic of chronic kidney disease (CKD) of unknown etiology in Central American workers.

**Objectives:** To investigate changes and job-specific differences in kidney function over a six-month sugarcane harvest season, explore the potential role of hydration, and measure proteinuria.

**Methods:** We recruited 284 Nicaraguan sugarcane workers performing seven distinct tasks. We measured urine albumin and serum creatinine and estimated glomerular filtration rate (eGFR).

**Results:** eGFR varied by job and decreased during the harvest in seed cutters (-8.6 mL/min/1.73 $m^2$), irrigators (-7.4 mL/min/1.73 $m^2$), and cane cutters (-5.0 mL/min/1.73 $m^2$), as compared to factory workers. The number of years employed at the company was negatively associated with eGFR. Fewer than 5% of workers had albumin-to-creatinine ratio >30 mg/g.

**Conclusions:** The decline in kidney function during the harvest and the differences by job category and employment duration provide evidence that one or more risk factors of CKD are occupational.

## INTRODUCTION

In Central America, there is an epidemic of chronic kidney disease (CKD) and the prevalence has been increasing over the past two decades (Cuadra et al. 2006; Wesseling et al. 2013b). The etiology of this disease, also referred to as Mesoamerican Nephropathy

(MeN) and CKD of nontraditional etiology ($CKD_{nt}$), is unknown and traditional CKD risk factors are not implicated (Orantes et al. 2014; Ordunez et al. 2014b; Raines et al. 2014; Ramirez-Rubio et al. 2013a; Ramirez-Rubio et al. 2013b; Weiner et al. 2013; Wesseling et al. 2013a; Wesseling et al. 2014). In Nicaragua, Honduras, and El Salvador, the age-adjusted mortality rates due to CKD are among the ten highest in the world, with El Salvador having the highest at 54 deaths per 100,000 people (WHO 2008). In the provinces of Chinandega and León in western Nicaragua, 73 and 77 deaths per 100,000 people are attributed to CKD, respectively (MINSA 2011). Cross-sectional studies in affected communities in Nicaragua and El Salvador have found the prevalence of reduced kidney function (as defined by glomerular filtration rate <60 mL/min/1.73$m^2$) to range between 12 and 18% in the general population and between 14 and 26% in men (O'Donnell et al. 2011; Orantes et al. 2011; Peraza et al. 2012; Sanoff et al. 2010; Torres et al. 2010).

In more industrialized countries, CKD occurs almost equally in older men and women (>60 years old) with antecedent diabetes and/or hypertension, and with proteinuria as a frequent clinical manifestation (Coresh et al. 2005; Coresh et al. 2007; Levey et al. 2007a). Studies in Nicaragua indicate that men are three to five times more likely to be affected than women, and that the disease develops at younger ages, often before the age of 40 (Cuadra et al. 2006; O'Donnell et al. 2011; Torres et al. 2010). In one study, the prevalence of reduced kidney function among men aged 31–40 was nearly 15 times higher in Quezalguaque, Nicaragua than in the United States (O'Donnell et al. 2011). Little evidence of diabetes or hypertension, and the lack of proteinuria, suggest

that the kidney disease is primarily tubulointerstitial in nature (Almaguer et al. 2014; Cuadra et al. 2006; O'Donnell et al. 2011; Orantes et al. 2011; Peraza et al. 2012; Raines et al. 2014; Sanoff et al. 2010; Torres et al. 2010).

Agricultural workers are a high risk population, two to three times more likely to develop CKD compared to individuals who have never worked in agriculture (Orantes et al. 2014; Peraza et al. 2012; Raines et al. 2014; Sanoff et al. 2010). A history of working in the sugarcane, cotton, corn, rice, and banana industries is associated with increased CKD risk, though increased risk has also been observed in communities of miners, subsistence farmers, and fishermen (Peraza et al. 2012; Sanoff et al. 2010; Torres et al. 2010). These findings have primarily been based on descriptive surveys and cross-sectional studies, with most studies focusing on current occupation rather than lifetime occupational history. Many occupational and non-occupational risk factors have been hypothesized, including heat stress, agrichemicals, heavy metals, aristolochic acid, nephrotoxic medications, systemic infections (e.g. leptospirosis), and genetic factors (Correa-Rotter et al. 2014; Cuadra et al. 2006; Weiner et al. 2013; Wesseling et al. 2013a). Several papers suggest that heat stress, described as repeated exposure to heat and strenuous labor with associated repeated volume depletion and dehydration, may play an important role (Brooks et al. 2012; Johnson and Sanchez-Lozada 2013; Peraza et al. 2012; Torres et al. 2010; Wesseling et al. 2013a). Most researchers believe that the causal mechanism of this MeN is multifactorial, though the specific risk factors remain unknown (Brooks et al. 2012; Ramirez-Rubio et al. 2013a; Wesseling et al. 2013a).

Given the high prevalence of CKD in Central America and limited availability of treatment, this epidemic has been significantly understudied. Analyses to date have focused on between- industry comparisons, but not within-industry (i.e. job-level) comparisons. There is a need for repeated measures studies and an improved approach to exposure assessment (Wesseling et al. 2014). Accordingly, the primary aims of this study were to: (1) investigate changes in kidney function among sugarcane workers in northwestern Nicaragua during the six-month harvest season; (2) evaluate differences in kidney function by job category; (3) explore associations between self-reported hydration and kidney function, and (4) measure presence of proteinuria as an indicator of location of injury within the kidney.

## METHODS

### *Study Design*

The study population included sugarcane workers employed by one company in northwestern Nicaragua. All participants were at least 18 years of age and provided informed consent. The Institutional Review Boards at the Boston University Medical Center and the Nicaraguan Ministry of Health approved this study.

Prior to each harvest season, the company conducts a medical exam, which includes screening for elevated serum creatinine, to determine whether workers are healthy for employment. During the study period, workers with serum creatinine levels ≥1.4 mg/dL were not hired. Study participants (n=1249) were enrolled and provided pre-harvest samples between October and December 2010. The baseline assessment included

collection of blood and urine samples and information about personal characteristics and occupational history.

Approximately 4–6 months later, toward the end of the harvest season (March to May 2011), we obtained a second sample from 506 workers at their respective work locations (Figure 2.1). All workers were sampled during the morning hours, which occurred prior to starting the work shift for all field workers, prior to or during the work shift for factory workers, and at the end of the night work shift for drivers. The follow-up assessment collected blood and urine samples and information about work practices, hydration, and alcohol consumption. Regarding hydration, workers were queried on the quantity of water and electrolyte solution packets (100 mL, distributed by the company) consumed during a typical workday.

A subset of the 506 workers sampled at pre- and late-harvest was included in the final study population. First, participants who performed more than one job during the harvest (n=34) were excluded from analysis. Next, we included all cane cutters, seed cutters, seeders, and agrichemical applicators. A random sample of irrigators, drivers, and factory workers was selected, as there were a larger number of workers in these jobs. Workers who reported performing jobs other than these seven were excluded due to small sample sizes. The final study population included 284 workers, representing seven jobs, with biological samples from pre- and late-harvest (Figure 2.1). Our goal was to select job categories that were emblematic of certain exposures hypothesized to cause MeN and to include enough workers in each job to have adequate power. For each job, assessment of the likelihood of exposure to putative causal agents, relative to other jobs, was

determined based on a prior industrial hygiene assessment (Table 2.1) (McClean et al. 2010).

*Biomarkers of Kidney Function*

Immediately following collection, blood and urine samples were processed at the local health center and stored at -20°C. Within 1 week, samples were transported to the ISO certified *Centro Nacional de Diagnóstico y Referencia* (CNDR) in Managua, a division of the Ministry of Health (MINSA), and stored at -80°C.

Serum creatinine was measured at CNDR using a kinetic-rate Jaffe method; 0.2 mg/dL was subtracted from creatinine results to calibrate to an isotopic dilution mass spectrometry (IDMS) standard. Urine samples were shipped to the Division of Nephrology and Hypertension at the Cincinnati Children's Hospital Medical Center (Cincinnati, Ohio, USA) for analysis of urine creatinine and albumin (to assess proteinuria). Urine albumin and creatinine were measured by immunoturbidimetry and a colorimetric modification of the Jaffe reaction, respectively. The limit of detection (LOD) for urine albumin was 1.3 mg/L.

*Data Analysis*

Data were analyzed using Statistical Analysis Software (SAS version 9.3, Cary, NC). The distribution of each biomarker was examined using histograms, graphical displays, and summary statistics, to determine if eGFR and serum creatinine were normally distributed. For albumin values below the LOD, the LOD/$\sqrt{2}$ was substituted.

To account for urine concentration, albumin was normalized to urine creatinine concentration and expressed as albumin-to-creatinine ratio (ACR) (mg/g). Serum creatinine (mg/dL) was used to estimate glomerular filtration rate (eGFR) (mL/min/1.73 $m^2$) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. (Levey et al. 2009) Lower eGFR and higher serum creatinine levels are indicative of worse kidney function. Race was considered "non-black" for purposes of calculating the CKD-EPI equation.

Paired t-tests were performed on unadjusted data to determine if eGFR and serum creatinine changed from pre-harvest to late-harvest by job category. Using multiple linear regression models, the association between job category and eGFR at pre-harvest, late-harvest, and change-during-harvest (calculated by subtracting each pre-harvest measurement from the corresponding late-harvest measurement) was evaluated. In the first set of models, 'field worker' (yes/no) was the primary predictor of interest (reference: non-field worker). In the second set of models, the 'job category' variable was the independent variable (reference: factory workers) (Table 2.1). Sensitivity analyses, restricted to men and to field workers, were performed to test for residual confounding by exposures associated with sex or field worker status. Because the CKD-EPI equation is not as accurate at higher levels of eGFR, sensitivity analyses were performed both truncating any eGFR values >120 mL/min/1.73 $m^2$ to 120 mL/min/1.73 $m^2$, as well as restricting analyses to workers with eGFR ≤120 mL/min/1.73 $m^2$.

Additional predictors of interest included years worked at the company, self-reported daily water/electrolyte solution intake, and weekly alcohol consumption. We

explored the effects of these variables on kidney function overall and within categories of job and field worker status. Sex and age were included in all adjusted models. For the two workers missing information on the number of years worked, data were imputed using age, sex, and job. Though age was correlated with years worked at the company ($r=0.67$; $p<0.001$), the two variables were not collinear (i.e. tolerance greater than 0.1 and variance inflation factor less than 10). To evaluate previous employment, we analyzed pre-harvest eGFR by job according to whether participants had worked at the company during the previous year. In separate models, we assessed predictors of hydration to explore how water and electrolyte solution consumption differs by job category and sex.

## RESULTS

*Study population and biomarkers of kidney function*

Figure 2.1 summarizes the derivation of the final study population. The majority of workers were men, with women only employed as seed cutters, seeders, and factory workers. The mean age of workers was 33.6 years (Table 2.2), and on average, drivers and factory workers were older than field workers (Table S2.1). The number of years worked at the company ranged from less than 1 to 40, with a mean of 9.4 years (Table 2.2).

Unadjusted values of serum creatinine and eGFR, stratified by job, are presented in Table 2.3. Both variables were normally distributed. Mean eGFR was lowest for drivers at pre-harvest and for cane cutters at late-harvest. Comparisons of pre-harvest to late-harvest measurements indicated that, on average, serum creatinine increased and

eGFR decreased during the harvest for seed cutters, irrigators, cane cutters, and to a lesser degree, agrichemical applicators.

Thirteen workers (4.6%) had serum creatinine concentrations that increased during the harvest by at least 0.3 mg/dL, a magnitude unlikely to be consistent with random variability. The number of workers with eGFR <60 mL/min/1.73 $m^2$ (the cutoff for Stage 3 CKD) increased from one worker at pre-harvest (0.4%) to eight workers at late-harvest (2.8%). All eight were field workers and included three cane cutters, three seeders, one seed cutter, and one irrigator.

Urine ACR was generally low in all workers at pre-harvest (median=2.4 mg/g) and late-harvest (median=2.1 mg/g). At both time points, fewer than 5% of workers had ACR >30 mg/g, the clinical threshold for abnormal urine ACR. Of the eight workers who had eGFR <60 mL/min/1.73 $m^2$ at late-harvest, only one had ACR >30 mg/g. Two of 29 workers (6.9%) with eGFR <90 mL/min/1.73 $m^2$ at late-harvest had ACR >30 mg/g.

*Job Category and eGFR*

At pre-harvest, there was no difference in eGFR between field workers and non-field workers (Table 2.4, Model 1). When analyzed by job category, cane cutters had the lowest mean eGFR at pre-harvest, an average of 9.2 mL/min/1.73 $m^2$ (95% CI: -14.9, -3.4) less than factory workers (Table 2.4, Model 2). In a sub-analysis of cane cutters, those who had worked in the previous year had a mean pre-harvest eGFR that was 13.1 mL/min/1.73 $m^2$ (95% CI: -24.6, -1.7) lower than those who had not worked (age adjusted). Similar sub-analyses were not possible for additional job categories due to an

insufficient number of new employees. In Model 2, years worked at the company was a predictor of eGFR, such that eGFR decreased by 0.3 mL/min/1.73 $m^2$ (95% CI: -0.6, -0.04) for each additional year of employment. The effect of employment duration did not differ by job.

At late-harvest, field workers had a mean eGFR that was 7.8 mL/min/1.73 $m^2$ (95% CI: -12.3, -3.2) lower than non-field workers (Table 2.4, Model 1). eGFR among cane cutters, seed cutters, seeders, and irrigators was lower than factory workers (Table 2.4, Model 2). The association between years worked at the company and late-harvest eGFR, though of lesser magnitude, followed a similar trend as at pre-harvest and did not vary by job.

Finally, we evaluated the change in eGFR during the 6-month harvest (late-harvest eGFR minus pre-harvest eGFR). Field workers experienced a 6.9 mL/min/1.73 $m^2$ (95% CI: -10.6, -3.2) decrease in eGFR during the harvest compared to non-field workers (Table 2.4, Model 1). Seed cutters (-8.6 mL/min/1.73 $m^2$; 95% CI: -16.7, -0.5), irrigators (-7.4 mL/min/1.73 $m^2$; 95% CI: -12.6, -2.1), and cane cutters (-5.0 mL/min/1.73 $m^2$; 95% CI: -10.5, 0.6) experienced the largest mean decreases in eGFR during the harvest, compared to factory workers. The number of years worked at the company was not a predictor of change in eGFR during the harvest. As an alternative to evaluating change-during-harvest, similar analyses were conducted using general regression models accounting for repeated measures with an unstructured covariance matrix, and results were similar to those presented (not shown).

For the pre- and late-harvest models, differences by job category were still

observed when the sample was restricted to males or field workers (not shown). In the models evaluating change in eGFR, differences were observed when restricted to males. When the sample was restricted to field workers, the trends and differences by job category were in a similar direction but of lesser magnitude (not shown). Trends and differences by job category were similar when eGFR values >120 mL/min/1.73 $m^2$ were truncated to 120 mL/min/1.73 $m^2$ and when restricting to workers with eGFR ≤120 mL/min/1.73 $m^2$ (not shown).

*Hydration and eGFR*

Overall, workers reported consuming a mean of 5.1 liters of water and 2.4 electrolyte solution packets (100mL each) while at work each day (Table 2.2). Seed cutters, cane cutters, and irrigators reported drinking the most water each workday, on average consuming 2.9, 2.3, and 2.3 liters more than factory workers each day, respectively, after adjusting for sex. On average, women reported drinking less water (difference=-2.2 L) than men, while controlling for job (Table S2.2). Cane cutters and agrichemical applicators reported consuming the most electrolyte solution packets each day, at an average of nearly 4 per day. There was no difference in electrolyte solution consumption by sex, after controlling for job (Table S2.2).

Each additional liter of water consumed during the workday was associated with an average 0.8 mL/min/1.73 $m^2$ lower eGFR at late-harvest (95% CI: -1.7, 0.2) and a 0.5 mL/min/1.73 $m^2$ decrease in eGFR during the harvest (95% CI: -1.3, 0.3), after controlling for sex, age, years worked, and electrolyte solution consumption (Table 2.5,

Model 1). These associations with water consumption disappeared after also adjusting for job category (Table 2.5, Model 2).

We did not find an association between eGFR and electrolyte solution consumption, after controlling for sex, age, years worked, and water consumption (Table 2.5). When stratified by job, electrolyte solution consumption appeared protective only for cane cutters. For every additional electrolyte solution packet consumed by cane cutters during the workday, mean late-harvest eGFR was 6.1 mL/min/1.73 $m^2$ higher (95% CI: -0.06, 12.2), and eGFR increased during the harvest by a mean of 7.0 mL/min/1.73 $m^2$ (95% CI: 1.9, 12.1).

Self-reported alcohol consumption was low, with 67% of workers reporting that they did not drink at all and 10% reporting > 5 drinks per week (Table 2.2). Alcohol is generally not an independent predictor of CKD and was not associated with eGFR, so was excluded from final models.

## DISCUSSION

This is the first longitudinal study, to our knowledge, to assess kidney function in a population at risk for MeN. We found that the kidney function of Nicaraguan sugarcane workers decreased during the harvest season, varied by job category, and was associated with the number of years worked at the company. These findings suggest that one or more of the underlying risk factors is related to occupational exposure. We also found that self-reported hydration with water and electrolyte solution varied by job category and sex and was not associated with kidney function, with the exception of electrolyte

solution consumption among cane cutters. Additionally, given that albuminuria was rare, our results suggest that the disease is likely tubulointerstitial rather than glomerular in nature.

Field workers were at greater risk of kidney function decline over one harvest season compared to non-field workers (factory workers and drivers). This finding suggests that fieldwork is associated with reduced kidney function, though not all field workers were equally affected. Additionally, we cannot rule out non-occupational differences between field workers and non-field workers that may also play a role in the development of MeN in combination with occupational exposures. For example, it is possible that a community may experience low-level exposures to a nephrotoxic agent (via food or drinking water) that by itself is insufficient to cause disease, but in combination with occupational factors such as heat stress and dehydration causes CKD. These potential etiologic occupational exposures may be explored more closely by examining differences in kidney function by individual job categories, each of which require different tasks with varying exposures to putative causal agents (Table 2.1). Though future investigations will benefit from direct monitoring of such agents, this preliminary study uses job category as a proxy for exposure, improving on past exposure assessments conducted at the industry level.

Over the 6-month harvest season, the decline in kidney function was greatest among seed cutters, irrigators, and cane cutters. These jobs require strenuous labor in a hot and humid environment, and cane cutters and seed cutters (as well as seeders) are paid piecemeal, which is often more physically demanding due to the financial

incentives. During a previous industrial hygiene assessment, we measured wet-bulb globe temperatures (WBGT) ranging from 26.9 to 33.2°C (McClean et al. 2010). Other assessments in sugarcane fields in Nicaragua and Costa Rica have documented similar ambient conditions and found that sugarcane workers, particularly cane cutters, are at risk of heat stress during their work shift (Cortez 2009; Crowe et al. 2010; Crowe et al. 2013; Kjellstrom and Crowe 2009). The differences we found in eGFR by job category are consistent with the hypothesis that heat stress may be a contributing factor, and future studies should quantify personal exposure to heat and dehydration. However, other unmeasured factors that vary by job could also contribute to the observed results. For example, cane cutters and seeders work in dusty conditions, likely with exposure to metals, and irrigators would have the greatest exposure to agents in water (e.g. leptospirosis). Among the field workers, agrichemical applicators experienced the least decline in kidney function. Because these workers have the most direct contact with agrichemicals, this finding does not support the hypothesis that agrichemicals are a causal agent. However, agrichemicals are used extensively in the region and widely believed among community members as the cause of the epidemic; additionally, several studies have found an association between self-reported agrichemical use and CKD (Orantes et al. 2011; Orantes et al. 2014; Raines et al. 2014; Wesseling et al. 2013b). Furthermore, in September 2013, El Salvador's National Assembly voted to ban 53 agrichemicals, including 2,4-D, paraquat, DDT, and glyphosate, among others, due to the perceived link to CKD (Chavkin 2013). Agrichemicals, therefore, remain an important hypothesis and warrant further study.

Many researchers hypothesize that inadequate hydration, coupled with repeated volume depletion from exposure to heat and strenuous labor, is partially responsible for MeN (Brooks et al. 2012; Johnson and Sanchez-Lozada 2013; Peraza et al. 2012; Torres et al. 2010; Wesseling et al. 2013a). There is also a strong perception among community physicians and pharmacists that poor hydration and strenuous labor are the major occupational factors responsible for the disease, and that there may be a reluctance to hydrate during the workday due to fear of contaminated water (Ramirez-Rubio et al. 2013a). We found a modest association between water consumption and eGFR, with water consumption appearing harmful for kidney function, similar to a finding by Sanoff *et al.* (Sanoff et al. 2010). Although this finding could be interpreted as consistent with the presence of a nephrotoxin in water, increased water consumption may also indicate a heavy workload, suggesting that the increased risk might be associated with working in strenuous jobs. Consistent with this latter interpretation, the association between increased water consumption and lower eGFR disappeared after controlling for job. We did observe a protective effect of electrolyte solution consumption among cane cutters. This suggests that for the most high-risk jobs, rehydrating with electrolyte solutions during the workday may be protective against renal damage; however, additional research should confirm this observation. Our protective finding contrasts with a recent study that found electrolyte solution consumption during the workday to be harmful for renal function (Raines et al. 2014). It is possible, however, that the harmful effect observed in that study is an artifact and would have disappeared with an adjustment for job category (as did our similar finding for water consumption).

Prior to the harvest, cane cutters had the lowest eGFRs of any job. There are several possible explanations for this finding: (1) cane cutters have held the same job for multiple years and their job exposures reduce kidney function more than other positions, (2) cane cutters were more likely to work in occupations with similar conditions/exposures during the non-harvest season, (3) cane cutters have greater muscle mass leading to higher creatinine generation (rather than decreased clearance), or (4) there are other shared characteristics of individuals who apply for cane cutting jobs that account for the decreased eGFR. Sub-analyses restricted to cane cutters indicated that those who worked at the company the previous year had a mean eGFR that was 13 mL/min/1.73 $m^2$ lower than those who did not. This result is consistent with the explanation that exposures related to working as a cane cutter reduce kidney function more than other jobs. However, we did not have data to address the other possible explanations.

We also found that the number of years employed by the company was associated with reduced eGFR, after controlling for age. Pre-harvest eGFR decreased on average by 0.3 mL/min/1.73 $m^2$ for each additional year employed by the company. Therefore, ten years of employment would, on average, be associated with a reduction in eGFR of 3.0 mL/min/1.73 $m^2$. At late-harvest, the association followed a similar trend. This is consistent with the findings of Peraza *et al.*, in which years of coastal sugarcane or cotton work were associated with elevated serum creatinine levels (Peraza et al. 2012). Specifically, they found that for every ten years of employment in these industries, men (OR=3.1) and women (OR=2.3) had an increased risk of elevated serum creatinine levels,

after controlling for age and smoking history.

Several studies conducted in the region have found that proteinuria is uncommon in individuals with CKD, and, when present, is generally low-grade (O'Donnell et al. 2011; Orantes et al. 2011; Orantes et al. 2014; Peraza et al. 2012; Raines et al. 2014; Torres et al. 2010). Consistent with these findings, few workers in the current study (<5%) had albuminuria. Of those workers with reduced kidney function, only 13% with eGFR <60 mL/min/1.73 $m^2$ and 7% with at eGFR <90 mL/min/1.73 $m^2$ had low levels of albuminuria, suggesting that the disease process does not appear to be primarily affecting the glomerulus. Kidney biopsy specimens from CKD patients in El Salvador showed signs of damage to both glomerular and tubulointerstitial compartments; biopsies showed varying amounts of glomerular sclerotic lesions with extensive tubular atrophy and interstitial fibrosis (Lopez-Marin et al. 2014; Wijkstrom et al. 2013). Given the paucity of albuminuria, these biopsies support a primary tubulointerstitial disease with secondary glomerular manifestations.

A limitation of this study is the use of job category as a surrogate exposure metric. Though an improvement on previous exposure surrogates such as 'community of residence' or 'industry category,' job category is a surrogate for one or more unknown risk factors. These factors could include occupational or non-occupational exposures. Of most concern is that field workers may be different from non-field workers with respect to non-occupational risk factors. However, we found that kidney function was different by job category even when restricted to field workers, suggesting that there is an occupational component to the disease.

An additional limitation is the fact that the assessment of hydration and alcohol was based on self-reported consumption. We found no association between water consumption and eGFR after controlling for job. While it is possible that there is no association, it is also possible that the null finding is attributable to exposure misclassification.

There were two types of loss-to-follow-up (LTF) during the harvest: (1) 99 workers who were no longer working at the time of the late-harvest sampling, and (2) 499 workers who were not sampled even though they were considered active employees at the time of the late-harvest sampling (Figure 2.1). Regarding the first type of LTF, 53 of the 99 workers left their jobs voluntarily, one factory worker left due to CKD, and the reasons for the remaining 45 were unknown. The majority of these 45 were cane cutters (n=18) and seed cutters (n=10), and it is possible that the reason for leaving was related to kidney function. We found that cane cutters and seed cutters experienced decreases in kidney function; therefore, if LTF was related to kidney function, the effects we observed would more likely be an underestimate than an overestimate. Regarding the second type of LTF, financial and logistical constraints forced us to select a sample of those active in the same job at the late-harvest investigation. Each workday, field workers were dispersed across an area of 35,000 hectares, and it was not possible to visit each area to obtain samples from all workers. We were, however, able to confirm that these 499 workers LTF were actively working at late-harvest, and it is unlikely that the placement of workers in particular fields would be related to their kidney function. We would be more concerned about bias if these 499 workers were no longer employed by the

company at late-harvest.

Importantly, it was not a goal of this investigation to characterize the prevalence of CKD. The sugarcane company has a health surveillance program that is designed to identify workers with elevated serum creatinine. When workers are screened prior to the harvest, those with elevated creatinine are not hired. Accordingly, these screening procedures reduce the number workers with CKD, creating a healthy worker effect. However, the repeated measures study design addressed this issue by evaluating change in kidney function in the same workers over a 6-month harvest period. Because this investigation was only during one harvest, we had limited ability to assess incidence or progression of CKD; however 2.8% of workers did develop new occurrence of eGFR <60 mL/min/1.73 $m^2$, all of whom were field workers. This number could be an underestimate due to the first type of LTF described above.

## CONCLUSION

Our results suggest that the kidney disease is primarily tubulointerstitial, and not glomerular, in nature. This is important because the primary causes of CKD globally are diabetes and hypertension, which more often result in glomerular disease manifestations. The observed decline in kidney function during the harvest, as well as the differences in kidney function by job category and employment duration provide evidence that one or more risk factors are occupational. These results, as well as the protective effect of consuming electrolyte solution, are consistent with the hypothesis that heat stress and dehydration may play a role in MeN. Future studies that directly measure exposure to

heat and dehydration are needed. Our results are not consistent with the hypothesis of agrichemicals as causal agents. However, given the extent of use in the region and widely held belief in the community that agrichemicals play a role, future studies should characterize personal exposure to agrichemicals.

**Table 2.1.** Job category description and likelihood of exposure to putative causal agents

| Job Category | Description | Likelihood of Exposure | | | |
|---|---|---|---|---|---|
| | | **Heat Stress** | **Agri-chemicals** | **Metals (dust)** | **Lepto-spirosis** |
| **Field workers** | | | | | |
| Cane cutter | Manually harvests the cane using machetes | High | Medium | High | High |
| Seeder | Transports seed billets and plants seeds | High | Low | High | High |
| Seed cutter | Cuts sugarcane stalks to make seed billets | Medium | Low | Medium | Medium |
| Agrichemical applicator | Mixes and applies agrichemicals (primarily herbicides) using backpack sprayers | High | High | Medium | Medium |
| Irrigator | Diverts water using a gravity-fed approach to irrigate cane fields | Medium | Medium | Medium | High |
| **Non-field workers** | | | | | |
| Driver | Operates vehicles during all steps of the sugarcane process | Low | Low | Low | Low |
| Factory worker | Processes the cane to create sugar, ethanol, and biofuels | Low | Low | Low | Low |

*Note*: Likelihood of exposure to putative causal agents was determined relative to the other jobs

**Table 2.2.** Characteristics of study population (n=284)

| | N (%) |
|---|---|
| **Job** | |
| Cane cutter | 51 (18%) |
| Seeder | 36 (13%) |
| Seed cutter | 19 (7%) |
| Agrichemical applicator | 29 (10%) |
| Irrigator | 49 (17%) |
| Driver | 41 (14%) |
| Factory worker | 59 (21%) |
| **Age** | |
| 18–24 | 58 (20%) |
| 25–34 | 112 (39%) |
| 35–44 | 65 (23%) |
| 45–54 | 34 (12%) |
| 55–63 | 15 (5%) |
| **Sex** | |
| Female | 33 (12%) |
| Male | 251 (88%) |
| **Years Worked at Company** | |
| <1–4 | 117 (41%) |
| 5–9 | 62 (22%) |
| 10–19 | 57 (20%) |
| 20–29 | 38 (13%) |
| 30–40 | 10 (4%) |
| **Water per day (liters)** | |
| ≤1 | 1 (0.4%) |
| 2–3 | 66 (23%) |
| 4–5 | 121 (43%) |
| 6–7 | 41 (14%) |
| 8–9 | 34 (12%) |
| ≥10 | 14 (5%) |
| Missing | 7 (2%) |
| **Electrolyte solution packets per day** | |
| 0 | 65 (23%) |
| 1–2 | 82 (29%) |
| 3–4 | 87 (31%) |
| ≥5 | 43 (15%) |
| Missing | 7 (2%) |
| **Alcoholic drinks per week** | |
| 0 | 189 (67%) |
| 1–2 | 43 (15%) |
| 3–5 | 17 (6%) |
| >5 | 27 (10%) |
| Missing | 8 (3%) |

**Table 2.3.** Summary statistics for biomarkers of kidney function and paired t-tests by job

| | Pre-harvest | | Late-harvest | | Change during harvest[a] | | (95% CI)[b] |
|---|---|---|---|---|---|---|---|
| | **Mean** | **Range** | **Mean** | **Range** | **Mean** | **Range** | |
| **Serum creatinine (mg/dL)** | | | | | | | |
| Cane cutter | 0.89 | 0.6 – 1.5 | 0.96 | 0.5 – 2.7 | 0.07 | -0.5 – 2.0 | (-0.04, 0.2) |
| Seeder | 0.72 | 0.4 – 1.5 | 0.73 | 0.4 – 1.5 | 0.005 | -0.2 – 0.4 | (-0.03, 0.04) |
| Seed cutter | 0.73 | 0.5 – 1.1 | 0.79 | 0.5 – 1.5 | 0.06 | -0.04 – 0.4 | (0.004, 0.1) |
| Agrichemical applicator | 0.80 | 0.6 – 1.1 | 0.82 | 0.6 – 1.2 | 0.03 | -0.09 – 0.3 | (-0.005, 0.06) |
| Irrigator | 0.78 | 0.5 – 1.2 | 0.85 | 0.6 – 1.8 | 0.07 | -0.09 – 1.0 | (0.02, 0.1) |
| Driver | 0.86 | 0.5 – 1.2 | 0.81 | 0.5 – 1.1 | -0.06 | -0.4 – 0.1 | (-0.09, -0.02) |
| Factory worker | 0.82 | 0.4 – 1.4 | 0.79 | 0.3 – 1.1 | -0.02 | -0.7 – 0.4 | (-0.06, 0.02) |
| **eGFR (mL/min/1.73 $m^2$)** | | | | | | | |
| Cane cutter | 111 | 61 – 137 | 108 | 29 – 150 | -3.0 | -90 – 34 | (-9.1, 3.0) |
| Seeder | 118 | 50 – 148 | 117 | 50 – 148 | -0.2 | -34 – 23 | (-3.7, 3.4) |
| Seed cutter | 116 | 72 – 135 | 112 | 52 – 135 | -4.5 | -31 – 2.7 | (-8.7, -0.2) |
| Agrichemical applicator | 116 | 74 – 143 | 114 | 82 – 139 | -1.8 | -23 – 7.7 | (-4.1, 0.5) |
| Irrigator | 120 | 84 – 144 | 116 | 46 – 143 | -4.9 | -63 – 9.1 | (-8.3, -1.5) |
| Driver | 106 | 67 – 147 | 110 | 78 – 139 | 4.6 | -12 – 30 | (1.7, 7.4) |
| Factory worker | 113 | 69 – 161 | 115 | 76 – 181 | 2.1 | -41 – 60 | (-1.6, 5.8) |

[a] Calculated by subtracting each pre-harvest measurement from the corresponding late-harvest measurement

[b] 95% Confidence Interval for paired t-test comparing pre-harvest measurement to late-harvest measurement

Abbreviations: eGFR = estimated Glomerular Filtration Rate

**Table 2.4.** Multivariable analysis of eGFR (mL/min/1.73 $m^2$) by job

| Variables | Pre-harvest | | Late-harvest | | Change during harvest | |
|---|---|---|---|---|---|---|
| | Mean Difference | (95% CI) | Mean Difference | (95% CI) | Mean Difference | (95% CI) |
| **Model 1[a]** | | | | | | |
| **Job** | | | | | | |
| Field worker | -0.8 | (-4.7, 3.1) | -7.8 | (-12.3, -3.2) | -6.9 | (-10.6, -3.2) |
| Non-field worker | ref | | ref | | ref | |
| **Sex** | | | | | | |
| Female | 1.7 | (-3.8, 7.1) | 5.5 | (-0.9, 11.8) | 3.7 | (-1.4, 8.9) |
| Male | ref | | ref | | ref | |
| **Age** | | | | | | |
| Years (Continuous) | -0.9 | (-1.1, -0.6) | -1.0 | (-1.2, -0.7) | -0.1 | (-0.3, 0.1) |
| **Years worked** | | | | | | |
| Years (Continuous) | -0.1 | (-0.4, 0.2) | -0.05 | (-0.4, 0.3) | 0.06 | (-0.2, 0.3) |
| **Model 2[b]** | | | | | | |
| **Job** | | | | | | |
| Cane cutter | -9.2 | (-14.9, -3.4) | -14.1 | (-20.9, -7.3) | -5.0 | (-10.5, 0.6) |
| Seeder | -3.7 | (-10.9, 3.5) | -8.9 | (-17.5, -0.2) | -4.7 | (-11.9, 2.4) |
| Seed cutter | -5.2 | (-13.5, 3.1) | -14.0 | (-23.8, -4.1) | -8.6 | (-16.7, -0.5) |
| Agrichemical applicator | 1.6 | (-4.7, 7.9) | -2.2 | (-9.6, 5.2) | -3.8 | (-9.9, 2.3) |
| Irrigator | 1.8 | (-3.6, 7.3) | -5.5 | (-12.0, 0.9) | -7.4 | (-12.6, -2.1) |
| Driver | -3.4 | (-9.1, 2.3) | -0.2 | (-7.0, 6.5) | 3.2 | (-2.3, 8.7) |
| Factory worker | ref | | ref | | ref | |
| **Sex** | | | | | | |
| Female | 2.2 | (-4.8, 9.3) | 6.7 | (-1.7, 15.2) | 4.2 | (-2.8, 11.1) |
| Male | ref | | ref | | ref | |
| **Age** | | | | | | |
| Years (Continuous) | -0.8 | (-1.0, -0.5) | -0.9 | (-1.2, -0.6) | -0.1 | (-0.4, 0.1) |
| **Years worked** | | | | | | |
| Years (Continuous) | -0.3 | (-0.6, -0.04) | -0.2 | (-0.6, 0.1) | 0.08 | (-0.2, 0.3) |

[a] Model 1: individual job categories grouped into field workers (cane cutters, seeders, seed cutters, agrichemical applicators, and irrigators), and non-field workers (drivers and factory workers).

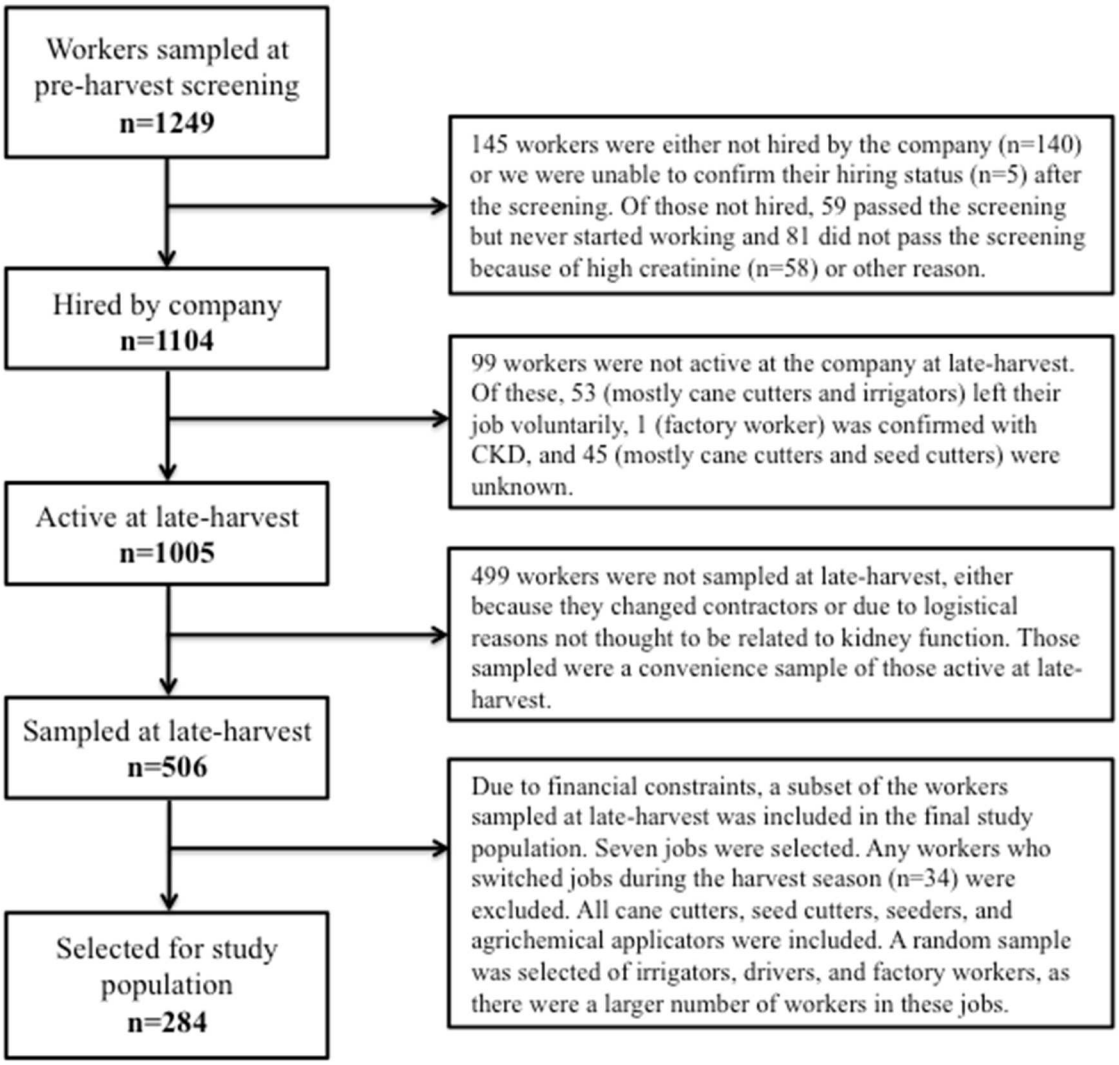
[b] Model 2: all job categories analyzed individually. Abbreviations: eGFR = estimated Glomerular Filtration Rate

**Table 2.5.** Multivariable analysis of eGFR (mL/min/1.73 $m^2$) by hydration

| Variables | Late-harvest | | Change during harvest | |
|---|---|---|---|---|
| | β[a] | (95% CI) | β[a] | (95% CI) |
| **Model 1[b]** | | | | |
| Water consumption (L/day) | -0.8 | (-1.7, 0.2) | -0.5 | (-1.3, 0.3) |
| Electrolyte solution consumption (n/day) | -0.4 | (-1.5, 0.7) | 0.02 | (-0.9, 0.9) |
| **Model 2[c]** | | | | |
| Water consumption (L/day) | -0.07 | (-1.1, 0.9) | 0.03 | (-0.8, 0.9) |
| Electrolyte solution consumption (n/day) | 0.3 | (-1.0, 1.6) | 0.7 | (-0.3, 1.7) |

[a] Estimate (β) shown is per unit increase of consumption.
[b] Adjusted for sex, age, and years worked
[c] Adjusted for sex, age, years worked, and job category
Abbreviations: eGFR = estimated Glomerular Filtration Rate

**Figure 2.1.** Derivation of study population (n=284)



## SUPPLEMENTAL MATERIAL

**Table S2.1.** Characteristics of study population by job (n=284)

| | Cane cutter | Seeder | Seed cutter | Agrichemical applicator | Irrigator | Driver | Factory worker |
|---|---|---|---|---|---|---|---|
| | (n=51) | (n=36) | (n=19) | (n=29) | (n=49) | (n=41) | (n=59) |
| **Age** | | | | | | | |
| 18–24 | 15 (29%) | 10 (28%) | 6 (32%) | 3 (10%) | 16 (33%) | 1 (2%) | 7 (12%) |
| 25–34 | 24 (47%) | 17 (47%) | 8 (42%) | 11 (38%) | 16 (33%) | 15 (37%) | 21 (36%) |
| 35–44 | 7 (14%) | 6 (17%) | 4 (21%) | 12 (41%) | 13 (27%) | 9 (22%) | 14 (24%) |
| 45–54 | 1 (2%) | 2 (6%) | 0 (0%) | 3 (10%) | 4 (8%) | 9 (22%) | 15 (25%) |
| 55–63 | 4 (8%) | 1 (3%) | 1 (5%) | 0 (0%) | 0 (0%) | 7 (17%) | 2 (3%) |
| **Sex** | | | | | | | |
| Female | 0 (0%) | 21 (58%) | 10 (53%) | 0 (0%) | 0 (0%) | 0 (0%) | 2 (3%) |
| Male | 51 (100%) | 15 (42%) | 9 (47%) | 29 (100%) | 49 (100%) | 41 (100%) | 57 (97%) |
| **Years Worked at Company** | | | | | | | |
| <1–4 | 37 (73%) | 23 (64%) | 14 (74%) | 6 (21%) | 17 (35%) | 6 (15%) | 14 (24%) |
| 5–9 | 9 (18%) | 8 (22%) | 4 (21%) | 7 (24%) | 13 (27%) | 9 (22%) | 12 (20%) |
| 10–19 | 4 (8%) | 4 (11%) | 0 (0%) | 11 (38%) | 12 (24%) | 14 (34%) | 12 (20%) |
| 20–29 | 1 (2%) | 1 (3%) | 1 (5%) | 4 (14%) | 6 (12%) | 9 (22%) | 16 (27%) |
| 30–40 | 0 (0%) | 0 (0%) | 0 (0%) | 1 (3%) | 1 (2%) | 3 (7%) | 5 (8%) |
| **Water per day (liters)** | | | | | | | |
| ≤1 | 0 (0%) | 1 (2.8%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| 2–3 | 6 (12%) | 12 (33%) | 3 (16%) | 7 (24%) | 1 (2%) | 9 (22%) | 28 (47%) |
| 4–5 | 16 (31%) | 15 (42%) | 7 (37%) | 17 (59%) | 20 (41%) | 24 (59%) | 22 (37%) |
| 6–7 | 7 (14%) | 4 (11%) | 5 (26%) | 3 (10%) | 11 (22%) | 5 (12%) | 6 (10%) |
| 8–9 | 6 (12%) | 3 (8%) | 2 (11%) | 1 (3%) | 16 (33%) | 3 (7%) | 3 (5%) |
| ≥10 | 9 (18%) | 1 (3%) | 2 (11%) | 1 (3%) | 1 (2%) | 0 (0%) | 0 (0%) |
| Missing | 7 (14%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| **Electrolyte solution packets per day** | | | | | | | |
| 0 | 1 (2%) | 7 (19%) | 1 (5%) | 3 (10%) | 4 (8%) | 36 (88%) | 13 (22%) |
| 1–2 | 7 (14%) | 17 (47%) | 10 (53%) | 5 (17%) | 28 (57%) | 2 (5%) | 13 (22%) |
| 3–4 | 26 (51%) | 10 (28%) | 6 (32%) | 11 (38%) | 11 (22%) | 2 (5%) | 21 (36%) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ≥5 | 10 (20%) | 2 (6%) | 2 (11%) | 10 (34%) | 6 (12%) | 1 (2%) | 12 (20%) |
| Missing | 7 (14%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| **Alcoholic drinks per week** | | | | | | | |
| 0 | 28 (55%) | 31 (86%) | 17 (89%) | 15 (52%) | 37 (76%) | 33 (80%) | 28 (47%) |
| 1–2 | 5 (10%) | 2 (6%) | 0 (0%) | 8 (28%) | 7 (14%) | 4 (10%) | 17 (29%) |
| 3–5 | 4 (8%) | 1 (3%) | 0 (0%) | 3 (10%) | 2 (4%) | 3 (7%) | 4 (7%) |
| >5 | 7 (14%) | 2 (6%) | 2 (11%) | 3 (10%) | 2 (4%) | 1 (2%) | 10 (17%) |
| Missing | 7 (14%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (2%) | 0 (0%) | 0 (0%) |

**Table S2.2.** Adjusted means of daily hydration by job and sex

| Variable | Water (L/day) | | | Electrolyte solution (n/day) | | |
|---|---|---|---|---|---|---|
| | Adjusted Mean[a] | (95% CI) | p-value[b] | Adjusted Mean[a] | (95% CI) | p-value[b] |
| **Job** | | | <0.0001 | | | <0.0001 |
| Cane cutter | 5.2 | (4.4, 6.0) | | 3.8 | (3.2, 4.4) | |
| Seeder | 4.6 | (4.0, 5.3) | | 1.9 | (1.4, 2.5) | |
| Seed cutter | 5.8 | (4.9, 6.7) | | 2.5 | (1.7, 3.2) | |
| Agrichemical applicator | 3.5 | (2.6, 4.3) | | 3.7 | (3.0, 4.4) | |
| Irrigator | 5.2 | (4.5, 6.0) | | 2.5 | (1.9, 3.1) | |
| Driver | 3.5 | (2.7, 4.3) | | 0.6 | (-0.02, 1.2) | |
| Factory worker | 2.9 | (2.2, 3.6) | | 2.9 | (2.4, 3.5) | |
| **Sex** | | | <0.0001 | | | 0.25 |
| Female | 3.3 | (2.4, 4.1) | | 2.8 | (2.1, 3.5) | |
| Male | 5.5 | (5.2, 5.8) | | 2.3 | (2.1, 2.6) | |

[a] Adjusted mean results predicted from a linear regression model accounting for job category and sex

[b] p-value for overall variable as a predictor of water or electrolyte solution consumption

## CHAPTER THREE. BIOMARKERS OF KIDNEY INJURY AMONG NICARAGUAN SUGARCANE WORKERS

Rebecca L. Laws[1], Daniel R. Brooks[2], Juan José Amador[2], Daniel E. Weiner[3], James S. Kaufman[4], Oriana Ramírez-Rubio[2,5], Alejandro Riefkohl[2], Madeleine K. Scammell[1], Damaris López-Pilarte[2], José Marcel Sánchez[2], Chirag R. Parikh[6,7], Michael D. McClean[1]

[1]Department of Environmental Health, Boston University School of Public Health, Boston, MA, USA
[2]Department of Epidemiology, Boston University School of Public Health, Boston, MA, USA
[3]Division of Nephrology, Department of Medicine, Tufts Medical Center and Tufts University School of Medicine, Boston, MA, USA
[4]Research Service, VA New York Harbor Healthcare System and Department of Medicine, New York, NY, USA
[5]Preventive Medicine and Public Health Department, Universidad Autónoma de Madrid, Madrid, Spain
[6]Section of Nephrology, Department of Medicine, Yale University and VA Medical Center, New Haven, CT, USA
[7]Program of Applied Translational Research, Department of Medicine, Yale University, New Haven, CT, USA

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Dr. Parikh is listed as a co-inventor on IL-18 patent granted to University of Colorado (no monetary value).

**ABSTRACT**

**Background:** In Central America, an epidemic of chronic kidney disease of unknown etiology disproportionately affects young, male agricultural workers.

**Study Design:** Longitudinal cohort study.

**Setting & Participants:** 284 sugarcane workers in seven jobs were recruited from one company in northwestern Nicaragua. Blood and urine samples were collected before and near the end of the six-month harvest season.

**Predictors:** Job category (cane cutter, seeder, seed cutter, agrichemical applicator, irrigator, driver, factory worker); self-reported water and electrolyte solution intake.

**Outcomes & Measurements:** Change in urinary kidney injury biomarkers normalized to urine creatinine, including neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), N-acetyl-D-glucosaminidase (NAG), and albumin; estimated glomerular filtration rate (eGFR) from serum creatinine.

**Results:** Cane cutters, a job with high heat exposure and manual exertion, had consistently greater increases in both NGAL and IL-18 during the harvest than other jobs. Compared to factory workers, these increases were 2.57 (95% CI: 1.54, 4.27) and 1.89 (95% CI: 1.08, 3.29) times as high, respectively. Due to loss-to-follow-up, true differences may be greater than observed. In stratified models at late-harvest, cane cutters had lower mean NGAL and NAG, and seed cutters had lower mean IL-18, with each additional electrolyte solution packet consumed. On average, workers with the largest increases in NGAL and NAG during the harvest (upper tertile compared to lower two tertiles) had declines in eGFR of 4.6 mL/min/1.73 $m^2$ (95% CI: -8.2, -1.0) and 3.1

mL/min/1.73 $m^2$ (95% CI: -6.7, 0.6), respectively. Few workers had albuminuria.

**Limitations:** Surrogate exposure measure, loss-to-follow-up.

**Conclusions:** Results are consistent with the hypothesis that occupational heat stress and volume depletion may be associated with development of kidney disease, and future studies should directly measure these occupational factors. The presence of urine tubular injury markers supports a tubulointerstitial disease process that could occur with repeated tubular injury.

## INTRODUCTION

In Central America, there is an epidemic of chronic kidney disease (CKD), referred to as Mesoamerican Nephropathy (Wesseling et al. 2014). This disease disproportionately affects young, male agricultural workers, and is not associated with traditional causes of CKD such as diabetes and hypertension (Orantes et al. 2014; Raines et al. 2014; Ramirez-Rubio et al. 2013a; Ramirez-Rubio et al. 2013b; Weiner et al. 2013; Wesseling et al. 2013a). The infrequent presence of high levels of proteinuria suggests that the disease is primarily tubulointerstitial in nature (Laws et al. 2015b; O'Donnell et al. 2011; Orantes et al. 2011; Peraza et al. 2012; Raines et al. 2014; Sanoff et al. 2010; Torres et al. 2010). Age-adjusted mortality rates from kidney disease in El Salvador and Nicaragua are among the highest in the world (WHO 2008). In these countries, the prevalence of reduced estimated glomerular filtration rate (eGFR) (<60 mL/min/1.73$m^2$) in affected communities is 12%–18% in the general population and 14%–26% in men (O'Donnell et al. 2011; Orantes et al. 2011; Peraza et al. 2012; Sanoff et al. 2010; Torres

et al. 2010), with age-specific rates among younger men up to 15 times higher than in the United States (O'Donnell et al. 2011).

Hypothesized causal agents include heat stress, agrichemicals, heavy metals, nephrotoxic medications, systemic infections (*e.g.* leptospirosis), and genetic factors (Correa-Rotter et al. 2014; Cuadra et al. 2006; Weiner et al. 2013; Wesseling et al. 2013a). The etiology of Mesoamerican Nephropathy is presumed to be multifactorial (Brooks et al. 2012; Ramirez-Rubio et al. 2013a; Wesseling et al. 2013a), and, though specific causes have not yet been identified, there is evidence that one or more risk factors are occupational (Laws et al. 2015b). Some studies have suggested that workers in occupations such as sugarcane, which require heavy manual labor in high ambient temperatures, may be particularly at risk (Laws et al. 2015b; Peraza et al. 2012; Torres et al. 2010).

We have previously shown that sugarcane workers performing the most strenuous tasks had the greatest increases in serum creatinine during one harvest (Laws et al. 2015b). However, serum creatinine is not a sufficiently sensitive marker of early kidney injury, since a measurable change may be evident only after substantial damage has occurred (Bonventre et al. 2010; Coca et al. 2008; Devarajan 2008; Vaidya et al. 2008). Several novel biomarkers of kidney injury have been identified, including neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), and N-acetyl-beta-D-glucosaminidase (NAG). NGAL, a 22 kD protein, is highly upregulated and released into plasma and urine following distal tubule injury (Bolignano et al. 2009; Charlton et al. 2014; Devarajan 2008; Obermuller et al. 2014). IL-18, a pro-inflammatory cytokine

produced by immune and non-immune cells, is excreted in the urine following ischemic proximal tubule injury (Charlton et al. 2014; Devarajan 2011; Liu et al. 2013; Parikh et al. 2005). Similarly, NAG, a lysosomal enzyme, is shed into the urine following proximal tubular epithelial cell injury and is indicative of structural damage (Charlton et al. 2014; Obermuller et al. 2014; Vaidya et al. 2008). These biomarkers may be useful tools for earlier diagnosis of clinically relevant acute kidney injury (AKI), as well as for localization of injury to a specific site in the nephron (Bonventre et al. 2010; Charlton et al. 2014; Vaidya et al. 2008). Furthermore, they may be useful for monitoring disease progression and severity (Bolignano et al. 2009; Chawla et al. 2014; Devarajan 2008; Vaidya et al. 2008).

The goal of this study was to assess whether job category and self-reported hydration are related to short-term changes in biomarkers of tubular injury in workers at risk for developing Mesoamerican Nephropathy. Accordingly, we: (1) evaluated changes in biomarkers of kidney injury among sugarcane workers in northwestern Nicaragua during the six-month harvest season; (2) assessed job-specific differences in changes in kidney injury; (3) evaluated relationships between changes in biomarkers of kidney injury and eGFR; and (4) explored associations between self-reported hydration and biomarkers of kidney injury.

## METHODS

*Study Design*

The study design and population have been previously described (Laws et al. 2015b). Briefly, the population included sugarcane workers employed by one company in northwestern Nicaragua. We enrolled participants (n=1249) prior to the harvest season (October to December 2010) at a screening conducted by the company, which aims to identify, and not hire, workers with serum creatinine levels ≥1.4 mg/dL. Four to six months later, near the end of the harvest season (March to May 2011), we re-sampled 506 of these workers (Figure S3.1). Both assessments included collection of blood and urine and information about personal characteristics, occupational history, and work practices. Information about typical hydration practices during the harvest was collected at late-harvest only; workers were asked to self-report the quantity of water and electrolyte solution packets (100 mL, distributed by the company) consumed during a typical workday.

As described previously (Laws et al. 2015b) and outlined in Figure S3.1, a subset of the 506 workers sampled at pre- and late-harvest was included in the final study population (n=284). These workers represented seven job categories: cane cutter, seeder, seed cutter, agrichemical applicator, irrigator, driver, and factory worker (Table S3.1). Our goal was to select jobs that reflected exposures hypothesized to cause Mesoamerican Nephropathy. For each job, we determined the likelihood of exposure to putative causal agents, relative to other jobs, based on an industrial hygiene assessment (Table S3.1) (McClean et al. 2010).

The Institutional Review Boards at the Boston University Medical Center and the Nicaraguan Ministry of Health approved study protocols. All participants provided informed consent prior to participation in research activities.

*Biomarkers of Kidney Function and Injury*

Immediately following collection, biological samples were processed and stored at -20°C. Within one week, samples were transported to the ISO certified Centro Nacional de Diagnóstico y Referencia (CNDR) in Managua, a division within the Ministry of Health (MINSA), and stored at -80°C until analysis. Samples collected at pre-harvest were stored for approximately 6–7 months, while those collected at late-harvest were stored for approximately 1–3 months prior to analysis.

Serum creatinine was measured at CNDR using a kinetic-rate Jaffe method; 0.2 mg/dL was subtracted from results to calibrate to an isotopic dilution mass spectrometry standard. Urine samples were analyzed at the Division of Nephrology and Hypertension at Cincinnati Children's Hospital Medical Center for creatinine, albumin, NGAL, IL-18, and NAG. Urine creatinine was measured using a colorimetric modification of the Jaffe reaction. The intra-assay coefficient of variation (CV) was 2.4%, and the inter-assay CV was 4.2%. Urine albumin was measured using immunoturbidimetry; intra- and inter-assay CV's were 2.9% and 5.9%, respectively. Commercially available ELISA kits were used to measure NGAL (Bioporto, Gentofte, Denmark) and IL-18 (MBL, Intl., Woburn, MA) per manufacturer's instructions. CV's for NGAL and IL-18 were intra (2.1%; 7.5%) and inter (9.1%; 7.3%). NAG activity was measured with a colorimetric assay (Roche

Diagnostics, USA) with method intra- and inter-assay CV's of 4.3% and 6.0%, respectively (Liangos et al. 2007). The detection limits were 1.3 mg/L for urine albumin, 1.6 pg/mL for NGAL, 4 pg/mL for IL-18, and 0.003 U/L for NAG.

*Statistical Analyses*

For values below the limit of detection (LOD), we substituted LOD/√2. To account for urine concentration, we normalized biomarkers of kidney damage to urinary creatinine concentration (g/L) and expressed as follows: albumin-to-creatinine ratio (ACR) (mg/g), NGAL (μg/g), IL-18 (ng/g), and NAG (U/g). We used the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation to calculate eGFR, considering race as "non-black" (Levey et al. 2009). We examined the distribution of each biomarker using histograms, graphical displays, and summary statistics. To satisfy normality assumptions, biomarkers that exhibited a lognormal distribution were natural log-transformed prior to analyses.

We evaluated the association between job category and each biomarker of kidney injury using linear mixed effects models with an unstructured covariance matrix. In the first set of models, the primary predictor of interest was 'field worker' (reference: non-field worker). In the second set of models, the 'job category' variable was the independent variable (reference: factory workers). To assess differences by job in the change in each biomarker during the harvest, we included an interaction term between the predictor of interest and time. We also tested whether the change in each biomarker was different by sex. To evaluate differences between biomarkers at pre- and late-harvest, we

used linear mixed effects models with time as a categorical variable, stratified by sex and job. We performed Spearman rank correlations to assess relationships between biomarkers of kidney injury and eGFR. We also performed multivariable linear regression models to determine whether change in eGFR (calculated by subtracting each pre-harvest measurement from the corresponding late-harvest measurement) was associated with change in biomarkers of kidney injury (categorized into tertiles).

To test for residual confounding by exposures associated with sex or field worker status, we performed sensitivity analyses restricted to men and to field workers. We also conducted sensitivity analyses further controlling for eGFR and ACR.

Additional predictors included self-reported daily water and electrolyte solution intake. We explored the effects of these variables at late-harvest on kidney injury overall and within job categories, using multivariable linear regression models. We included sex, age, and years worked at the company in all adjusted models. We analyzed data using Statistical Analysis Software (SAS version 9.3, Cary, NC).

## RESULTS

### *Description of Study Population*

Men comprised the majority of the study population (88%), with women only employed as seeders, seed cutters, and factory workers (Table 3.1). The mean age of workers was 33.6 years and ranged from 18–63, while mean duration of employment at the company was 9.4 years and ranged from <1–40 years. Workers reported drinking an average 5.1 liters of water and 2.4 electrolyte solution packets while at work each day (Table 3.1).

Biomarkers of kidney injury and function were log-normally distributed, with the exception of eGFR, which was normally distributed. The overall late-harvest geometric means of NGAL, IL-18, and NAG normalized to urine creatinine were, respectively, 10.4 μg/g, 8.8 ng/g, and 0.90 U/g in men and 25.0 μg/g, 25.1 ng/g, and 1.26 U/g in women (Table 3.2). Comparisons of pre-harvest to late-harvest measurements indicated that, on average, NGAL increased during the harvest in men; this was driven by increases in cane cutters and irrigators. NAG decreased during the harvest in men, driven primarily by the decrease in factory workers (Table 3.2). Summary statistics for non-normalized biomarkers, as well as for urine creatinine, are presented in Table S3.2.

The overall late-harvest mean eGFR was 112 mL/min/1.73 $m^2$ in men and 118 mL/min/1.73 $m^2$ in women (Table 3.2). At pre-harvest, one worker (0.4%) had eGFR <60 mL/min/1.73 $m^2$, compared to eight at late-harvest (2.8%). Urine ACR was generally low (Table 3.2); fewer than 5% of workers had levels greater than 30 mg/g at either time point (Table 3.1).

*Job Category and Biomarkers of Kidney Injury*

Field workers (cane cutters, seeders, seed cutters, agrichemical applicators, and irrigators) had a mean increase in NGAL concentration during the harvest that was 1.49 times as high as the change among non-field workers (drivers and factory workers) (relative mean (RM): 1.49; 95% CI: 1.06, 2.09 (Table 3.3, Model 1). Similarly, the change in IL-18 was 61% higher in field workers than non-field workers (RM: 1.61; 95% CI: 1.12, 2.31) (Table 3.3, Model 1).

When analyzed by individual job category, the mean increase in NGAL during the harvest was more than twice as high in cane cutters (RM: 2.57; 95% CI: 1.54, 4.27) and irrigators (RM: 2.07; 95% CI: 1.24, 3.47) as the change in factory workers (Table 3.3, Model 2). Compared to factory workers, cane cutters (RM: 1.89; 95% CI: 1.08, 3.29) and seeders (RM: 2.11; 95% CI: 1.14, 3.92) experienced the largest increases in IL-18 during the harvest (Table 3.3, Model 2).

Among all jobs, factory workers had the highest NAG concentrations at pre-harvest and the lowest NAG concentrations at late-harvest (Table 3.2). Changes in NAG during the harvest, compared to factory workers, were significant for all other six jobs (Table 3.3, Model 2). However, these relative increases were driven by a decrease in NAG among factory workers; therefore, no jobs were associated with increases in NAG during the harvest.

Men had concentrations of NGAL and IL-18 that were roughly one-third those of women (Table 3.3). For both biomarkers, the change during the harvest was not different by sex; this interaction term was therefore excluded from final models. There were no differences in NAG by sex (Table 3.3). For all models, there was no association between age or years worked and the biomarker of interest.

Differences by job category were maintained in sensitivity analyses restricted to men (Table 3.3) and to field workers. Results remained similar in models further adjusting for eGFR and ACR.

*Hydration and Biomarkers of Kidney Injury*

There was no association between self-reported daily intake of water or electrolyte solution during the harvest and NGAL, IL-18, or NAG at late-harvest (Table 3.4). However, when stratified by job, each electrolyte solution packet consumed by cane cutters during the workday was associated with a 23% decrease in mean late-harvest NGAL (RM: 0.77; 95% CI: 0.61, 0.97) and a 17% decrease in mean late-harvest NAG (RM: 0.83; 95% CI: 0.70, 0.99). Similarly, seed cutters had a 32% mean decrease in late-harvest IL-18 with each additional electrolyte solution packet consumed (RM: 0.68; 95% CI: 0.46, 1.01).

*Relationships Between Biomarkers of Kidney Injury and eGFR*

To determine whether marked increases in urinary biomarkers were associated with decreased eGFR, the change in each kidney injury biomarker was categorized into tertiles. During the six month harvest, workers with the largest increases in NGAL and NAG (upper tertile compared to lower two tertiles) had declines in eGFR of 4.6 mL/min/1.73 $m^2$ (95% CI: -8.2, -1.0) and 3.1 mL/min/1.73 $m^2$ (95% CI: -6.7, 0.6), respectively, while controlling for age, sex, and years worked. This relationship was not observed for IL-18. When examined as continuous variables, changes in biomarkers during the harvest season were not correlated with changes in eGFR, with the exception of NAG, which had a weak negative correlation with change in eGFR (Table 3.5). Although increases in all biomarkers were associated with increases in albuminuria, the

increases in albuminuria were small; during the harvest, ACR remained >30mg/g (microalbuminuria) in four workers and increased to >30 mg/g in five workers.

## DISCUSSION

In a population of Nicaraguan sugarcane workers at risk for Mesoamerican Nephropathy, urinary NGAL and IL-18, both biomarkers of kidney injury, increased during the harvest season in certain job tasks relative to others. These findings suggest that subclinical kidney injury is occurring in these workers during one harvest season, and the differences by job provide evidence that occupational exposures play a role. We found associations between increases in NGAL and NAG and decreases in eGFR. Finally, we found evidence that consumption of electrolyte solution may reduce biomarkers of kidney injury among high-risk jobs.

Our results indicate that, of the seven jobs studied, cane cutters are at highest risk of kidney injury during the harvest. These workers had consistently greater increases in both NGAL and IL-18 during the harvest season, suggesting more substantial kidney injury than workers in other jobs, though irrigators and seeders showed some evidence of injury as well. Cane cutters have the most physically strenuous sugarcane job; furthermore, they are paid based on their daily work output, a compensation structure that may increase physical strain. Previous studies in Central America have determined that cane cutters are at risk for heat stress, documenting wet-bulb globe temperature indices that exceed internationally accepted heat exposure limits (Cortez 2009; Crowe et al. 2010; Crowe et al. 2013; Kjellstrom and Crowe 2009; McClean et al. 2010). Seeders also

have a demanding job in hot ambient conditions, and are paid based on a similar structure (McClean et al. 2010). Our finding is consistent with the hypothesis that heat stress may be playing a role, although other factors that vary by job could contribute to the observed results (Table S3.1). Although agrichemicals have been suggested as an etiology of the CKD epidemic, compared to other field workers, agrichemical applicators did not experience increases in kidney injury biomarkers. Despite this finding, the potential role of agrichemical exposure should be investigated further given the extensive use in the region (Chavkin 2013; Wesseling et al. 2013b).

In combination with intense labor in high heat settings, repeated volume depletion may be an important contributor to kidney disease in the region (Brooks et al. 2012; Johnson and Sanchez-Lozada 2013; Peraza et al. 2012; Torres et al. 2010; Wesseling et al. 2013a). In this study, self-reported intake of water or electrolyte solution was generally not associated with biomarkers of kidney injury. However, when stratified by job, we observed a protective effect of electrolyte solution among cane cutters and seed cutters. Consistent with these findings, we previously noted in these cane cutters that late-harvest eGFR was higher by a mean of 6.1 mL/min/1.73 $m^2$ with each additional electrolyte solution packet consumed (Laws et al. 2015b). These findings suggest that for the most high-risk jobs such as cane cutting, rehydrating with electrolyte solutions during the workday may protect against kidney injury.

In this population, we previously reported mean decreases in eGFR during the harvest that varied by job (Laws et al. 2015b). Those results are consistent with findings from the present study and suggest that one or more risk factors for this kidney disease

are occupational. Few of these workers had substantially decreased eGFR, however, highlighting the importance of examining kidney injury biomarkers. An evolving body of literature suggests that these markers may be useful as early indicators of AKI (Bonventre et al. 2010; Charlton et al. 2014; Coca et al. 2014; Parikh et al. 2005; Vaidya et al. 2008). Less is known about their ability to predict subclinical AKI or future CKD; repeated incidents of subclinical kidney damage may increase CKD risk, but this relationship remains unclear (Bedford et al. 2012; Venkatachalam et al. 2010; Yang et al. 2011). The lack of overall correlation between changes in urinary biomarkers and changes in eGFR may reflect the possibility that changes in biomarkers precede changes in eGFR. However, workers with the greatest increases in NGAL and NAG during the harvest had greater declines in eGFR. Some biomarkers, particularly NGAL, may also be markers of disease severity and can predict CKD progression and mortality (Bolignano et al. 2009; Coca et al. 2014; Devarajan 2008; Smith et al. 2013). It is possible that among these workers, repeated subclinical kidney damage over multiple harvests leads to clinically apparent disease and progressive CKD.

Few workers had albuminuria; this is consistent with findings from studies in the region (O'Donnell et al. 2011; Orantes et al. 2011; Orantes et al. 2014; Peraza et al. 2012; Raines et al. 2014; Torres et al. 2010), but may be expected due to the relatively healthy nature of the study population. The presence of NGAL, IL-18, and NAG in the urine of these workers, however, provides evidence that the disease process may be primarily tubulointerstitial. While we cannot rule out systemic stress or inflammation as a cause of the elevated biomarkers (Bonventre et al. 2010; Devarajan 2011; Obermuller et al. 2014),

these individuals are overtly healthy and exposed to environmental conditions that are hypothesized to cause kidney injury and damage (Soderland et al. 2010). Accordingly, it is most likely that the changes in these biomarkers are consistent with tubular injury. Kidney biopsy specimens from CKD patients in El Salvador showed signs of glomerular lesions in addition to tubulointerstitial damage (Lopez-Marin et al. 2014; Wijkstrom et al. 2013), which may suggest a primary tubulointerstitial disease with secondary glomerular manifestations.

Studies in Nicaragua suggest that men are 3–5 times more likely to be affected by Mesoamerican Nephropathy than women (Cuadra et al. 2006; O'Donnell et al. 2011; Torres et al. 2010). We found that at both pre- and late-harvest, men had NGAL and IL-18 concentrations that were roughly one-third those of women, regardless of normalization for urine creatinine, but that the change in these biomarkers during the harvest was not different by sex. In other populations, there are inconsistent findings regarding inherent differences by sex, though one study reports higher NGAL levels in healthy female children and adults than in males of the same age (Pennemans et al. 2013). A previous study of Nicaraguan adolescents also found that biomarker levels were higher in girls than boys (Ramirez-Rubio et al. 2012). These findings require further investigation.

One limitation of this research is the lack of established kidney injury biomarker levels that represent subclinical damage. Furthermore, we do not know the short-term intra-individual variability of these biomarkers. We were able to evaluate concentrations within and between workers during the six-month harvest, but the magnitude of the

increase is difficult to interpret and the clinical relevance is unknown. When comparing our population to pre-operative cardiac surgery patients who did not develop AKI, mean and median concentrations of NGAL were similar, but concentrations of IL-18 were lower in our population (Parikh et al. 2011). There are limited comparable occupational cohorts that have measured these biomarkers in workers (Lacquaniti et al. 2012). To our knowledge, only one previous study in this region has measured kidney injury biomarkers; NGAL levels were increased in 26% of CKD cases in El Salvador (Herrera et al. 2014). However, it is difficult to compare our population of healthy workers to a population of CKD cases.

There are several additional limitations to this study. First, we used job category as a surrogate exposure metric. Job category may represent one or more unknown risk factors, which could include occupational and/or non-occupational exposures. Future studies should directly measure exposure to putative causal agents. Second, biomarker levels could potentially be affected by storage of biological specimens. Though NGAL and IL-18 levels do not seem to be greatly affected by short-term handling variations (Parikh et al. 2014), levels may decline over time in frozen samples. In one study, NAG was shown to decline 28-fold over one year while stored at -80°C (Nauta et al. 2012), though this finding has not been confirmed. It is possible that this degradation issue influenced our null findings for NAG. Finally, the loss-to-follow-up in this study population is a potential issue and has been previously described in detail (Laws et al. 2015b). There were two types of loss-to-follow-up during the harvest: (1) 99 workers who were no longer working at the time of the late-harvest sampling, and (2) 499 workers

who were actively working but not sampled at late-harvest (Figure S3.1). If the reason for leaving work was related to kidney injury, it is possible that biomarker levels would have been higher than what we observed, with more pronounced differences by job task, as many of these workers were in higher risk jobs. Financial and logistical constraints forced us to select a random sample of those workers still active and would not be expected to influence our results.

## CONCLUSION

In this population of Nicaraguan sugarcane workers, we found that biomarkers of kidney injury varied by job and increased during the harvest season, most notably among cane cutters. These findings suggest that occupational heat stress and volume depletion may play a role in Mesoamerican Nephropathy, and future studies that quantify these exposures are needed. Our results are consistent with tubular injury and support the hypothesis that in these workers, repeated subclinical kidney damage may lead to clinically apparent CKD over time. Our findings suggest that for high-risk jobs such as cane cutting, rehydrating with electrolyte solutions during the workday may protect against kidney injury; this should be evaluated with more quantitative measures of fluid balance in future studies.

**Table 3.1.** Characteristics and baseline kidney function of study population

| | **Job:** | **Overall** | **Cane cutter** | **Seeder** | **Seed cutter** | **Agrichemical applicator** | **Irrigator** | **Driver** | **Factory worker** |
|---|---|---|---|---|---|---|---|---|---|
| | **n (%)** | 284 (100%) | 51 (18%) | 36 (13%) | 19 (7%) | 29 (10%) | 49 (17%) | 41 (14%) | 59 (21%) |
| **Characteristics** | | | | | | | | | |
| Male sex | | 251 (88%) | 51 (100%) | 15 (42%) | 9 (47%) | 29 (100%) | 49 (100%) | 41 (100%) | 57 (97%) |
| Age (years) | | 33.6 ±10.4 | 30.5 ± 10.6 | 30.2 ± 8.7 | 30.3 ± 9.2 | 34.7 ± 8.2 | 30.3 ± 8.8 | 40.9 ± 11.2 | 36.5 ± 9.8 |
| Years worked at company | | 9.4 ± 9.0 | 3.6 ± 5.3 | 4.9 ± 4.8 | 3.7 ± 4.4 | 12.0 ± 8.0 | 9.7 ± 8.0 | 14.4 ± 8.9 | 14.2 ± 10.6 |
| Water per day (liters) | | 5.1 ± 2.2 | 6.3 ± 2.8 | 4.4 ± 2.2 | 5.8 ± 2.4 | 4.6 ± 1.8 | 6.3 ± 1.9 | 4.6 ± 1.6 | 4.0 ± 1.7 |
| Electrolyte solution packets per day (n)[a] | | 2.4 ± 1.9 | 3.6 ± 1.4 | 2.0 ± 1.5 | 2.5 ± 1.5 | 3.5 ± 1.9 | 2.3 ± 1.6 | 0.4 ± 1.2 | 2.7 ± 2.0 |
| **Kidney function at pre-harvest** | | | | | | | | | |
| eGFR (mL/min/1.73 $m^2$) | | 114 ± 17 | 111 ±18 | 118 ± 19 | 116 ± 18 | 116 ± 14 | 120 ± 14 | 106 ± 17 | 113 ± 16 |
| ACR (mg/g) | | 2.9 ± 3.5 | 3.8 ± 4.3 | 4.5 ± 4.4 | 2.9 ± 2.3 | 2.4 ± 4.5 | 2.2 ± 3.6 | 3.0 ± 2.7 | 2.5 ± 2.7 |
| eGFR <60 mL/min/1.73 $m^2$ | | 1 (0.4%) | 0 (0%) | 1 (3%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| ACR >30 mg/g | | 12 (4%) | 3 (6%) | 4 (11%) | 0 (0%) | 2 (7%) | 2 (4%) | 1 (2%) | 0 (0%) |

*Note*: Values for categorical variables are given as number (percentage). Values for continuous variables are given as mean ± standard deviation, with the exception of ACR, which is given as geometric mean ± geometric standard deviation.

Abbreviations: eGFR, estimated glomerular filtration rate; ACR, albumin-to-creatinine ratio.

[a] Each packet (n) is 100mL.

**Table 3.2.** Creatinine-normalized biomarkers of kidney injury [geometric mean] and function [mean] among sugarcane workers at pre- and late-harvest, stratified by sex

| | NGAL (µg/g)[a] | | | IL-18 (ng/g)[a] | | | NAG (U/g)[a] | | | eGFR (mL/min/1.73 m$^2$)[b] | | | ACR (mg/g)[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Pre** | **Late** | ***P*** | **Pre** | **Late** | ***P*** | **Pre** | **Late** | ***P*** | **Pre** | **Late** | ***P*** | **Pre** | **Late** | ***P*** |
| **Male** | | | | | | | | | | | | | | | |
| Overall | 7.5 | 10.4 | <0.001 | 9.0 | 8.8 | 0.8 | 1.32 | 0.90 | <0.001 | 113 | 112 | 0.3 | 2.8 | 2.4 | 0.07 |
| Job | | | | | | | | | | | | | | | |
| Cane cutter | 7.6 | 19.3 | <0.001 | 9.1 | 12.6 | 0.1 | 1.17 | 1.54 | 0.08 | 111 | 108 | 0.3 | 3.8 | 2.0 | 0.01 |
| Seeder | 10.4 | 15.3 | 0.2 | 5.9 | 14.8 | 0.05 | 1.22 | 1.61 | 0.3 | 115 | 110 | 0.4 | 4.3 | 6.6 | 0.2 |
| Seed cutter | 10.1 | 6.2 | 0.02 | 9.2 | 5.4 | 0.4 | 1.54 | 0.63 | 0.06 | 119 | 112 | 0.1 | 3.0 | 1.0 | 0.02 |
| Agrichemical app. | 7.0 | 6.9 | 0.9 | 9.7 | 8.5 | 0.6 | 1.09 | 0.93 | 0.7 | 116 | 114 | 0.1 | 2.4 | 2.7 | 0.7 |
| Irrigator | 7.2 | 14.7 | <0.001 | 6.8 | 7.5 | 0.6 | 0.92 | 0.90 | 0.9 | 120 | 116 | 0.006 | 2.2 | 4.2 | <0.001 |
| Driver | 6.9 | 7.5 | 0.6 | 14.0 | 9.9 | 0.2 | 1.27 | 0.69 | 0.06 | 106 | 110 | 0.003 | 3.0 | 2.0 | 0.08 |
| Factory worker | 7.2 | 6.8 | 0.8 | 8.8 | 6.4 | 0.07 | 2.23 | 0.60 | <0.001 | 112 | 115 | 0.2 | 2.4 | 1.6 | 0.02 |
| **Female** | | | | | | | | | | | | | | | |
| Overall | 28.1 | 25.0 | 0.7 | 22.9 | 25.1 | 0.7 | 1.29 | 1.26 | 0.9 | 118 | 118 | 0.7 | 3.9 | 4.7 | 0.4 |
| Job | | | | | | | | | | | | | | | |
| Cane cutter | - | - | | - | - | | - | - | | - | - | | - | - | |
| Seeder | 32.2 | 24.7 | 0.5 | 26.4 | 29.8 | 0.7 | 1.19 | 1.26 | 0.7 | 120 | 122 | 0.1 | 4.6 | 5.6 | 0.6 |
| Seed cutter | 26.2 | 25.1 | 0.9 | 15.3 | 15.1 | 0.9 | 1.26 | 1.37 | 0.8 | 114 | 112 | 0.3 | 2.7 | 3.7 | 0.3 |
| Agrichemical app. | - | - | | - | - | | - | - | | - | - | | - | - | |
| Irrigator | - | - | | - | - | | - | - | | - | - | | - | - | |
| Driver | - | - | | - | - | | - | - | | - | - | | - | - | |
| Factory worker | 9.3 | 28.4 | 0.6 | 38.1 | 51.4 | 0.5 | 3.32 | 0.80 | 0.005 | 116 | 113 | 0.1 | 4.2 | 2.4 | 0.6 |

*Note*: *P* is for comparison of pre- to late-harvest levels of each biomarker, using linear mixed effects models with time as a categorical variable. Overall models for each sex are adjusted for job, age, and years worked. Models further stratified by job are adjusted for age and years worked.

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18; NAG, N-acetyl-D-glucosaminidase; eGFR, estimated glomerular filtration rate; ACR, albumin-to-creatinine ratio.

[a] Geometric mean is presented; variable is log-normally distributed.

[b] Mean is presented; variable is normally distributed.

**Table 3.3.** Linear mixed effects models for change in urinary biomarkers of kidney injury among sugarcane workers

| Variable | All Workers (n=284) | | | | | | Restricted to Men (n=251) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ln_NGAL (µg/g) | | Ln_IL-18 (ng/g) | | Ln_NAG (U/g) | | Ln_NGAL (µg/g) | | Ln_IL-18 (ng/g) | | Ln_NAG (U/g) | |
| | Relative Mean | (95% CI) | Relative Mean | (95% CI) | Relative Mean | (95% CI) | Relative Mean | (95% CI) | Relative Mean | (95% CI) | Relative Mean | (95% CI) |
| ***Model 1***[a] | | | | | | | | | | | | |
| **Change in biomarker during harvest** | | | | | | | | | | | | |
| Field worker | 1.49 | (1.06, 2.09) | 1.61 | (1.12, 2.31) | 2.92 | (2.08, 4.10) | 1.73 | (1.22, 2.44) | 1.66 | (1.14, 2.42) | 2.88 | (1.99, 4.16) |
| Non-field worker | ref | | ref | | ref | | ref | | ref | | ref | |
| **Sex** | | | | | | | | | | | | |
| Male | 0.38 | (0.27, 0.53) | 0.38 | (0.27, 0.51) | 0.85 | (0.59, 1.22) | - | | - | | - | |
| Female | ref | | ref | | ref | | - | | - | | - | |
| ***Model 2***[b] | | | | | | | | | | | | |
| **Change in biomarker during harvest** | | | | | | | | | | | | |
| Cane cutter | 2.57 | (1.54, 4.27) | 1.89 | (1.08, 3.29) | 4.92 | (2.94, 8.23) | 2.67 | (1.62, 4.41) | 1.93 | (1.10, 3.37) | 4.90 | (2.86, 8.39) |
| Seeder | 1.00 | (0.57, 1.76) | 2.11 | (1.14, 3.92) | 4.38 | (2.47, 7.77) | 1.50 | (0.69, 3.23) | 3.44 | (1.46, 8.08) | 4.98 | (2.19, 11.4) |
| Seed cutter | 0.79 | (0.39, 1.58) | 1.05 | (0.49, 2.27) | 2.57 | (1.26, 5.23) | 0.65 | (0.25, 1.65) | 0.82 | (0.29, 2.33) | 1.54 | (0.56, 4.18) |
| Agrichemical applicator | 1.00 | (0.55, 1.84) | 1.19 | (0.61, 2.32) | 3.23 | (1.74, 5.97) | 1.04 | (0.57, 1.90) | 1.22 | (0.63, 2.37) | 3.21 | (1.69, 6.10) |
| Irrigator | 2.07 | (1.24, 3.47) | 1.50 | (0.86, 2.63) | 3.66 | (2.18, 6.17) | 2.16 | (1.30, 3.58) | 1.53 | (0.87, 2.70) | 3.65 | (2.12, 6.29) |
| Driver | 1.09 | (0.63, 1.88) | 0.96 | (0.53, 1.74) | 2.03 | (1.17, 3.52) | 1.13 | (0.66, 1.94) | 0.98 | (0.54, 1.79) | 2.02 | (1.14, 3.58) |
| Factory worker | ref | | ref | | ref | | ref | | ref | | ref | |
| **Sex** | | | | | | | | | | | | |
| Male | 0.41 | (0.26, 0.63) | 0.34 | (0.23, 0.51) | 0.94 | (0.58, 1.51) | - | | - | | - | |
| Female | ref | | ref | | ref | | - | | - | | - | |

*Note*: Relative mean (e^β) represents the fold-change in biomarker during the harvest compared to the reference group, as outcome variables were natural log-transformed prior to analyses. For example, field workers had a mean change in NGAL concentration during the harvest that was 1.49 times as high as the change among non-field workers.

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18; NAG, N-acetyl-D-glucosaminidase.

[a] Model 1: individual job categories were grouped into field workers (cane cutters, seeders, seed cutters, agrichemical applicators, and irrigators) and non-field workers (drivers and factory workers). Model parameters for each ln(outcome) include time, fieldworker status, time*fieldworker status, sex, age, and years worked. Results presented as "Change in biomarker during harvest" are the exponentiated beta coefficients of the time*fieldworker status variable. Full model results and p-values are presented in Table S3.3.

[b] Model 2: all job categories were analyzed individually. Model parameters for each ln(outcome) include time, job, time*job, sex, age, and years worked. Results presented as "Change in biomarker during harvest" are the exponentiated beta coefficients of the time*job variable. Full model results and p-values are presented in Table S3.4.

**Table 3.4.** Multivariable analysis of late-harvest biomarkers of kidney injury by hydration among sugarcane workers

| Variable | Ln_NGAL (µg/g) | | Ln_IL-18 (ng/g) | | Ln_NAG (U/g) | |
|---|---|---|---|---|---|---|
| | β | (95% CI) | β | (95% CI) | β | (95% CI) |
| Water consumption (L) | -0.01 | (-0.08, 0.06) | 0.01 | (-0.05, 0.08) | 0.04 | (-0.02, 0.10) |
| Electrolyte solution consumption (n)[a] | 0.01 | (-0.07, 0.10) | 0.05 | (-0.04, 0.13) | 0.002 | (-0.07, 0.08) |

*Note*: Estimate ( $\beta$ ) shown is difference in ln(outcome) per unit increase of consumption. Model parameters for each ln(outcome) include job, sex, age, and years worked.

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18; NAG, N-acetyl-D-glucosaminidase.

[a] Each packet (n) is 100mL.

**Table 3.5.** Spearman correlations between biomarkers of kidney injury and function among sugarcane workers

| Pre-harvest | **NGAL (µg/g)** | | **IL-18 (ng/g)** | | **NAG (U/g)** | |
|---|---|---|---|---|---|---|
| | r | *P* | r | *P* | r | *P* |
| **eGFR (mL/min/1.73 m$^2$)** | 0.02 | 0.8 | 0.14 | 0.02 | -0.12 | 0.05 |
| **ACR (mg/g)** | 0.29 | <0.001 | 0.23 | <0.001 | 0.20 | <0.001 |
| Change during harvest | **△ NGAL (µg/g)** | | **△ IL-18 (ng/g)** | | **△ NAG (U/g)** | |
| | r | *P* | r | *P* | r | *P* |
| **△ eGFR (mL/min/1.73 m$^2$)** | -0.11 | 0.08 | 0.01 | 0.8 | -0.12 | 0.05 |
| **△ ACR (mg/g)** | 0.22 | <0.001 | 0.21 | <0.001 | 0.28 | <0.001 |

*Note*: r represents the Spearman correlation coefficient, and *P* is its associated p-value.

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18; NAG, N-acetyl-D-glucosaminidase; eGFR, estimated glomerular filtration rate; ACR, albumin-to-creatinine ratio.

## SUPPLEMENTAL MATERIAL

**Table S3.1.** Description of sugarcane job categories and likelihood of exposure to putative causal agents

| Job Category | Description | Likelihood of Exposure | | | |
|---|---|---|---|---|---|
| | | **Heat stress** | **Agri-chemicals** | **Metals (dust)** | **Lepto-spirosis** |
| **Field workers** | | | | | |
| Cane cutter | Manually harvests the cane using machetes | High | Medium | High | High |
| Seeder | Transports seed billets and plants seeds | High | Low | High | High |
| Seed cutter | Cuts sugarcane stalks to make seed billets | Medium | Low | Medium | Medium |
| Agrichemical applicator | Mixes and applies agrichemicals (primarily herbicides) using backpack sprayers | High | High | Medium | Medium |
| Irrigator | Diverts water using a gravity-fed approach to irrigate cane fields | Medium | Medium | Medium | High |
| **Non-field workers** | | | | | |
| Driver | Operates vehicles during all steps of the sugarcane process | Low | Low | Low | Low |
| Factory worker | Processes the cane to create sugar, ethanol, and biofuels | Low | Low | Low | Low |

*Note*: Likelihood of exposure to putative causal agents was determined relative to the other jobs.

**Table S3.2.** Non-normalized urinary biomarkers of kidney injury [geometric mean] among sugarcane workers at pre- and late-harvest, stratified by sex

| | NGAL (ng/mL) | | | IL-18 (pg/mL) | | | NAG (mU/mL) | | | Urine creatinine (mg/mL) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Pre** | **Late** | ***P*** | **Pre** | **Late** | ***P*** | **Pre** | **Late** | ***P*** | **Pre** | **Late** | ***P*** |
| **Male** | | | | | | | | | | | | |
| Overall | 6.4 | 9.9 | <0.001 | 7.7 | 8.3 | 0.7 | 1.08 | 0.85 | <0.001 | 0.86 | 0.95 | 0.1 |
| Job | | | | | | | | | | | | |
| Cane cutter | 7.9 | 16.7 | <0.001 | 9.4 | 10.9 | 0.4 | 1.21 | 1.33 | 0.1 | 1.04 | 0.86 | 0.1 |
| Seeder | 12.0 | 11.1 | 0.2 | 6.4 | 10.7 | 0.05 | 0.91 | 1.17 | 0.07 | 1.15 | 0.73 | 0.09 |
| Seed cutter | 9.2 | 7.3 | 0.1 | 8.4 | 6.4 | 0.5 | 1.40 | 0.75 | 0.07 | 0.91 | 1.18 | 0.4 |
| Agrichemical app. | 6.9 | 8.2 | 0.9 | 9.4 | 10.1 | 0.9 | 1.06 | 1.11 | 0.5 | 0.97 | 1.19 | 0.2 |
| Irrigator | 5.0 | 10.3 | <0.001 | 4.7 | 5.2 | 0.6 | 0.64 | 0.63 | 0.9 | 0.70 | 0.70 | 0.9 |
| Driver | 5.1 | 8.1 | 0.3 | 10.0 | 10.7 | 0.4 | 0.81 | 0.74 | 0.1 | 0.74 | 1.08 | 0.002 |
| Factory worker | 6.2 | 7.6 | 0.7 | 7.6 | 7.0 | 0.3 | 1.91 | 0.66 | <0.001 | 0.86 | 1.11 | 0.06 |
| **Female** | | | | | | | | | | | | |
| Overall | 22.8 | 14.6 | 0.5 | 18.6 | 14.6 | 0.8 | 1.04 | 0.73 | 0.9 | 0.81 | 0.58 | 0.01 |
| Job | | | | | | | | | | | | |
| Cane cutter | - | - | | - | - | | - | - | | - | - | |
| Seeder | 24.9 | 14.1 | 0.2 | 20.4 | 17.0 | 0.8 | 0.92 | 0.72 | 0.9 | 0.77 | 0.57 | 0.05 |
| Seed cutter | 21.9 | 15.0 | 0.9 | 12.8 | 9.0 | 0.6 | 1.05 | 0.82 | 0.7 | 0.84 | 0.60 | 0.3 |
| Agrichemical app. | - | - | | - | - | | - | - | | - | - | |
| Irrigator | - | - | | - | - | | - | - | | - | - | |
| Driver | - | - | | - | - | | - | - | | - | - | |
| Factory worker | 10.6 | 18.2 | <0.001 | 43.5 | 32.9 | <0.001 | 3.79 | 0.51 | <0.001 | 1.14 | 0.64 | DNC[a] |

*Note*: *P* is for comparison of pre- to late-harvest levels of each biomarker, using linear mixed effects models with time as a categorical variable. Overall models for each sex are adjusted for job, age, and years worked. Models further stratified by job are adjusted for age and years worked. Models for NGAL, IL-18, and NAG include urine creatinine as a predictor.

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18; NAG, N-acetyl-D-glucosaminidase; DNC, did not converge.

[a] Model did not converge due to low n.

**Table S3.3.** Model output for linear mixed effects models evaluating change in biomarkers of kidney injury by fieldworker status

| Variable | All Workers (n=284) | | | | | | Restricted to Men (n=251) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ln_NGAL (µg/g) | | Ln_IL-18 (ng/g) | | Ln_NAG (U/g) | | Ln_NGAL (µg/g) | | Ln_IL-18 (ng/g) | | Ln_NAG (U/g) | |
| | β | *P* | β | *P* | β | *P* | β | *P* | β | *P* | β | *P* |
| **Intercept** | 2.56 | <0.001 | 3.55 | <0.001 | 0.42 | 0.2 | 1.59 | <0.001 | 2.51 | <0.001 | 0.26 | 0.3 |
| **Time** | | | | | | | | | | | | |
| Late-harvest | 0.02 | 0.9 | -0.32 | 0.03 | -1.03 | <0.001 | 0.001 | 0.9 | -0.33 | 0.03 | -1.02 | <0.001 |
| Pre-harvest | ref | | ref | | ref | | ref | | ref | | ref | |
| **Job** | | | | | | | | | | | | |
| Field worker | 0.22 | 0.2 | -0.36 | 0.01 | -0.42 | 0.008 | 0.15 | 0.3 | -0.35 | 0.02 | -0.40 | 0.01 |
| Non-field worker | ref | | ref | | ref | | ref | | ref | | ref | |
| **Job*Time** | | | | | | | | | | | | |
| Field worker*Late-harvest | 0.40 | 0.02 | 0.48 | 0.01 | 1.07 | <0.001 | 0.55 | 0.002 | 0.51 | 0.009 | 1.06 | <0.001 |
| Non-field worker*Late-harvest | ref | | ref | | ref | | ref | | ref | | ref | |
| **Sex** | | | | | | | | | | | | |
| Male | -0.98 | <0.001 | -0.98 | <0.001 | -0.17 | 0.4 | - | | - | | - | |
| Female | ref | | ref | | ref | | - | | - | | - | |
| **Age** | | | | | | | | | | | | |
| (Continuous, per 5 y) | 0.01 | 0.1 | -0.002 | 0.8 | 0.01 | 0.4 | 0.01 | 0.2 | 0.00 | 0.9 | 0.01 | 0.5 |
| **Years worked** | | | | | | | | | | | | |
| (Continuous, per 5 y) | -0.002 | 0.8 | -0.01 | 0.2 | 0.005 | 0.6 | -0.001 | 0.9 | -0.01 | 0.2 | 0.01 | 0.5 |

*Note*: individual job categories were grouped into field workers (cane cutters, seeders, seed cutters, agrichemical applicators, and irrigators), and non-field workers (drivers and factory workers).

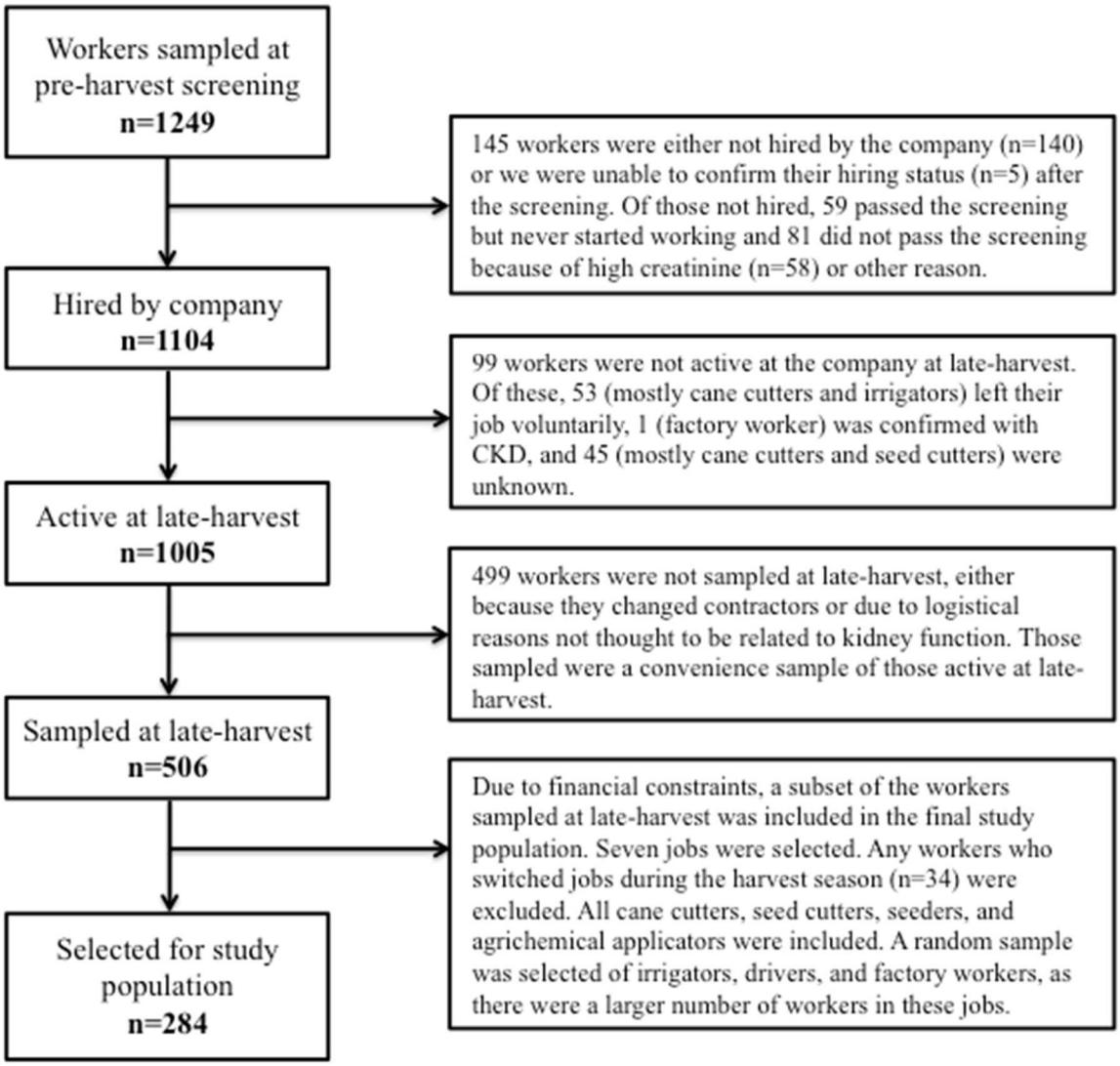
Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18; NAG, N-acetyl-D-glucosaminidase.

**Table S3.4.** Model output for linear mixed effects models evaluating change in biomarkers of kidney injury by job

| Variable | All Workers (n=284) | | | | | | Restricted to Men (n=251) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ln_NGAL (µg/g) | | Ln_IL-18 (ng/g) | | Ln_NAG (U/g) | | Ln_NGAL (µg/g) | | Ln_IL-18 (ng/g) | | Ln_NAG (U/g) | |
| | β | *P* | β | *P* | β | *P* | β | *P* | β | *P* | β | *P* |
| **Intercept** | 1.56 | <0.001 | 2.49 | <0.001 | 0.47 | 0.08 | 1.59 | <0.001 | 2.47 | <0.001 | 0.49 | 0.07 |
| **Time** | | | | | | | | | | | | |
| Late-harvest | -0.01 | 0.9 | -0.31 | 0.1 | -1.32 | <0.001 | -0.05 | 0.8 | -0.33 | 0.09 | -1.31 | <0.001 |
| Pre-harvest | ref | | ref | | ref | | ref | | ref | | ref | |
| **Job** | | | | | | | | | | | | |
| Cane cutter | 0.17 | 0.5 | -0.06 | 0.8 | -0.50 | 0.03 | 0.16 | 0.5 | -0.04 | 0.8 | -0.48 | 0.04 |
| Seeder | 0.63 | 0.02 | -0.23 | 0.4 | -0.54 | 0.07 | 0.48 | 0.1 | -0.44 | 0.2 | -0.51 | 0.1 |
| Seed cutter | 0.48 | 0.1 | -0.35 | 0.3 | -0.37 | 0.3 | 0.48 | 0.2 | -0.06 | 0.9 | -0.18 | 0.7 |
| Agrichemical applicator | 0.01 | 0.9 | 0.06 | 0.8 | -0.70 | 0.009 | -0.02 | 0.9 | 0.08 | 0.8 | -0.69 | 0.01 |
| Irrigator | 0.09 | 0.7 | -0.34 | 0.1 | -0.82 | <0.001 | 0.07 | 0.7 | -0.32 | 0.1 | -0.81 | <0.001 |
| Driver | -0.05 | 0.8 | 0.48 | 0.04 | -0.60 | 0.01 | -0.07 | 0.8 | 0.49 | 0.03 | -0.58 | 0.02 |
| Factory worker | ref | | ref | | ref | | ref | | ref | | ref | |
| **Job*Time** | | | | | | | | | | | | |
| Cane cutter*Late-harvest | 0.94 | <0.001 | 0.64 | 0.03 | 1.59 | <0.001 | 0.98 | <0.001 | 0.66 | 0.02 | 1.59 | <0.001 |
| Seeder*Late-harvest | 0.00 | 0.9 | 0.75 | 0.02 | 1.48 | <0.001 | 0.40 | 0.3 | 1.23 | 0.005 | 1.61 | <0.001 |
| Seed cutter*Late-harvest | -0.24 | 0.5 | 0.05 | 0.9 | 0.94 | 0.01 | -0.44 | 0.4 | -0.20 | 0.7 | 0.43 | 0.4 |
| Agrichemical app.*Late-harvest | 0.00 | 0.9 | 0.18 | 0.6 | 1.17 | <0.001 | 0.04 | 0.9 | 0.20 | 0.6 | 1.17 | <0.001 |
| Irrigator*Late-harvest | 0.73 | 0.006 | 0.41 | 0.2 | 1.30 | <0.001 | 0.77 | 0.003 | 0.43 | 0.1 | 1.29 | <0.001 |
| Driver*Late-harvest | 0.09 | 0.8 | -0.04 | 0.9 | 0.71 | 0.01 | 0.12 | 0.6 | -0.02 | 0.9 | 0.70 | 0.02 |
| Factory worker*Late-harvest | ref | | ref | | ref | | ref | | ref | | ref | |
| **Sex** | | | | | | | | | | | | |
| Male | -0.90 | <0.001 | -1.08 | <0.001 | -0.07 | 0.8 | - | | - | | - | |
| Female | ref | | ref | | ref | | - | | - | | - | |
| **Age** | | | | | | | | | | | | |
| (Continuous, per 5 y) | 0.05 | 0.2 | -0.03 | 0.3 | 0.03 | 0.5 | 0.04 | 0.2 | -0.03 | 0.3 | 0.02 | 0.7 |
| **Years worked** | | | | | | | | | | | | |
| (Continuous, per 5 y) | 0.02 | 0.7 | -0.02 | 0.7 | 0.06 | 0.2 | 0.02 | 0.6 | -0.02 | 0.7 | 0.07 | 0.2 |

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18; NAG, N-acetyl-D-glucosaminidas.

**Figure S3.1.** Derivation of study population (n=284)



# CHAPTER FOUR. KIDNEY FUNCTION AND INJURY ASSOCIATED WITH METALS EXPOSURE AMONG NICARAGUAN WORKERS

Rebecca L. Laws[1], Daniel R. Brooks[2], Juan José Amador[2], Daniel E. Weiner[3], James S. Kaufman[4], Oriana Ramírez-Rubio[2,5], Alejandro Riefkohl[2], Madeleine K. Scammell[1], Damaris López-Pilarte[2], José Marcel Sánchez[2], Chirag R. Parikh[6,7], Michael D. McClean[1]

[1]Department of Environmental Health, Boston University School of Public Health, Boston, MA, USA
[2]Department of Epidemiology, Boston University School of Public Health, Boston, MA, USA
[3]Division of Nephrology, Department of Medicine, Tufts Medical Center and Tufts University School of Medicine, Boston, MA, USA
[4]Research Service, VA New York Harbor Healthcare System and Department of Medicine, New York, NY, USA
[5]Preventive Medicine and Public Health Department, Universidad Autónoma de Madrid, Madrid, Spain
[6]Section of Nephrology, Department of Medicine, Yale University and VA Medical Center, New Haven, CT, USA
[7]Program of Applied Translational Research, Department of Medicine, Yale University, New Haven, CT, USA

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Dr. Parikh is listed as a co-inventor on IL-18 patent granted to University of Colorado (no monetary value).

**ABSTRACT**

**Background:** There is an epidemic of chronic kidney disease of non-traditional etiology in Central America, often referred to as Mesoamerican Nephropathy (MeN). One hypothesized causal agent is metals, which are naturally occurring in the environment and known to be nephrotoxic.

**Objectives:** The aims of this study were to characterize exposure to metals (*i.e.* arsenic, cadmium, lead, uranium) among sugarcane workers and miners in northwestern Nicaragua; evaluate differences by industry and job; and examine the relationship between metals and biomarkers of kidney function and injury.

**Methods:** We sampled 78 sugarcane workers in four jobs at two time points (before and near the end of the harvest season) and 20 miners at one time point. In urine, we measured total arsenic, cadmium, uranium, neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), N-acetyl-D-glucosaminidase (NAG), albumin, and creatinine. We measured lead in whole blood and creatinine in serum, and estimated glomerular filtration rate (eGFR). We evaluated metals as predictors of kidney function (eGFR) and kidney injury (NGAL, IL-18, and NAG).

**Results:** Among workers believed to be at high risk of MeN, metal biomarkers were not elevated and did not increase during the harvest season, though there were some differences by job. Total urinary arsenic concentrations were higher among outdoor workers, while blood lead levels were higher among factory workers. When metals were evaluated categorically (≥ median compared to <median), the overall pattern indicated increased NGAL, IL-18, and NAG with higher concentrations of cadmium, lead, and

uranium, but not arsenic. Associations were most pronounced between cadmium and IL-18 (relative mean (RM): 1.55; 95% CI: 1.10, 2.17), lead and IL-18 (RM: 1.45; 95% CI: 0.98, 2.16), and uranium and NGAL (RM: 1.66; 95% CI: 1.09, 2.54). Additionally, higher lead concentrations were associated with decreased eGFR, when compared to lower concentrations (mean difference: -5.57 mL/min/1.73m$^2$; 95% CI: -12.2, 1.08). There was some evidence that exposure to a greater number of metals at higher concentrations was associated with decreased kidney function and increased injury, suggesting a possible combined effect.

**Conclusions:** Our findings do not provide evidence that metals are a primary causal agent of Mesoamerican Nephropathy. However, it is possible that low-level environmental exposure to metals, most likely in combination with one or more other agents, may play a role in the etiology of this disease. More studies are needed in larger populations to further explore this hypothesis.

## INTRODUCTION

An epidemic of chronic kidney disease (CKD) in Central America, commonly referred to as the Mesoamerican Nephropathy (MeN), disproportionately affects young, male laborers. This disease is atypical in that it is not caused by traditional risk factors, such as diabetes or hypertension (Cuadra et al. 2006; Orantes et al. 2011; Orantes et al. 2014; Raines et al. 2014; Ramirez-Rubio et al. 2013a; Ramirez-Rubio et al. 2013b; Sanoff et al. 2010; Weiner et al. 2013; Wesseling et al. 2013a). MeN concentrates in the low-lying, Pacific coastal regions of multiple Central American countries. For example,

the provinces of Chinandega and León on the Pacific coast of Nicaragua have CKD mortality rates of 73 and 77 per 100,000, respectively, compared to 19.6 per 100,000 for the country as a whole (MINSA 2011). These regions and the affected populations are largely comprised of agricultural workers, particularly sugarcane workers (Orantes et al. 2014; Peraza et al. 2012; Raines et al. 2014; Sanoff et al. 2010; Torres et al. 2010), though other at-risk occupations such as mining have been identified (Torres et al. 2010). Heat stress is a likely important factor, probably in combination with one or more other exposures (Brooks et al. 2012; Johnson and Sanchez-Lozada 2013; Laws et al. 2015b; Peraza et al. 2012; Torres et al. 2010; Wesseling et al. 2013a).

Metals and metalloids are one such hypothesized etiologic agent, and may be elevated throughout the Pacific region of Central America due to a series of active volcanoes (Lopez et al. 2012). Arsenic is of particular concern, given that high concentrations (>50 µg/L) have been detected in multiple Central American countries in surface and groundwater, both common sources of drinking water (Bundschuh et al. 2012; Lopez et al. 2012; McClintock et al. 2012). Although environmental data for cadmium, lead, and uranium are lacking in the region, these metals have been identified in high concentrations in soils of other volcanic areas (Bubach et al. 2014; Mora 2003; Queirolo et al. 2000).

Notably, exposure to heavy metals (*i.e.* cadmium, lead, and uranium) is associated with acute and chronic nephrotoxicity, most directly affecting the proximal tubule cells (Gonick 2008; Sabath and Robles-Osorio 2012; Soderland et al. 2010; Vicente-Vicente et al. 2010). Though the mechanism of action may slightly vary by metal, it likely involves

some combination of local oxidative stress with associated lipid peroxidation, apoptosis, and necrosis, and manifests clinically as reduced glomerular filtration rate (GFR) with low molecular weight proteinuria (Gonick 2008; Sabath and Robles-Osorio 2012; Sabolic 2006; Vicente-Vicente et al. 2010). The relationship between arsenic and kidney damage is less clear, though a growing body of literature suggests an association (Zheng et al. 2014), possibly with a similar oxidative stress mechanism (Jomova et al. 2011; Majhi et al. 2011; Prabu and Muthumani 2012). Mesoamerican Nephropathy is characterized by infrequent, low-grade proteinuria, suggesting a tubulointerstitial disease (Brooks et al. 2012; Correa-Rotter et al. 2014; Laws et al. 2015b; O'Donnell et al. 2011; Orantes et al. 2011; Ordunez et al. 2014b; Peraza et al. 2012; Torres et al. 2010), which is consistent with the type of nephrotoxic damage expected from exposure to metals.

Because of their ubiquity in the region and known relationship with tubular kidney damage, metals are commonly hypothesized as a cause of MeN. However, due to their resource-intensive nature to study, there is a dearth of data addressing this hypothesis. In fact, the Pan American Health Organization (PAHO) recently recognized this data gap and recommended more research into the potential role of heavy metals (Ordunez et al. 2014a). Importantly, epidemics of CKD with similar characteristics to MeN have been identified in Sri Lanka and India, and leading hypotheses in these regions include exposure to cadmium and arsenic, possibly in combination with other agents (Jayasumana et al. 2014a; Jayasumana et al. 2014b; Jayasumana et al. 2013; Jayatilake et al. 2013).

In a population of Nicaraguan sugarcane workers, we previously reported a

decrease in kidney function and an increase in kidney injury during the harvest season that varied by job category, providing evidence that there is an occupational component to MeN (Laws et al. 2015a, b). Cane cutters, who perform the most strenuous tasks in a hot ambient environment, had the most consistent evidence of kidney injury, suggesting that heat stress may play a role in the disease. This study, however, utilized job category as a surrogate exposure measure, and did not directly measure exposure to potential etiologic agents.

The current study was conducted in a subset of these sugarcane workers, as well as a population of miners. This study quantitatively assessed exposure to metals, improving upon previous surrogate exposure measures, and provides the first comprehensive evaluation of this hypothesis as it relates to MeN. Accordingly, the primary goals were to (1) characterize exposure to nephrotoxic metals (*i.e.* arsenic, cadmium, lead, uranium) among sugarcane workers and miners in northwestern Nicaragua; (2) evaluate differences in metal biomarkers by job category to provide information about exposure pathways; and (3) examine the relationship between metals exposure and biomarkers of kidney function and injury.

## METHODS

### *Study population*

The study population included sugarcane workers and miners from northwestern Nicaragua that were at least 18 years of age. Previously, we described the enrollment of 284 sugarcane workers, representing seven different job categories, from one company in

the province of Chinandega (Laws et al. 2015b). To be eligible for this study, sugarcane workers had to be employed in one of four of these jobs (cane cutter, seeder, irrigator, and factory worker). Based on our prior industrial hygiene assessment (Laws et al. 2015b; McClean et al. 2010), we included cane cutters and seeders due to their high potential for exposure to metals in soil/dust, and irrigators due to their high potential for exposure to metals in water. Factory workers were included as a group of non-field workers, at lower risk for MeN than field workers (Laws et al. 2015a, b). Factory workers likely have lower exposure to metals in soil or water than field workers, but potentially higher exposure to metals through machining operations in the factory.

In addition to the sugarcane workers, we recruited 51 miners from one company in the province of León who had not previously been employed in the sugarcane industry. These workers were mostly laborers and operators who worked in and around an underground mine that primarily produces gold. Miners were included because of their potential for exposure to metals through soil/dust, and because mining activities can cause environmental contamination that may further expose workers and the surrounding community to elevated metals levels (Barenys et al. 2014; Cui et al. 2005; Li et al. 2014; Navarro et al. 2008; Saunders et al. 2013). Additionally, the majority of studies on Mesoamerican Nephropathy have focused on sugarcane workers, but other industries in the region are affected, including mining (Torres et al. 2010).

For this assessment of metals, we randomly selected 20 sugarcane workers each from the four job categories (cane cutter, seeder, irrigator, and factory worker). Two workers were later determined to be ineligible and were excluded; therefore the number

of sugarcane workers totaled 78. We also randomly selected 20 of the 51 miners, so the final study population included 98 workers. We collected samples from sugarcane workers prior to the harvest season (October to December 2010) and again four to six months later, near the end of the harvest season (March to May 2011). We sampled miners around the same time as the late-harvest sampling of sugarcane workers (May to June 2011). Each assessment included the collection of blood and urine and questionnaires about personal characteristics and occupational history. Sugarcane workers were sampled twice, while miners were sampled once; therefore, we collected a total of 176 blood and urine samples. Immediately following collection, biological samples were processed and stored at -20°C. Within one week, samples were transported to the ISO certified *Centro Nacional de Diagnóstico y Referencia* (CNDR) in Managua, a division within the Ministry of Health (MINSA), and stored at -80°C until analysis.

The Institutional Review Boards at the Boston University Medical Center and the Nicaraguan Ministry of Health approved study protocols. All participants provided informed consent prior to participation in research activities.

*Laboratory analysis of metals*

Urine and blood samples were analyzed for metals at the *Centre de toxicologie of the Institut national de santé publique du Québec* (INSPQ) in Quebec, Canada. The laboratory is accredited under ISO 17025 and uses numerous external quality control programs. Arsenic (total), cadmium, and uranium were analyzed in urine. A 500 μL aliquot was diluted twenty-fold in dilute nitric acid and analyzed by inductively coupled

plasma mass spectrometry (ICP-MS). An internal standard was added for improved precision, and calibration was performed in a urine-based matrix. The detection limits were 0.22 µg/L for arsenic, 0.09 µg/L for cadmium, and 0.01 µg/L for uranium. Lead was analyzed in whole blood. A 500 µL aliquot was diluted twenty-fold in a dilute ammonium hydroxide / Triton-X solution and analyzed by ICP-MS. An internal standard was added for improved precision, and calibration was performed in a blood-based matrix. The detection limit for blood lead was 0.22 µg/L.

*Laboratory analysis of biomarkers of kidney function and injury*

Serum creatinine was measured at CNDR in Managua using a kinetic-rate Jaffe method; 0.2 mg/dL was subtracted from results to calibrate to an isotopic dilution mass spectrometry standard. Urine samples were analyzed for creatinine, albumin, neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), and N-acetyl-D-glucosaminidase (NAG) at the Division of Nephrology and Hypertension at the Cincinnati Children's Hospital Medical Center (Cincinnati, Ohio, USA). Urine creatinine was measured using a colorimetric modification of the Jaffe reaction, and urine albumin was measured using immunoturbidimetry. The intra-assay coefficient of variation (CV) was 2.4% for creatinine and 2.9% for albumin; inter-assay CVs were 4.2% and 5.9%, respectively. ELISA was used to measure NGAL (Bioporto, Gentofte, Denmark) and IL-18 (MBL, Intl., Woburn, MA). CVs for NGAL and IL-18 were intra (2.1%; 7.5%) and inter (9.1%; 7.3%). NAG activity was measured with a colorimetric assay (Roche Diagnostics, USA), with method intra- and inter-assay CVs of 4.3% and 6.0%,

respectively (Liangos et al. 2007). The detection limits were 1.3 mg/L for urine albumin, 1.6 pg/mL for NGAL, 4 pg/mL for IL-18, and 0.003 U/L for NAG.

*Statistical analyses*

To account for urine concentration, we normalized biomarkers of metals and kidney injury to urinary creatinine concentration (g/L) and expressed as follows: arsenic (μg/g), cadmium (μg/g), uranium (μg/g), albumin-to-creatinine ratio (ACR) (mg/g), NGAL (μg/g), IL-18 (ng/g), and NAG (U/g). Lead in blood was reported as micrograms per deciliter (μg/dL) to facilitate comparison with reference values. We estimated glomerular filtration rate (eGFR) from serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, considering race as "non-black" (Levey et al. 2009). We examined the distribution of each biomarker using histograms, graphical displays, and summary statistics. To satisfy normality assumptions, biomarkers that exhibited a lognormal distribution were natural log-transformed when evaluated as outcomes. This included all biomarkers except eGFR, which was normally distributed. Values below the limit of detection (LOD) were substituted with LOD/√2.

Geometric mean concentrations of each metal in this population were compared to those of the United States adult population (males and females, 20 years and older) using data from the 2011–2012 National Health and Nutrition Examination Survey (NHANES), conducted by the U.S. Centers for Disease Control and Prevention to characterize the distribution of chemical exposures in a random sample of the U.S. general population (CDC 2015). We assessed correlations among metals using Spearman

rank coefficients and assessed potential collinearity by calculating variance inflation factors and tolerances. We evaluated job category as a predictor of arsenic, cadmium, and lead using linear mixed effects models with an unstructured covariance matrix. For uranium, given the low percentage of detected values, we evaluated job category as a predictor of detection (yes/no) using a generalized linear model accounting for repeated measures. In each model, we tested the interaction between time and job category to determine if, among sugarcane workers, the change during the harvest season was different by job. All models were adjusted for age and sex.

We assessed metals as predictors of kidney function (eGFR) and kidney injury (NGAL, IL-18, NAG) using linear mixed effects models with an unstructured covariance matrix. Metals were evaluated both continuously and categorically (dichotomized at the median and 90$^{th}$ percentile values and categorized into tertiles) to explore linear and/or nonlinear relationships. For each model, all metals were included together as predictors, although sensitivity analyses were performed with each individual metal in separate models. Finally, we estimated the joint effect of exposure to multiple metals, defined as the number of metals to which an individual was exposed at levels greater than or equal to the median concentration. This variable was evaluated both continuously and categorically as a predictor of biomarkers of kidney injury and function. All models were adjusted for age, sex, time, and job category. Data were analyzed using Statistical Analysis Software (SAS version 9.3, Cary, NC).

## RESULTS

*Population characteristics and exposure to metals*

The majority of participants were men (84%); women were employed only in seeding, factory, and mining jobs (Table 4.1). Mean age was 32.9 ± 10.0 years; on average, miners and factory workers were older than cane cutters, seeders, and irrigators (Table 4.1). All samples had detectable levels of total arsenic and lead. Twenty-nine samples (16%) had concentrations below the LOD for cadmium, and 90 samples (51%) had concentrations below the LOD for uranium. Metals were not correlated with each other.

For each metal, the overall geometric mean (GM) was higher in this population than the general U.S. adult population, though only marginally so (Figure 4.1). Overall and job-specific GMs of cadmium and uranium were generally consistent with U.S. geometric mean concentrations (cadmium: 0.22 μg/g; uranium: 0.007 μg/g); GMs for arsenic and lead were closer to the 75$^{th}$ percentile values of U.S. concentrations (arsenic: 14.8 μg/g; lead: 1.7 μg/dL) (Table 4.2) (CDC 2015). When compared to occupational exposure guidelines (available for lead and uranium), all blood lead levels were below the American Conference of Governmental Industrial Hygienists (ACGIH) biological exposure index (BEI) of 30 μg/dL (ACGIH 2004), and all urine uranium levels were well below the U.S. Nuclear Regulatory Commission guideline of 15 μg/L (NRC 1978).

*Job category and metals*

Total urinary arsenic concentrations were consistently higher among outdoor workers (cane cutters, seeders, irrigators, and miners), ranging between 20% and 52% higher than concentrations among factory workers (Table 4.3). Blood lead concentrations were higher among factory workers than all other jobs, while urinary cadmium was not different by job (Table 4.3). Compared to factory workers, concentrations of uranium in urine were less likely to be detected in miners (odds ratio (OR): 0.18; 95% CI: 0.05, 0.65) (results not shown). Urinary cadmium was lower in males than females (relative mean (RM): 0.65; 95% CI: 0.43, 0.98), while blood lead was twice as high among males than females (RM: 2.04; 95% CI: 1.19, 3.49) (Table 4.3). Among sugarcane workers, concentrations of metals did not increase during the harvest and in fact were lower at late-harvest than pre-harvest for cadmium and lead (Table 4.3). There was no difference in the effect of time by job; this interaction term was therefore excluded from final models. Age was not associated with any metal biomarkers (Table 4.3).

*Association between metals and kidney function and injury*

Details regarding biomarkers of kidney function and injury in these sugarcane workers have been previously described (Laws et al. 2015a, b). Miners were sampled only once, corresponding to late-harvest; measurements for these workers are therefore included at this time point but not at pre-harvest. In general, this was a healthy population, as all participants were current workers; overall and job-specific eGFR means were above 100 mL/min/1.73m$^2$ (Table 4.2). No workers had eGFR <60 mL/min/1.73m$^2$

at pre-harvest, while five (5%) did at late-harvest. Six workers (8%) at pre-harvest and sixteen workers (16%) at late-harvest had eGFR <90 mL/min/1.73m$^2$. No workers had ACR >300 mg/g at either time point; five workers (6%) at pre-harvest and ten workers (10%) at late-harvest had ACR >30 mg/g. Because ACR was generally low (Table 4.2), with 43% of samples below the detection limit, it was not included in statistical analyses.

Metal biomarkers were evaluated as continuous and categorical predictors of eGFR, NGAL, IL-18, and NAG; uranium was not included in continuous models due to low percent detect. When assessing the relationship between metals and kidney function, we found some evidence that higher lead concentrations (≥ median) were associated with decreased eGFR, when compared to lower concentrations (<median) (mean difference: -5.57 mL/min/1.73m$^2$; 95% CI: -12.2, 1.08) (Table 4.4, Model 2). This association was not observed when lead was evaluated as a continuous predictor and was not seen for the other metals (Table 4.4, Model 1). We next explored potential relationships between metals and kidney injury biomarkers. When evaluated categorically, the overall pattern indicated a trend of increased NGAL, IL-18, and NAG with higher concentrations of cadmium, lead, and uranium (Table 4.4, Model 2). Though not consistent across each metal and kidney injury biomarker, associations were most pronounced between cadmium and IL-18 (RM: 1.55; 95% CI: 1.10, 2.17), lead and IL-18 (RM: 1.45; 95% CI: 0.98, 2.16), and uranium and NGAL (RM: 1.66; 95% CI: 1.09, 2.54) (Table 4.4, Model 2). This pattern was not observed for arsenic. These associations remained when comparing the upper tertile of metals concentrations to the lower two tertiles, but were attenuated when comparing the upper 10$^{th}$ percentile to the lower 90$^{th}$ percentile (results

not shown). When metals were evaluated continuously, the relationship between cadmium and IL-18 was the only to persist; every additional 0.1 μg/g of cadmium was associated with a relative increase in IL-18 of 1.15 (95% CI: 1.03, 1.28) (Table 4.4, Model 1). Results did not materially change when each metal was evaluated individually rather than together in models.

Finally, we explored a possible joint effect of exposure to multiple metals, a variable defined as the number of metals to which an individual is exposed at levels greater than or equal to the median concentration (0 through 4) (Table 4.5). When evaluated as a continuous predictor, we found a negative association with eGFR and positive associations with NGAL, IL-18, and NAG. On average, a one-unit increase in the number of metals at or above the median was associated with a 1.89 mL/min/1.73m$^2$ decrease in eGFR (95% CI: -4.51, 0.73), a 24% increase in NGAL (RM: 1.24; 95% CI: 1.02, 1.51), a 26% increase in IL-18 (RM: 1.26; 95% CI: 1.08, 1.49), and a 17% increase in NAG (RM: 1.17; 95% CI: 0.99, 1.39) (Table 4.5, Model 1). When this joint metal exposure variable was evaluated categorically (reference: 0 metals at or above the median value), there appeared to be an increased trend in each biomarker of kidney injury and a decreased trend in eGFR for every additional metal at or above the median value (Table 4.5, Model 2). After testing in statistical models, this linear trend was only significant for IL-18 (eGFR: p=0.2; NGAL: p=0.7; IL-18: p=0.05; NAG; p=0.6). However, eGFR was significantly lower, and NGAL, IL-18, and NAG were significantly higher, among individuals who had elevated exposure to all 4 metals compared to individuals who had elevated exposure to 0 metals (Table 4.5, Model 2).

## DISCUSSION

This is the first comprehensive assessment of exposure to metals and their relationship with kidney function and injury in a population of workers at risk for Mesoamerican Nephropathy. We found that metal concentrations were not elevated in this population and did not increase during the harvest season, though there were some differences by job. There was an overall pattern of increased NGAL, IL-18, and NAG with higher concentrations of cadmium, lead, and uranium, but this was not consistent across each metal and kidney injury biomarker. We also observed decreased kidney function with higher lead concentrations. There was some evidence that exposure to a greater number of metals at higher concentrations was associated with decreased kidney function and increased injury, suggesting a possible combined effect. These findings do not provide strong evidence that metals are a primary causal agent of MeN; however, it is possible that low-level exposure to metals may play a role in the etiology of this disease.

Arsenic, cadmium, lead, and uranium are naturally occurring in the environment, and it is thought that these metals, particularly arsenic, are elevated in northwestern Nicaragua due to volcanic activity (Bundschuh et al. 2012; Lopez et al. 2012; McClintock et al. 2012). They may also enter the environment through anthropogenic activities such as application of arsenical pesticides or fertilizers and mining (Barenys et al. 2014; Cui et al. 2005; Li et al. 2014; Navarro et al. 2008; Saunders et al. 2013). In this population, there is the potential for occupational exposure to metals through contact with soil, air, or water while at work. Factory workers, who are less likely exposed through these media, may have increased exposure through machining operations in the factory.

Workers also have the potential for exposure through non-occupational activities including consumption of contaminated food and water.

Among sugarcane workers in this population, we previously found that eGFR decreased and biomarkers of kidney injury increased during the harvest season. These changes varied by sugarcane job, with kidney injury biomarkers being most notably elevated among cane cutters, suggesting that occupational exposures play a role in MeN (Laws et al. 2015a, b). In this study, we assessed both the absolute levels of metals and their variability by job, as well as their relation to markers of kidney function and injury. One goal was to determine if differences in metals exposure by job could explain our previous findings of differences in kidney function and injury by job. Concentrations of metals in this population were consistent with those among adults in the United States; although not an ideal comparison population, this suggests that on average, levels were not elevated in these workers. Metal biomarkers did not increase from pre- to late-harvest, but there were some differences by job. Urinary arsenic was higher among outdoor workers, which is consistent with the pattern of increased kidney injury biomarkers we previously found. Blood lead, however, was higher among factory workers, a job that has been identified as having lower risk for MeN (Laws et al. 2015a, b). Together, these findings suggest that while there may be some differences in exposure by job category, metal concentrations in this population do not appear to be increased due to occupation. With the exception of arsenic, the exposure pattern is not what we would expect for a key causal agent of MeN.

Only a handful of studies have investigated the potential role of metals in this

epidemic of CKD, with inconsistent results. O'Donnell *et al.*, the only peer-reviewed study, measured blood lead in 299 individuals (87 CKD cases and 212 controls) from Quezalguaque, Nicaragua and found that levels were generally low (<3 μg/dL) and not associated with CKD (O'Donnell et al. 2011). Three unpublished case-control studies among Nicaraguan sugarcane workers, however, found positive results. The first reported that 28% of sick workers and 2.5% of healthy workers had a positive lead test (OR: 18.9) (Marin Ruiz and Berroteran 2002), while a second study found elevated blood lead levels in CKD patients compared to controls in León and Chinandega (Zelaya 2001). A third study found that cadmium levels were higher among cases than controls, though this difference may have been related to cigarette smoking, another source of cadmium exposure (Uriarte Barrera 2000). These positive findings are difficult to interpret and may in part be due to both small sample size and the timing of the testing. Individuals with CKD were prevalent cases, and therefore it was not possible to determine whether higher levels of lead and cadmium among cases were a cause of CKD or an effect of impaired renal function (*i.e.* increased metal concentrations due to decreased renal excretion). Our finding of decreased eGFR with higher lead concentrations is consistent with the classic presentation of chronic lead nephrotoxicity (Soderland et al. 2010; Wedeen et al. 1979), and could indicate that lead plays a role in MeN. However, these results could also be attributable to reverse causality, although this may be less of a problem in our population because it is comprised of generally healthy individuals.

Low-molecular weight proteinuria, particularly NAG, may be the most sensitive and reliable biomarker of cadmium and lead-induced renal injury, and may be evident

before decreased eGFR (Lin and Tai-Yi 2007; Prozialeck and Edwards 2010). Less is known about markers of early renal dysfunction as a result of uranium exposure (Kurttio et al. 2002; Kurttio et al. 2006; Selden et al. 2009; Vicente-Vicente et al. 2010; Zamora et al. 1998), and evidence of arsenic-induced nephrotoxicity is most consistent with albuminuria outcomes, with mixed evidence for associations with NAG and other kidney injury biomarkers (Chen et al. 2011; Zheng et al. 2014). Because NGAL and IL-18 are more novel biomarkers of kidney injury, there are limited studies evaluating their relationships with these metals. In this study, we found an overall pattern of increased kidney injury biomarkers with higher concentrations of cadmium, lead, and uranium, but not arsenic. This was most pronounced for associations between cadmium and IL-18, lead and IL-18, and uranium and NGAL, but there was also more evidence than not of associations between these three metals and NAG. One problem with these findings is that concentrations of metals in this population were much lower than established benchmark doses associated with renal dysfunction (Hong et al. 2004; Lin and Tai-Yi 2007; Suwazono et al. 2006; Woo et al. 2015). However, there is growing evidence that exposure to these metals at low levels, much lower than previously thought, may contribute to nephrotoxicity (Said and Hernandez 2015; Weaver et al. 2011). This is most notable for blood lead, which may be associated with progression of CKD even at levels <5 μg/dL (Ekong et al. 2006; Lin et al. 2003; Lin et al. 2006). So, while it is unlikely that metal concentrations at these levels are solely responsible for this CKD epidemic, they may be contributing in some way, possibly acting as initiating or progressing factors of disease.

It is also possible that co-exposure to one or more metals, even at low levels, may have combined effects on the kidney, acting in an additive or synergistic manner (Hambach et al. 2013; Hong et al. 2004; Huang et al. 2009; Navas-Acien et al. 2009; Nordberg et al. 2005; Trzeciakowski et al. 2014). Supporting this idea, we found that exposure to a greater number of metals at higher concentrations was associated with increased kidney injury and decreased kidney function. This suggests that these metals may cause kidney damage via similar mechanisms, such that the combined approach better captures their effect than when evaluating each metal individually. It is also possible that one metal could potentiate the effect of another, resulting in greater kidney damage than exposure to each metal alone (Hambach et al. 2013; Hong et al. 2004; Nordberg et al. 2005). However, another possible explanation for this finding is that exposure to multiple metals at elevated concentrations is acting as a surrogate for contact with a particular media (*i.e.* soil or water), which contains both metals and the actual etiologic agent of interest.

There are some limitations to this study. First, this was a relatively healthy population, since by definition all participants were well enough to be working. Additionally, sugarcane workers underwent serum creatinine screening prior to the harvest, reducing the number of workers with CKD. Though a limitation in some ways, this was also a strength in that it decreased the possibility of reverse causation due to metals. Another limitation was the relatively small study size. Although the majority of workers had two measurements, there were only 98 participants, making it difficult to examine finer divisions of exposure categories or jobs. Future studies investigating this

hypothesis should be conducted in larger populations. Third, there could be residual confounding by other potential etiologic agents (*i.e.* heat stress, agrichemicals) that vary by job. In models assessing metals as predictors of kidney function and injury, we controlled for job category, but this could be a proxy for one or more other unknown exposures. Finally, we found no association between total urinary arsenic and biomarkers of kidney function or injury. Total arsenic includes all species of organic and inorganic arsenic (Hughes et al. 2011), but the inorganic fraction and its associated metabolites are of primary concern with respect to kidney damage (Zheng et al. 2015). Therefore, while it is possible that there truly is no association, it is also possible that we were simply unable to capture exposure to the species of concern. Due to reports of arsenic contaminated drinking water in the region (Bundschuh et al. 2012; Lopez et al. 2012), and the growing body of literature supporting its association with CKD (Zheng et al. 2014), future studies that speciate urinary arsenic should be conducted.

## CONCLUSION

In this population of sugarcane workers and miners, we found that metal concentrations were generally consistent with those in the U.S. population and did not increase during the harvest. Though this does not suggest that metals are increased due to occupation, there were some differences by job; most notably, total urinary arsenic was higher among outdoor workers and blood lead was higher among factory factors. There was an overall pattern of increased kidney injury with higher concentrations of cadmium, lead, and uranium, but not arsenic. Higher blood lead levels were also associated with

decreased kidney function, and there was some evidence of a possible combined effect of multiple metals. Both the exposure pattern and absolute levels of these metals do not suggest that they are a primary causal agent of Mesoamerican Nephropathy. However, it is possible that low-level environmental exposure to metals, most likely in combination with one or more other agents, may play a role in the etiology of this disease. More studies are needed in larger populations to further explore this hypothesis, particularly evaluating species of arsenic.

**Table 4.1.** Characteristics of study population, overall and by job category

| | Overall (n=98) | Cane cutter (n=20) | Seeder (n=19) | Irrigator (n=20) | Factory worker (n=19) | Miner (n=20) |
|---|---|---|---|---|---|---|
| **Age** | | | | | | |
| 18–24 | 21 (21%) | 6 (30%) | 6 (32%) | 4 (20%) | 5 (26%) | 0 (0%) |
| 25–34 | 39 (40%) | 8 (40%) | 9 (47%) | 8 (40%) | 6 (32%) | 8 (40%) |
| 35–44 | 21 (21%) | 4 (20%) | 2 (11%) | 6 (30%) | 2 (11%) | 7 (35%) |
| 45–54 | 15 (15%) | 1 (5%) | 1 (5%) | 2 (10%) | 6 (32%) | 5 (25%) |
| 55–63 | 2 (2%) | 1 (5%) | 1 (5%) | 0 (0%) | 0 (0%) | 0 (0%) |
| **Sex** | | | | | | |
| Male | 82 (84%) | 20 (100%) | 5 (26%) | 20 (100%) | 18 (95%) | 19 (95%) |
| Female | 16 (16%) | 0 (0%) | 14 (74%) | 0 (0%) | 1 (5%) | 1 (5%) |

*Note:* 176 samples were collected from 98 workers. Values are given as number (percentage).

**Table 4.2.** Summary statistics [geometric mean and range] of biomarkers of metals and kidney damage at pre- and late-harvest (N=98, n=176)

| | Metals | | | | Kidney Function and Injury | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Arsenic (µg/g)** | **Cadmium (µg/g)** | **Lead (µg/dL)** | **Uranium (µg/g)** | **eGFR (mL/min/1.73m²)** | **ACR (mg/g)** | **NGAL (µg/g)** | **IL-18 (ng/g)** | **NAG (U/g)** |
| **Overall** | | | | | | | | | |
| Pre-harvest | 14.0 (2.8–82.5) | 0.27 (0.05–0.66) | 1.9 (0.3–24.0) | ND (0.003–0.41) | 116 (61–143) | 3.6 (0.5–174.7) | 11.6 (0.9–659.9) | 11.2 (1.1–280.7) | 1.43 (0.01–21.0) |
| Late–harvest | 15.8 (2.8–92.8) | 0.24 (0.05–0.84) | 1.7 (0.3–15.0) | ND (0.001–0.75) | 112 (29–181) | ND (0.3–182.6) | 14.2 (0.1–334.5) | 10.6 (1.0–136.5) | 1.01 (0.002–5.94) |
| **Cane cutter** | | | | | | | | | |
| Pre-harvest | 13.4 (2.9–82.5) | 0.22 (0.05–0.51) | 2.0 (0.8–24.0) | ND (0.003–0.05) | 108 (61–137) | 5.0 (0.5–174.7) | 10.3 (1.9–79.7) | 7.0 (1.1–51.6) | 1.74 (0.50–11.2) |
| Late-harvest | 18.0 (6.4–54.2) | 0.26 (0.14–0.77) | 1.8 (0.8–8.7) | 0.016 (0.005–0.07) | 101 (29–150) | ND (0.3–16.3) | 27.9 (5.7–116.1) | 13.3 (1.9–78.9) | 1.85 (0.44–5.64) |
| **Seeder** | | | | | | | | | |
| Pre-harvest | 15.1 (5.0–33.0) | 0.31 (0.12–0.66) | 1.2 (0.3–14.0) | ND (0.003–0.06) | 123 (92–143) | 5.0 (0.5–128.5) | 21.1 (3.5–244.7) | 19.2 (1.7–280.7) | 0.90 (0.01–7.42) |
| Late-harvest | 18.8 (6.3–80.1) | 0.23 (0.05–0.84) | 1.0 (0.3–9.8) | ND (0.006–0.03) | 124 (83–146) | ND (0.6–126.8) | 19.7 (0.1–187.0) | 25.8 (2.6–136.5) | 1.15 (0.004–5.81) |
| **Irrigator** | | | | | | | | | |
| Pre-harvest | 15.2 (2.8–80.3) | 0.27 (0.10–0.62) | 1.7 (1.0–4.1) | 0.012 (0.006–0.04) | 120 (96–137) | ND (0.5–138.7) | 7.9 (0.9–659.9) | 8.5 (1.6–34.7) | 0.84 (0.01–3.91) |
| Late-harvest | 15.6 (6.2–47.5) | 0.25 (0.10–0.57) | 1.4 (0.8–3.4) | ND (0.006–0.75) | 113 (46–139) | 4.5 (0.5–141.0) | 20.8 (0.5–334.5) | ND (1.6–130.2) | 0.79 (0.002–5.94) |
| **Factory worker** | | | | | | | | | |
| Pre-harvest | 12.4 (6.0–29.6) | 0.28 (0.14–0.66) | 2.9 (1.2–15.0) | ND (0.004–0.41) | 113 (69–134) | 3.5 (0.5–11.3) | 10.9 (3.0–174.5) | ND (1.9–104.0) | 3.20 (0.45–21.0) |
| Late-harvest | 12.2 (2.8–46.2) | 0.21 (0.09–0.80) | 2.6 (1.1–15.0) | 0.013 (0.004–0.24) | 116 (76–181) | ND (0.4–16.3) | 5.2 (0.1–32.8) | 7.2 (1.3–47.6) | 0.75 (0.26–2.29) |
| **Miner** | | | | | | | | | |
| Late-harvest | 15.3 (6.5–92.8) | 0.24 (0.08–0.70) | 1.8 (0.7–13.0) | ND (0.001–0.02) | 104 (58–132) | 5.7 (0.3–182.6) | 9.4 (2.1–42.9) | 7.3 (1.0–37.3) | 0.81 (0.30–1.73) |

*Note*: Values are given as geometric mean (range), with the exception of eGFR, which is given as mean (range). Non-detect (ND) is reported instead of geometric mean if the percent detect is <60%.

Abbreviations: eGFR, estimated glomerular filtration rate; ACR, albumin-to-creatinine ratio; NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18; NAG, N-acetyl-D-glucosaminidase; ND: non-detect.

**Table 4.3.** Results of linear mixed effects models for metal biomarkers by job category

| Variable | Arsenic (µg/g) | | Cadmium (µg/g) | | Lead (µg/dL) | |
|---|---|---|---|---|---|---|
| | Relative Mean | 95% CI | Relative Mean | 95% CI | Relative Mean | 95% CI |
| **Time** | | | | | | |
| Late-harvest | 1.14 | (0.93, 1.40) | 0.88 | (0.78, 0.99) | 0.86 | (0.80, 0.93) |
| Pre-harvest | ref | | ref | | ref | |
| **Job** | | | | | | |
| Cane cutter | 1.21 | (0.90, 1.63) | 1.03 | (0.76, 1.40) | 0.65 | (0.43, 0.97) |
| Seeder | 1.52 | (1.01, 2.29) | 0.85 | (0.56, 1.30) | 0.66 | (0.38, 1.16) |
| Irrigator | 1.21 | (0.90, 1.63) | 1.10 | (0.81, 1.50) | 0.54 | (0.36, 0.82) |
| Miner | 1.20 | (0.83, 1.74) | 1.03 | (0.74, 1.43) | 0.71 | (0.47, 1.08) |
| Factory worker | ref | | ref | | ref | |
| **Sex** | | | | | | |
| Male | 1.24 | (0.83, 1.86) | 0.65 | (0.43, 0.98) | 2.04 | (1.19, 3.49) |
| Female | ref | | ref | | ref | |
| **Age** (Years, continuous) | 0.99 | (0.98, 1.00) | 1.01 | (1.00, 1.02) | 1.00 | (0.98, 1.01) |

*Note*: Uranium was not included in linear mixed effects models due to low percentage of detected values.

**Table 4.4.** Results of linear mixed effects models for kidney function and injury associated with metals, evaluated continuously and categorically [dichotomized at median value]

| Variable | eGFR (mL/min/1.73m$^2$) | | NGAL (µg/g) | | IL-18 (ng/g) | | NAG (U/g) | |
|---|---|---|---|---|---|---|---|---|
| | Mean Difference | (95% CI) | Relative Mean | (95% CI) | Relative Mean | (95% CI) | Relative Mean | (95% CI) |
| ***Model 1*** **(continuous)**[a] | | | | | | | | |
| Arsenic (µg/g) | -0.003 | (-0.18, 0.17) | 1.00 | (0.99, 1.02) | 1.00 | (0.99, 1.01) | 1.00 | (0.99, 1.01) |
| Cadmium (µg/g) | 0.98 | (-0.81, 2.77) | 1.09 | (0.96, 1.25) | 1.15 | (1.03, 1.28) | 1.01 | (0.90, 1.14) |
| Lead (µg/dL) | 0.06 | (-0.92, 1.05) | 1.04 | (0.96, 1.11) | 1.01 | (0.96, 1.07) | 1.00 | (0.94, 1.07) |
| ***Model 2*** **(categorical)**[b] | | | | | | | | |
| Arsenic (µg/g) | -2.68 | (-8.10, 2.75) | 1.10 | (0.73, 1.65) | 0.93 | (0.67, 1.31) | 1.03 | (0.72, 1.48) |
| Cadmium (µg/g) | -1.06 | (-6.72, 4.60) | 1.28 | (0.84, 1.95) | 1.55 | (1.10, 2.17) | 1.25 | (0.86, 1.81) |
| Lead (µg/dL) | -5.57 | (-12.2, 1.08) | 0.98 | (0.60, 1.60) | 1.45 | (0.98, 2.16) | 1.22 | (0.79, 1.90) |
| Uranium (µg/g) | 0.86 | (-4.79, 6.51) | 1.66 | (1.09, 2.54) | 1.18 | (0.83, 1.66) | 1.22 | (0.84, 1.77) |

*Note*: All models are adjusted for age, sex, time, and job category.

Abbreviations: eGFR, estimated glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18; NAG, N-acetyl-D-glucosaminidase.

[a] Metals evaluated as continuous predictors of each outcome. Uranium was excluded due to large percent non-detect. The beta coefficient represents the mean difference or relative mean in each biomarker of kidney function or injury, for a 1 µg/g increase in arsenic (range: 2.8–92.8 µg/g), a 0.1 µg/g increase in cadmium (range: 0.05–0.84 µg/g), and a 1 µg/dL increase in lead (range: 0.3–24.0 µg/dL).

[b] Metals evaluated as categorical predictors of each outcome, dichotomized at the median value. The beta coefficient represents the mean difference or relative mean in each biomarker of kidney function or injury, for metal values ≥ median, compared to values < median. For uranium, this corresponds to values detected compared to values not detected.

**Table 4.5.** Results of linear mixed effects models for kidney function and injury associated with joint exposure to metals

| Variable | eGFR (mL/min/1.73m$^2$) | | NGAL (ug/g) | | IL-18 (ng/g) | | NAG (U/g) | |
|---|---|---|---|---|---|---|---|---|
| | Mean Difference | (95% CI) | Relative Mean | (95% CI) | Relative Mean | (95% CI) | Relative Mean | (95% CI) |
| ***Model 1***[a] | | | | | | | | |
| **Number of metals ≥ median** | | | | | | | | |
| Number (continuous) | -1.89 | (-4.51, 0.73) | 1.24 | (1.02, 1.51) | 1.26 | (1.08, 1.49) | 1.17 | (0.99, 1.39) |
| ***Model 2***[b] | | | | | | | | |
| **Number of metals ≥ median** | | | | | | | | |
| 0 | ref | | ref | | ref | | ref | |
| 1 | -10.7 | (-23.4, 2.08) | 1.27 | (0.49, 3.31) | 3.11 | (1.43, 6.77) | 1.11 | (0.48, 2.56) |
| 2 | -10.9 | (-23.3, 1.48) | 1.22 | (0.48, 3.08) | 2.82 | (1.33, 5.96) | 1.20 | (0.53, 2.72) |
| 3 | -9.8 | (-22.9, 3.46) | 1.57 | (0.58, 4.21) | 3.59 | (1.62, 7.95) | 1.23 | (0.51, 2.93) |
| 4 | -15.8 | (-30.1, -1.54) | 2.91 | (0.99, 8.50) | 5.02 | (2.09, 12.1) | 2.24 | (0.88, 5.73) |

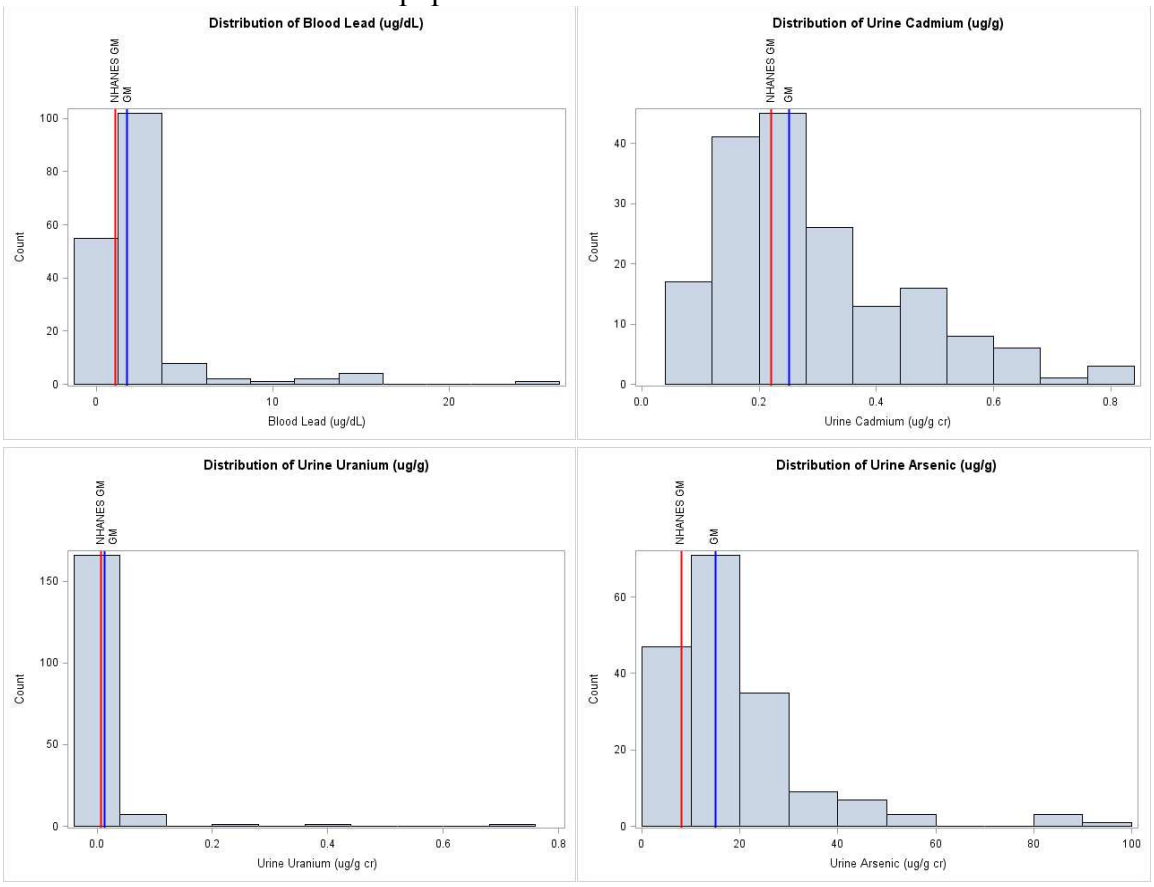
*Note*: All models are adjusted for age, sex, time, and job category. The joint metal exposure variable is defined as the number of metals to which an individual is exposed to at levels greater than or equal to the median concentration.

Abbreviations: eGFR, estimated glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18; NAG, N-acetyl-D-glucosaminidase.

[a] Joint metal exposure variable evaluated as a continuous predictor of each outcome. The beta coefficient represents the mean difference or relative mean in each biomarker of kidney function or injury, for a one-unit increase in joint metal exposure.

[b] Joint metal exposure evaluated as a categorical predictor of each outcome. The beta coefficient represents the mean difference or relative mean in each biomarker of kidney function or injury, for each category of joint metal exposure compared to individuals with 0 metals at or above the median value.

**Figure 4.1.** Distribution of metal biomarkers and comparison of geometric mean (GM) concentrations with U.S. adult population




*Note:* Blue line indicates population geometric mean (GM). Red line indicates GM of United States adult population (males and females, age 20 and over), gathered from 2011–2012 National Health and Nutrition

## CHAPTER FIVE. CONCLUSION

This research aimed to evaluate changes in biomarkers of kidney function and injury during the six-month sugarcane harvest season and assess differences in these biomarkers by job category. The research was designed to determine which, if any, occupational factors may be important in the etiology of Mesoamerican Nephropathy. We also assessed relationships between self-reported hydration and biomarkers of kidney function and injury, and between biomarkers of metals and kidney function and injury. We conducted analyses in a cohort of sugarcane workers, recruited from one company in northwestern Nicaragua, and miners from the same region, both populations at risk for MeN. To our knowledge, this is the most comprehensive occupational investigation of this epidemic to date. In the context of MeN, it is the first study to utilize a longitudinal design, the first to assess job-level comparisons, the first to evaluate novel biomarkers of tubular injury, and the first to quantitatively assess exposure to metals and their relationship with kidney function and injury.

*Kidney function decreased during the harvest and was different by job*

We assessed changes in eGFR (estimated from serum creatinine) during the harvest season, with the particular interest of evaluating differences by job category. We found that field workers had greater decline in eGFR during the harvest than non-field workers, suggesting that some aspect of fieldwork is associated with reduced kidney function. In particular, seed cutters, irrigators, and cane cutters were the jobs with the greatest decline in kidney function. The most obvious shared exposure between these

jobs is heat stress, as each job requires strenuous labor in a hot environment; there could, however, be other differences by job that contribute to the observed results. Notably, among field workers, agrichemical applicators experienced the least decline in eGFR, which does not support the hypothesis that agrichemicals are a causal agent of MeN. Longer employment duration at the company was associated with reduced eGFR, after controlling for age. This finding, in conjunction with the observed differences in kidney function decline by job, provides evidence that one or more of the underlying risk factors is related to occupational exposure, potentially heat stress.

*Kidney injury increased during the harvest and was different by job*

A measurable decrease in eGFR often is not evident until substantial renal damage has occurred; in fact, serum creatinine has been described as not sufficiently sensitive to measure early kidney injury (Bonventre et al. 2010; Coca et al. 2008; Devarajan 2008; Vaidya et al. 2008). For these reasons, we measured several novel urinary biomarkers of kidney injury (NGAL, IL-18, NAG), which may be useful both for earlier diagnosis of overt AKI and localization of injury in the kidney (Bonventre et al. 2010; Charlton et al. 2014; Vaidya et al. 2008), and evaluated job-specific changes in these biomarkers during the harvest season. Cane cutters, who perform a job widely acknowledged to be the most strenuous, had consistently greater increases in both NGAL and IL-18 during the harvest than other jobs. These findings strengthen the evidence that occupational exposures are involved in the etiology MeN and suggest that heat stress may play a role. Future studies should directly measure exposure to this agent.

The limited albuminuria in combination with the presence of NGAL, IL-18, and NAG in the urine of these workers, is most consistent with tubular injury. We found associations between increases in NGAL and NAG and decreases in eGFR, raising the possibility that repeated subclinical kidney injury over multiple harvests leads to clinically apparent disease and progressive CKD.

*Increased hydration associated with better kidney function and less kidney injury*

Among cane cutters, increased consumption of electrolyte solution, but not water, was protective against decreased eGFR and was associated with decreased NGAL and NAG (*i.e.* less injury). These findings strengthen the evidence that occupational exposures are involved in the etiology MeN and suggest that repeated exposure to heat and strenuous labor with associated volume depletion and dehydration may play a role.

*Some evidence that exposure to metals may play a role*

In a subset of the sugarcane worker participants and a population of miners, we analyzed urine for total arsenic, cadmium, and uranium, and whole blood for lead. Compared to the general U.S. adult population, metal concentrations were not elevated. They also did not increase during the harvest, suggesting that there is not elevated occupational exposure to metals in this population.

Increased blood lead levels were associated with decreased eGFR, and there was an overall pattern of increased kidney injury with higher concentrations of cadmium, lead, and uranium, but not arsenic. However, concentrations were much lower than

established benchmark doses associated with renal dysfunction, making it unlikely that metals are a primary causal agent of MeN. We did find a potential combined effect of metals, suggesting that low-level environmental exposure to metals, most likely in combination with one or more other agents, may play a role. However, it is also possible that the estimate of combined exposure to multiple metals is acting as a surrogate for contact with a particular media (*e.g.* soil or water), which contains the actual etiologic agent of interest. This finding requires further investigation.

*Study Limitations*

Limitations for each study are outlined in *Chapters 2–4*. There were some limitations that were common across studies, which we will review here. First, job category was used as a surrogate exposure measure. Assessing comparisons between jobs allowed us to explore the role of occupational exposures within the sugarcane industry. This is an improvement upon previous studies, which have used broader surrogates such as community of residence or major industry of employment. Job category, however, is still acting as a surrogate for one or more unmeasured exposures; therefore, we cannot rule out the possibility that non-occupational risk factors that vary by job may be contributing to the differences we found. Of most concern are non-occupational differences that may exist between field workers and non-field workers; however, we also found differences by job even when restricted to field workers, providing evidence that the differences observed between jobs are likely due to occupational factors.

Loss-to-follow-up (LTF) among sugarcane workers has potential impacts on all of

our findings. There were two types of LTF during the harvest: (1) 99 workers who were no longer working at the time of the late-harvest sampling and (2) 499 workers who were not sampled even though they were considered active employees at the time of the late-harvest sampling (Figure 2.1). When considering the first type of LTF, roughly half of the 99 workers left their jobs voluntarily and the other half, mostly cane cutters and seed cutters, were unknown. Because those who left their jobs for unknown reasons were employed in higher risk jobs, it is possible that the reason for leaving was related to kidney function or injury. If so, it is possible that kidney injury would have been greater than what we observed, with more pronounced differences by job task. When considering the second type of LTF, financial and logistical constraints forced us to select a random sample of those workers still active. This selection, therefore, would not be expected to influence our results.

This population was relatively healthy, as all participants were well enough to be currently working. Additionally, sugarcane workers underwent serum creatinine testing prior to the harvest season, reducing the number of workers with CKD. Though we gained valuable information studying kidney injury and function in a population at risk for MeN, we were not studying actual cases of Mesoamerican Nephropathy. We also were unable to follow workers for longer than one harvest, limiting our ability to assess incidence of CKD or determine whether the findings represent irreversible damage.

*Public Health Implications*

Worldwide, chronic diseases have now surpassed communicable diseases as the leading causes of morbidity and premature mortality (Couser et al. 2011; Levey et al. 2007b). These diseases, including CKD, create an enormous cost to the healthcare system. Additionally, CKD increases the risk of other chronic diseases such as cardiovascular disease, which further burden healthcare systems. Developing countries are increasingly and disproportionately bearing the burden of these diseases, as 80% of chronic disease deaths are occurring in low- and middle-income countries (WHO 2005). This burden comes in the form of premature death, effects on quality of life, and severe economic impacts to families, communities, societies, and countries (WHO 2005). Compounding this burden, prevention and treatment are often neglected in developing countries due to both lack of awareness and a focus on infectious diseases (Nugent et al. 2011).

The Mesoamerican Nephropathy is just one of these chronic diseases, but in some regions of Central America, it is a severe public health crisis. Governments and health systems are unequipped to properly handle the epidemic, and the limited access to renal replacement therapy means that the majority of those diagnosed with CKD will die. The personal, social, and economic impact of the epidemic is magnified by the fact that so many of the victims provide the main support for their families.

The research conducted is the most comprehensive investigation to date, in a population at risk for developing MeN. Notably, we found that one or more of the underlying risk factors are related to occupational exposure. This represents as significant

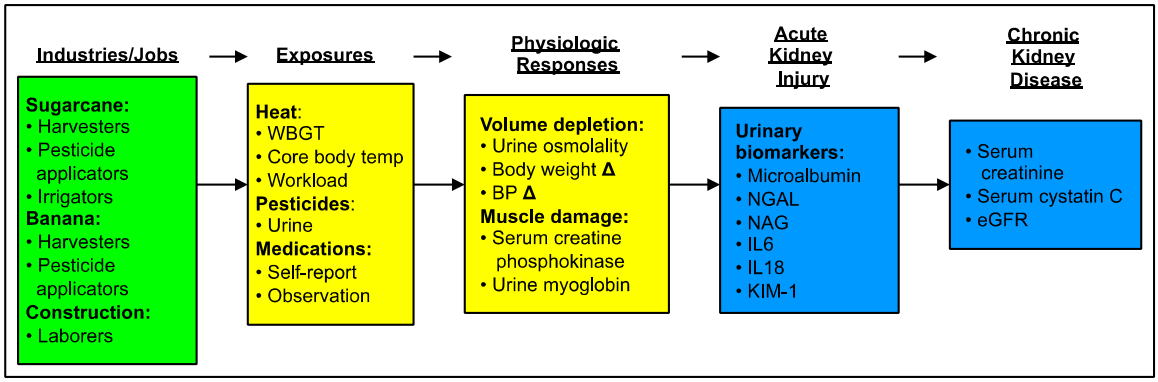
advancement in our knowledge of MeN, as previous studies have been unable to disentangle whether observed findings were due to occupational or non-occupational factors. Our results are consistent with the hypothesis that heat stress and associated volume depletion may be playing a role in MeN, and suggest that there may be some role for low-level environmental exposure to metals. Both of these findings require more research.

Although this research is focused in one geographical region, the potential implications of its results could be far more reaching. Most notably, our findings will inform research in communities in Sri Lanka and India that are being affected by CKD epidemics with strikingly similar characteristics (Jayatilake et al. 2013; Singh et al. 2013). Furthermore, if exposure to heat is in fact an important modifying factor as hypothesized, then we may be observing a previously unrecognized human health effect of climate change. It is plausible that such effects would first be observable in low latitude, tropical countries such as Nicaragua, Sri Lanka, and India that are particularly vulnerable to the effects of local warming due to a low adaptive capacity (Mahlstein et al. 2011). Finally, better understanding this disease process could have relevance to developed countries as well, particularly addressing whether repeated incidents of subclinical kidney injury increase the risk for CKD (Bedford et al. 2012; Venkatachalam et al. 2010; Yang et al. 2011).

*Directions for Future Research*

The findings from this work will help inform the design of future studies conducted in the region. Most directly following this study of sugarcane workers should come a larger, prospective, longitudinal study of workers from multiple industries, followed for a longer period of time to monitor incidence and/or progression. This study should include more detailed observations of workers and quantitative measurements of potential etiologic agents, particularly heat (using ingestible sensors capable of measuring core body temperature), volume depletion, and agrichemicals. The inclusion of manual laborers in other industries would allow for the assessment of commonalities in characteristics and exposures. This study is currently in the planning phase and is slated to begin recruitment within the next year. A detailed overview of the study design is available in Figure 5.1.

**Figure 5.1.** Overview of study design for longitudinal investigation of CKD among Central American workers




Though the research described here has focused on occupational exposures, there is some evidence that adolescents who have never worked in sugarcane may be at risk for MeN (Ramirez-Rubio et al. 2012). Another potentially important area of research, therefore, is a focus on early-life exposures that may damage the kidneys. If this is

occurring, it is possible that some individuals are more susceptible to etiologic occupational exposures. Also important to consider is the potential role of genetic factors. A well-designed study may have the ability to determine if genetics play an important role in who develops MeN, and if so, knowledge of the specific genetic variant may help identify important non-genetic etiologic agents. Studies of adolescents and children, to investigate early-life exposures, and genetics are currently underway or in planning.

Critically, workplace evaluations and well-designed intervention studies in both the workplace and community could have important implications for work practice improvements, reduction of heat stress, improved hydration, and/or greater understanding of pharmaceuticals. If any these interventions are successful in reducing the incidence of Mesoamerican Nephropathy, we could more rapidly identify a key causal agent. Finally, in addition to these research priorities, it is essential to improve surveillance of CKD in the region and strengthen healthcare systems to improve care and treatment for the affected community.

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## CURRICULUM VITAE