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Occupational heat stress and risk factors for kidney injury among outdoor workers in El Salvador and Nicaragua

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OCCUPATIONAL HEAT STRESS AND RISK FACTORS FOR KIDNEY INJURY AMONG OUTDOOR WORKERS IN EL SALVADOR AND NICARAGUA

by

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DEDICATION

Dedicated to the workers who participated in the
MesoAmerican Nephropathy Occupational Study and to
everyone affected by Mesoamerican nephropathy.

OCCUPATIONAL HEAT STRESS AND RISK FACTORS FOR KIDNEY INJURY AMONG OUTDOOR WORKERS IN EL SALVADOR AND NICARAGUA

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ABSTRACT

There is an epidemic of chronic kidney disease of unknown etiology, also referred to as Mesoamerican Nephropathy (MeN), in Central America [1,2]. Researchers studying this epidemic believe the disease etiology likely has an occupational component, with a growing body of evidence to support the hypothesis that heat stress and dehydration play an important role. Previous research has focused extensively on sugarcane workers, but there are limited data describing the heat strain experienced at work for these and other workers. There are no established early indicators of disease onset and the role of other exposures in the disease's etiology are still uncertain. This dissertation aims to address some of these gaps using data from two occupational cohort studies in Central America.

The first is a cohort of Nicaraguan sugarcane workers who were monitored across the 2010-2011 harvest season. The second is the MesoAmerican Nephropathy Occupational Study (MANOS)—a cohort of 569 workers in El Salvador and Nicaragua from the sugarcane, corn, plantain, brick manufacturing, and construction industries.

We found that dipstick leukocyte esterase at the end of the harvest, which was relatively common among cane cutters (33%), seed cutters (22%), and seeders/reseeders

(21%), was associated with a 12.9 ml/min per 1.73 m^2 (95% CI: −18.7 to −7.0) lower mean eGFR and 2.8 times (95% CI: 1.8 to 4.3) higher mean neutrophil gelatinase-associated lipocalin (NGAL). We also found that workers who reported symptoms (e.g., flank pain, fever/chills, and dysuria) had higher mean kidney injury biomarker levels.

Among MANOS participants, we found that sugarcane workers, especially cane cutters and Nicaraguan agrichemical applicators, had the highest estimated work rates, core temperatures (T_c), and heart rates (HR), but workers in other industries occasionally reached high T_c (> 39°C) as well. We found that workers with low eGFR had higher average Tc and HR values and that spending more time on break was associated with lower average HR. We report a higher incidence of cross-shift, serum creatinine-defined kidney injury among sugarcane workers, particularly at one Nicaraguan company, and found evidence that core body temperature and work rate were risk factors for this outcome.

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LIST OF ABBREVIATIONS

ACR	Albumin-to-Creatinine Ratio
AKI	Acute Kidney Injury
BUN	Blood Urea Nitrogen
CI	Confidence Interval
CKD	Chronic Kidney Disease
CKDu	Chronic Kidney Disease of Unknown Etiology
CPK	Creatine Phosphokinase
eGFR	Estimated Glomerular Filtration Rate
GWAS	Genome-Wide Association Study
HR	Heart Rate
IL-18	Interleukin-18
KDIGO	Kidney Disease: Improving Global Outcomes
LOESS	Local Regression
MAD	Median Absolute Deviation
MANOS	MesoAmerican Nephropathy Occupational Study
MeN	Mesoamerican Nephropathy
NAG	N-acetyl-β-D Glucosaminidase
NGAL	Neutrophil Gelatinase-Associated Lipocalin
NHANES	National Health and Nutrition Examination Survey
NIOSH	National Institute for Occupational Safety and Health
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OSHA	U.S. Occupational Safety and Health Administration
REL	Recommended Exposure Limit
SCr	Serum Creatinine

SD	Standard Deviation
T_c	Core Body Temperature
UTI	Urinary Tract Infection
WBGT	Wet Bulb Globe Temperature

CHAPTER ONE: INTRODUCTION

Epidemic of Chronic Kidney Disease of Unknown Etiology

There is an epidemic of chronic kidney disease of unknown etiology (CKDu), also referred to as Mesoamerican Nephropathy (MeN), in Central America [1,2]. This disease affects primarily young males employed in physically demanding, outdoor jobs. While agricultural workers are especially affected [3–6], the disease has also been documented among manual laborers in other industries, including construction and brickmaking [7–9]. The disease is a leading cause of death in El Salvador and Nicaragua, where mortality rates are much higher than nearby countries (Figure 1.1, orange and red lines, respectively). [1,10,11].

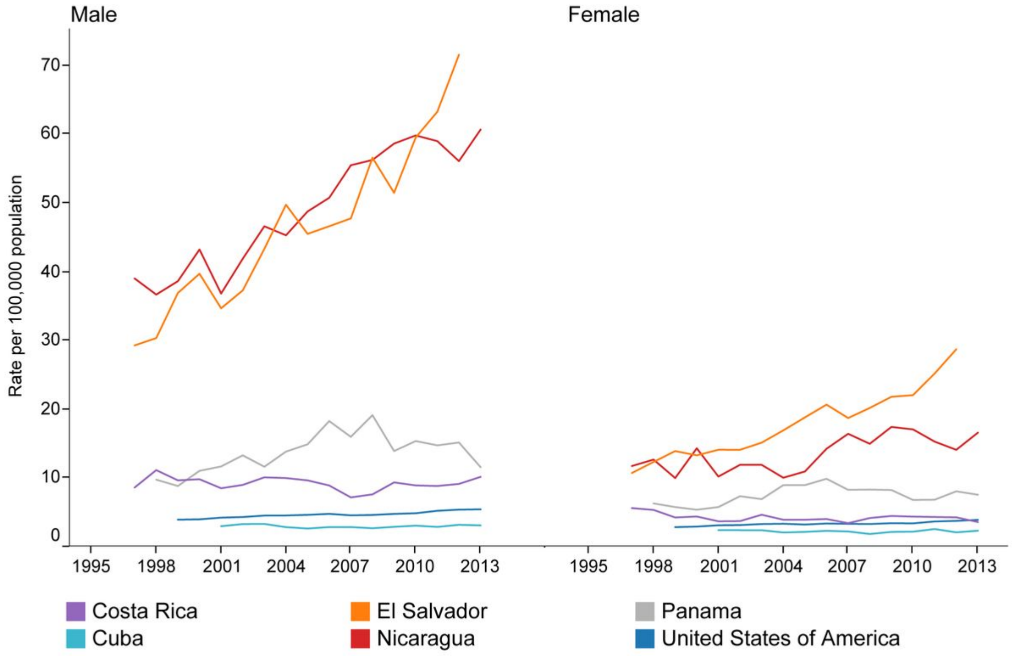

Figure 1.1. Age-standardized mortality rates for chronic kidney disease, by sex, in Central American countries, Cuba, and the U.S. from 1997-2013 (Ordunez et al., 2018)

As indicated in Figure 1.1, the age-standardized mortality rates for kidney disease in both of these countries have been sharply increasing since the late 1990s, with rates increasing almost 250% in El Salvador from 1997 to 2013 and over 150% in Nicaragua in the same time period [10]. Other studies have also found that CKD mortality in these countries has increased over time: a 1.5-fold increase in El Salvador between 2000–2009 and a 2.5-fold and 5-fold increase in Chinandega and León, Nicaragua, respectively, between 1992–2005 [12–14]. However, these data may be biased due to a heightened awareness of the disease in certain countries and regions and among certain workers (e.g., sugarcane workers).

Similar epidemics have been described in Egypt, India, and Sri Lanka [15–17], however, the research community is still uncertain whether these are the same disease with the same pathology or etiology [18]. While no such epidemic has been documented in the U.S., anecdotal reports by physicians in areas with large populations of Central American immigrants have heightened awareness of the possibility [19]. A case report of CKDu in a Salvadoran immigrant in California has been published and there is documentation of cross-shift kidney injury in agricultural populations in the U.S. [19–21]. Epidemiologists and nephrologists have been investigating MeN since it was first described in the peer-reviewed literature in 2002 [22], however, no cause has yet been confirmed. There are numerous hypothesized causes, including chronic heat stress, chronic volume depletion, rhabdomyolysis, nephrotoxic medications, heavy metals, agrichemicals, and infectious agents.

MeN is not associated with traditional causes of CKD, such as diabetes [4–6,8,23]. Patients with early stage MeN also do not exhibit high levels of proteinuria [4–6,23–26], nor active urine sediment [27], but do have increased levels of tubular injury biomarkers [28,29], suggesting that the disease is primarily the result of tubulointerstitial damage. Renal biopsies have revealed interstitial fibrosis and tubular atrophy, with evidence of secondary glomerular damage, further suggesting that this is a tubulointerstitial disease [28,30]. Hyperuricemia, dysuria, and sterile pyuria have also been commonly found [2,27,31–34], but more research is needed to understand if these are related to the disease etiology (e.g., hyperuricemia may indicate low-grade rhabdomyolysis, a risk factor for kidney injury that can result from strenuous exercise in hot, humid conditions [35–39]).

Hypothesized Causes and Evidence To-Date

Participants at the 2015 workshop on MeN, which convened experts on the epidemic, declared that the disease etiology likely has an occupational component, with a growing body of evidence to support the hypothesis that heat stress and dehydration are important risk factors. The conference report also recommended that the roles of other nephrotoxicants (e.g., medications and heavy metals) and genetic susceptibility be explored further, as the evidence is still limited [40]. Since many of the hypothesized causes of MeN are known or suspected to cause acute kidney injury (AKI), it is possible that two or more of these exposures are acting in an additive or synergistic manner, and most researchers believe that the etiology of MeN is likely multifactorial [40]. However, epidemics are usually due to a change in a single exposure, so the hypothesis of a multifactorial etiology should not be the only hypothesis taken into consideration [41].

Agrochemicals

Prevalence of the disease appears to be highest among agricultural workers [5,6,23,42,43], which has prompted hypotheses that agrichemical exposures (particularly glyphosate [44–46]) are involved in MeN, supported by evidence of pesticide nephrotoxicity at high doses in animal models and case studies [47–57], as well as in epidemiologic studies in agricultural workers in the United States [58]. While some studies have found self-reported pesticide use to be associated with CKDu [6,23,25,33,42,46,59], a literature review by Valcke et al. found mixed evidence for the agrichemical hypothesis, concluding that many of the studies were limited by cross-sectional design, uncontrolled confounding, and recall bias [60]. A 2018 meta-analysis of epidemiologic studies on MeN additionally found no significant association between CKDu and pesticide use (yes versus no) [61].

Metals

Another hypothesized cause is heavy metals, as many heavy metals are known to be nephrotoxic [49] and there are reports of heavy metal contamination in drinking water and soil in some of the regions with high rates of MeN. One early biomonitoring study among Nicaraguan sugarcane workers, however, found low levels of urinary metal concentrations relative to National Health and Nutrition Examination Survey (NHANES) levels [8]. A 2019 study conducted in Nicaragua by Smpokou et al. found no differences in urinary metal concentrations between sugarcane workers with declining and stable kidney function [62]. Recent evidence from Guatemala, on the other hand, suggests that sugarcane workers who consume well water and municipal water have higher exposure to lead and arsenic (and glyphosate) compared to workers who consume chlorinated water provided by their employer, and employer-provided drinking water appeared to be protective against increases in serum creatinine across the work shift [63].

Studies measuring urinary metals concentrations in this population, however, are potentially subject to differential exposure misclassification, where impaired filtration through the kidneys results in lower urinary metals concentrations. Spot urine samples taken at a single time point may also be inadequate for characterizing chronic exposures to heavy metals [64]. Other differences between workers, specifically which workers drink chlorinated or otherwise filtered water, also need to be considered to avoid confounding.

Heat Stress

One of the leading hypotheses focuses on the roles of hyperthermia and extracellular fluid volume depletion resulting from physically demanding work in hot, humid environments [40,65–68]. This hypothesis is supported by epidemiological evidence of worse kidney function among workers in more physically demanding industries (e.g., sugarcane) and job tasks (e.g. cane cutting, seed cutting) [8,24,69–71] and in warmer, low-altitude regions [5,72], as well as evidence that workplace temperatures in this region are consistently above health-protective guidelines [33,73–78]. The apparent increase in MeN mortality over the past few decades [11–14] may also be explained in part by climate change-related temperature increases in the region [79–81]. Intervention studies that provide sugarcane workers with longer breaks and increased access to shade and water have found reductions in cross-shift and cross-harvest changes in serum creatinine [82,83]. This finding has been confirmed in a controlled, experimental setting [84].

Researchers suspect that hyperthermia and chronic volume depletion are leading to repeated clinical or subclinical AKI events through a number of hypothesized mechanisms [85], including vasopressin-induced fibrosis and inflammation [86], uric acid crystalluria [87], activation of the polyol-fructokinase pathway [88], and reduced excretion of nephrotoxicants [65]. The hypothesis further suggests that repeated AKI events eventually lead to CKD, potentially via subclinical damage that persists despite clinical indications of recovery [89,90]. However, there is limited research so far on the relationship between heat-related AKI and development of MeN, even though AKI is a risk factor for CKD in non-CKDu populations [91]. More work is also needed to understand the direction of effect between AKI and CKDu—whether AKI increases risk of kidney disease or whether there is undetected kidney disease that is increasing individuals' risk of AKI.

Medications

Nephrotoxic medications (e.g., non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics) are commonly used in these populations, often to manage pain or treat urinary tract infections, which are commonly diagnosed by physicians in the region [27,32,34]. In addition to the potential for direct nephrotoxicity, certain medications can also impair thermoregulation [92] or volume regulation (e.g., diuretics).

Fructose-Sweetened Beverages

In vivo evidence from toxicological studies conducted in rats have suggested that rehydrating with fructose-sweetened beverages following exposure to heat stress and dehydration may further exacerbate kidney damage, likely through increases in vasopressin and activation of the polyol-fructokinase pathway [85,88,93]. This has been validated in controlled experiments with humans [94,95].

Gene-Environment Interaction

There is evidence suggesting that genetic susceptibility may be a factor in the development of MeN, including the absence of CKD epidemics among agricultural populations in other tropical regions, differing outcomes among workers with similar exposure profiles and job tasks, and evidence of familial clustering of the disease [31,61,96]. A genome-wide association study (GWAS) conducted in Sri Lanka identified a statistically significant locus on chromosome 10 that was significantly associated with CKDu, but this has yet to be replicated in another population [97]. Further research into genetic risk variants associated with MeN is important for identifying environmental contributors to disease incidence and progression, both through an understanding of the role of these variants in altered cellular function and through improving our understanding of which individuals may be at greatest risk for the effects of environmental exposures [98].

Limitations of Prior Research and Gaps in the Literature

One limitation of many prior studies is the use of self-reported exposures or exposure surrogates, such as industry, job title, or geography. These proxies can introduce bias that masks true associations or creates misleading associations. Additionally, many of the studies to date have been cross-sectional in design, limiting the ability to draw causal conclusions. Other studies, while not cross-sectional, have largely been limited in the length of follow-up, ranging from one day [33,70] to one harvest season [24,29,71,82,83,99], which has hindered the ability to understand pre-clinical decline in kidney function, short- and long-term fluctuations in serum creatinine, and factors affecting disease progression.

For instance, while a leading hypothesis is that repeated AKI events are causing CKD, it has been largely untested in this population, with a couple exceptions. A 2018 study followed Nicaraguan sugarcane workers with AKI for 12 months to observe incidence of CKD and found that ~50% of those workers developed CKD, while others showed improved kidney function [69]. This study did not have a reference population (e.g. workers without AKI) and had a small sample size (n=29). A 2019 community-based cohort study of 263 Nicaraguan men found that neutrophil gelatinase-associated lipocalin (a kidney injury biomarker) and serum creatinine at 6 and 12 months after baseline were predictive of whether individuals experienced rapid eGFR decline over two years [100]. Additionally, much attention has been given to sugarcane workers in Central America, but workers in other industries (e.g., brickmaking, construction) appear to be at risk of MeN as well given their working conditions. More research is needed to understand similarities and differences in the exposure profiles of these workers, specifically heat strain (e.g., elevated core body temperature and heart rate, dehydration), metabolic heat loads, medication use, and hydration practices.

Finally, we know that ambient heat and metabolic heat are the largest contributors to heat strain, but there are still outstanding questions about the importance of certain mediators, such as medication use, consumption of fructose-sweetened beverages, and genetic predisposition, on the relationship between heat stress and heat strain (e.g., heart rate, core body temperature), as well as on the relationship between heat strain and kidney injury, among workers at risk of MeN. Recent intervention studies in this region have demonstrated the role of water, rest, and shade programs in preventing kidney function decline at a population-level [82,83]. More research to confirm these findings in individuals at risk of MeN and elucidate the specific mechanism of effect will further inform best practices for preventing MeN.

Dissertation Objectives and Specific Aims

This research attempts to address some of these gaps in the MeN literature using data from two occupational cohorts in Central America. The first is a cohort of Nicaraguan sugarcane workers who were monitored across the 2010-2011 harvest season. Data were collected on serum creatinine, as well as urine dipstick parameters, self-reported urinary tract diagnoses, symptoms, and medication use. These data have been previously described in the literature[8,24,29]. The second cohort is the MesoAmerican Nephropathy Occupational Study (MANOS)—a cohort of 569 workers in El Salvador and Nicaragua. MANOS participants represent multiple industries with high rates of reported MeN—sugarcane, corn, plantain, brick manufacturing, and construction. As part of the MANOS study, the research team conducted an extensive exposure assessment over the course of three days at baseline in early 2018, which included core body temperature and heart rate monitoring throughout a work shift, biomarkers of heavy metal and glyphosate exposure, and measures of dehydration and muscle damage following a work shift. Questionnaire data were also collected, which included information on medication use, hydration practices, diet, work history, and family history of CKD. These participants were followed every 6 months afterwards for 6 rounds to examine trends in kidney health and to update occupational and behavioral information.

Chapter 2 presents self-reported symptoms and urine dipstick results among the cohort of Nicaraguan sugarcane workers from 2010-2011, comparing rates among different job tasks with different exposure profiles. We examine whether symptoms and urine dipstick results predict measures of kidney injury and function at the end of the harvest. We also report findings of urine culture analyses and patterns of participant medication use and medication recommendations from physicians.

Chapter 3 describes heat stress, heat strain, physical activity, and other work shift characteristics among MANOS participants during the three workdays observed at baseline. We examine differences between job tasks and industries of employment. We also seek to answer whether medication use, kidney dysfunction, hydration practices, break duration, and/or genetic risk (via family history of kidney disease) are important predictors of heat strain among these workers.

Chapter 4 presents cross-shift findings of acute kidney injury, dehydration, and muscle damage among MANOS participants at baseline. We assess the relationships between heat strain and muscle damage and measures of kidney injury, with an additional focus on whether hydration practices, break duration, family history of kidney disease, and medication use affected risk of kidney injury.

Finally, *Chapter 5* describes the limitations and implications of this research and places these findings in the broader context of the MeN epidemic and global worker safety and health. This chapter also includes a discussion of future directions for research in this area.

CHAPTER TWO: KIDNEY FUNCTION, SELF-REPORTED SYMPTOMS, AND URINE FINDINGS IN NICARAGUAN SUGARCANE WORKERS

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Abstract

Background: An epidemic of chronic kidney disease in Central America predominantly affects males working in certain industries including sugarcane. Urinary tract infections are commonly diagnosed among men in Nicaragua, who often receive antibiotics and nonsteroidal anti-inflammatory drugs for urinary symptoms.

Methods: We followed 251 male Nicaraguan sugarcane workers in seven job tasks over one harvest and measured urine dipstick parameters, kidney injury biomarkers, and estimated glomerular filtration rate (eGFR). We administered a questionnaire about urinary symptoms, health-related behaviors, and medication history. We cultured urine in a subset of workers.

Results: The study population was composed of factory workers (22.7%), cane cutters (20.3%), irrigators (19.5%), drivers (16.3%), agrichemical applicators (11.6%), seeeders/reseeders (6.0%), and seed cutters (3.6%). The mean age was 33.9 years and mean employment duration was 10.1 years. Cane cutters reported higher proportions of urinary-related symptoms compared with agrichemical applicators, irrigators, and seeders/reseeders. Seed cutters were more likely to take antibiotics (22.2%), while drivers and seeders/reseeders were more likely to take pain medications (26.8% and 26.7%, respectively). Proteinuria was uncommon, while dipstick leukocyte esterase was relatively common, especially among cane cutters, seed cutters, and seeders/reseeders (33.3%, 22.2%, and 21.4% at late-harvest, respectively). Dipstick leukocyte esterase at late-harvest was associated with a 12.9 mL/min/1.73m^2 (95% CI: -18.7, -7.0) lower mean eGFR and 2.8 times (95% CI: 1.8, 4.3) higher mean neutrophil gelatinase-associated lipocalin (NGAL). In general, workers who reported urinary-related symptoms had higher mean kidney injury biomarker levels at late harvest. None of the workers had positive urine cultures, including those reporting urinary symptoms and/or with positive leukocyte esterase results. Amoxicillin, ibuprofen, and acetaminophen were the most commonly used medications.

Conclusions: Job task is associated with urinary symptoms and dipstick leukocyte esterase. Urinary tract infection is misdiagnosed based on leukocyte esterase, which may be an important predictor of kidney outcomes.

Introduction

An epidemic of chronic kidney disease (CKD) of uncertain etiology, often referred to as either CKDu or Mesoamerican nephropathy (MeN), has been affecting Central America for more than two decades. Traditional risk factors for CKD, such as hypertension and diabetes, are not implicated in CKDu in this region [4,5,23,101,102]. This disease most often affects younger men employed in certain industries, including agriculture, mining, and brickmaking [4–7,9]. Nicaragua has one of the highest CKD mortality rates in the world, particularly among younger men [10]. Possible causes include known and potential nephrotoxins, such as heavy metals, agrichemicals, infectious agents, nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, and other etiologies of intrinsic kidney injury such as sequelae of volume-related acute tubular necrosis.

CKDu patients rarely exhibit high levels of proteinuria, but do appear to have elevated levels of tubular injury biomarkers, suggesting early tubulointerstitial damage [4–6,23,25–27,29,103], and kidney biopsies show interstitial fibrosis and tubular atrophy[28,30,104,105]. Hyperuricemia, dysuria, and sterile pyuria have also been reported in these populations [2,27,31]. Interviews with physicians and pharmacists in two agricultural regions of Nicaragua indicate that patients frequently report symptoms consistent with urinary tract infections (UTIs) (e.g. dysuria, back pain) [32]. In a sample of 61 Nicaraguan sugarcane workers, review of their medical records revealed that 69% received one or more UTI diagnoses, which were accompanied by urinalyses showing 75% had evidence of white blood cells, 35% had leukocyte casts, and 53% had crystalluria [106]. A cross-sectional study among Costa Rican sugarcane workers found that harvesters were more likely than non-harvesters to experience dysuria once per week (28.3% vs 3.2%) [107] and a retrospective cohort study of Nicaraguan sugarcane workers reported that the odds of dysuria was 2.5-fold in cane cutters compared with other field jobs (95% CI 1.6-4.0) [108]. These findings suggest a relationship between urinary symptoms and job task. Dysuria is so common in Nicaragua that a colloquial term, *chistata*, has emerged to describe a set of symptoms including painful urination, burning during urination, and back pain[32]. In El Salvador, the same common symptoms are referred to as *mal de orina*. Physicians in Nicaragua acknowledge that they frequently prescribe NSAIDs and antibiotics for patients with these symptoms, and many report diagnosing a UTI in men based on urine dipstick results alone [32], using urine cultures infrequently because of clinical protocols and limited resources. Positive urine dipstick tests, which test for presence of leukocyte esterase and nitrites, may suggest infection, but are not specific. As UTIs are relatively uncommon in men compared to women, the routine diagnosis of UTIs in this predominantly male population at risk for CKDu is surprising and requires further investigation. The use of NSAIDs to treat dysuria may be a common practice throughout Central America, as indicated by a study in El Salvador that found that NSAID use (41.3%) and dysuria (39.1%) were common among a population largely working in agriculture [27].

It has been hypothesized that the frequent use of nephrotoxic medications for these UTI-like symptoms may lead to or exacerbate kidney damage [1]. It has also been suggested that kidney injury in these populations could be the result of chronic volume depletion due to occupational conditions, which could result in crystal-related symptoms due to high concentration of urinary urates [85,87]. Diuretics are commonly prescribed to treat *chistata* [32], which could further increase volume depletion, thereby resulting in higher concentrations of urinary urates and other substances during the volume challenge experienced during heavy manual labor in excessive heat. Kidney damage could also result from the combination of these two pathways, along with other nephrotoxic exposures (e.g., agrichemicals, heavy metals).

The objectives of this study, conducted among male Nicaraguan sugarcane workers, were to: (1) assess differences in self-reported symptoms and urine dipstick results across job categories, (2) determine the associations among self-reported symptoms, urine dipstick results, late-harvest kidney function, and kidney injury biomarkers, (3) describe patterns of medication use, and (4) determine whether workers with UTI-like symptoms and positive urine leukocyte esterase results have positive urine cultures.

Methods

Study Participants

The study population and data collection methods have been described previously [24,29]. Briefly, during October-December 2010 at the beginning of the harvest season ("pre-harvest"), sugarcane workers in Nicaragua were recruited and data were collected after a screening and hiring process conducted by the employer. Job applicants with serum creatinine $\geqq$1.4 mg/dl were not hired and therefore were not included in the study population. A second round of data collection occurred near the end of the harvest season ("late-harvest") during March-May 2011 among a subset of workers selected by convenience. Of the workers who participated in both rounds of data collection (n=506), a random sample (n=284) of workers was selected for analyses after excluding workers with more than one job and workers in certain job categories (e.g., maintenance workers, machine operators). This sample was primarily male (88.4%). The 33 women were not equally distributed amongst the job categories—2 factory workers, 10 seed cutters, and 21 seeders/reseeders—and did not include any cane cutters, the work category previously identified to be associated with the greatest risk of CKDu. Thus, the analyses in this study were restricted to men only (n=251) (see Supplemental Material for data on female participants). All participants were at least 18 years old.

The final population represented workers from seven different job categories: drivers, factory workers, cane cutters, irrigators, agrichemical applicators, seed cutters, and seeders/re-seeders. Factory workers are employees of the sugarcane company who perform a variety of jobs, including operators, mechanics, and technicians. They typically work 12-hour shifts. Cane cutters are contracted laborers who use machetes to cut the sugarcane at the base and stack the stalks into a pile for collection. Cane cutters typically cut 4-6 tons of sugarcane per day over a 6-hour shift and are paid based on the amount of sugarcane cut each day. Irrigators are employees of the sugarcane company who manually divert water from main water conductors into the furrows of individual fields using sticks and plastic or removal/addition of soil. They typically handle 1,000 gallons/field/day over 9-hour shifts. Agrichemical applicators are also contracted and apply agrichemicals (primarily herbicides) to the ground and base of the sugarcane using backpack pumps (the majority of which require manual force to control the pressure). The most commonly used herbicide mixture is 2,4-dichlorophenoxyacetic acid, terbutryn, and ametryn. Applicator shift durations vary between 5-12 hours per day. Seed cutters are contracted laborers who use machetes to cut the sugarcane into 20–22 inch pieces and tie them together into packages (typically preparing 80-100 packages per day). They are paid by the package and work up to eight hours per day. Seeders/re-seeders are contracted laborers who distribute the cut sugarcane stalks evenly in 204-meter furrows and cover them with soil using shovels or hoes. They typically plant 2–4 furrows a day over a 5–6 hour shift and are paid by the furrow.

Data Collection

At pre-harvest, workers provided blood and urine samples and were administered questionnaires by trained interviewers about their work history and demographics. Similar data were collected at late-harvest, with additional questions regarding symptoms experienced, hydration practices, and alcohol consumption. Symptoms included abdominal, back, and flank pain; burning or pain during urination; periods of frequent urination; fever or chills; and *chistata*. Workers also reported whether they sought medical treatment during the harvest season and whether they were diagnosed with a UTI during the harvest season. Separate questions asked about general use of pain medication and antibiotics (i.e., yes/no in previous 3 months) versus specific treatments recommended by health professionals and used by workers (discussed in further detail under “Medications Recommended and Used”).

Serum creatinine was measured at the *Centro Nacional de Diagnóstico y Referencia* (Managua, Nicaragua) using a kinetic-rate Jaffe method (IDMS-traceable). Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation[109]. Urine creatinine, albumin, neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), and N-acetyl-β-D glucosaminidase (NAG) were analyzed at the Division of Nephrology and Hypertension at the Cincinnati Children's Hospital Medical Center (Cincinnati, Ohio). Urine creatinine was measured using immunoturbidimetry. Albumin was measured using a colorimetric modification of the Jaffe reaction. Albumin-to-creatinine ratio (ACR) was calculated by dividing urine albumin by urine creatinine. NGAL and IL-18 were measured with commercially-available, enzyme-linked immunosorbent assay kits (Bioporto, Gentofte, Denmark; MBL, Intl., Woburn, MA, USA). NAG was measured with a colorimetric assay (Roche Diagnostics, Basel, Switzerland). 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. Urine dipstick analyses were performed using a Combur 10UX® dipstick (Roche Diagnostics) and Urisys 1100 strip reader (Roche Diagnostics)[24,29].

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

Urine Cultures

During late-harvest, urine samples were collected from 70 workers for urine cultures, which were initiated within 3 hours of specimen collection at *Universidad Nacional Autónoma de Nicaragua-León*. This subset of workers was selected based on combinations of urinary symptoms in the past 24 hours and/or a positive leukocyte esterase test result from a dipstick analysis (see Supplemental Material for selection criteria and demographic information). Positive cultures were defined as either having a growth of at least 100,000 CFU/ml or having a growth of at least 20,000 CFU/ml in the presence of positive urine dipstick for nitrates or leukocytes.

Statistical Analyses

Summary statistics were calculated for all participants (n=251). For normally distributed variables, the mean and standard deviation were calculated. For log-normally distributed variables, the geometric mean and geometric standard deviation were calculated. Histograms were used to assess the normality of continuous outcome variables. All urine dipstick results were dichotomized into any positive (e.g., 1+, 2+, 3+) versus negative to avoid small counts.

To examine differences in self-reported symptoms and urine dipstick parameters between job tasks, chi-square tests for crude analyses and logistic regression models to adjust for age and employment duration were used. Factory workers were used as the reference population. For rare outcomes, Firth's bias correction was used to avoid quasi-complete separation [110]. Additional analyses were conducted examining the differences between field workers and non-field workers by combining drivers and factory workers into a "non-field workers" category and combining cane cutters, irrigators, agrichemical applicators, seed cutters, and seeders/re-seeders into a “field workers” category.

To examine crude associations among self-reported symptoms, urine dipstick results, and kidney outcomes [kidney function (eGFR) and kidney injury biomarkers (NGAL, NAG, IL-18, ACR)], mean differences were calculated and two-sample t-tests (using equality of variances F tests to determine whether equal variances could be assumed) were applied. Adjusted mean differences, controlling for age, employment duration, and job category, were calculated using multivariable linear regression models. For all models with injury biomarkers as the dependent variable, we also adjusted for urine creatinine concentration (except for ACR, which is urine creatinine normalized). The injury biomarkers were natural log-transformed to account for non-normal distributions, therefore the parameter estimates reported are relative mean differences derived from exponentiation of the parameter estimates. For models predicting late-harvest eGFR, we truncated all eGFR values > 120 mL/min/1.73m^2 at 120 mL/min/1.73m^2 given the imprecision of GFR estimates at these high levels.

Medications Recommended and Used

For workers who reported receiving treatment recommendations or prescriptions from medical care providers, we asked them to list these prescriptions and/or treatments, as well as the specific antibiotics and pain medications they actually took in the three months prior to the late-harvest data collection. As these answers contain large percentages of missing values or instances when workers could not remember the name of a medication, results provided for these questions are descriptive only. Questions pertaining to general use of antibiotics and pain medication (i.e. yes/no in prior 3 months), as well as seeking treatment and being diagnosed with a UTI, were not subject to the same levels of missingness and were therefore included in our statistical analyses.

All analyses were conducted using SAS Statistical Software (SAS Version 9.4, Cary, NC).

Results

Study Cohort Characteristics

Our study population was primarily composed of factory workers (22.7%), cane cutters (20.3%), irrigators (19.5%), drivers (16.3%), and agrichemical applicators (11.6%). There were relatively few seed cutters (3.6%) and seeeders/reseeders (6.0%) after excluding female workers from these analyses. The mean age was 33.9 ± 10.5 years (Table 2.1). There were differences in mean age by job category, with drivers being the oldest, followed by factory workers and agrichemical applicators. Drivers, factory workers, agrichemical applicators, irrigators, and seeders/reseeders worked for the sugarcane company longer than cane cutters and seed cutters—in many cases more than two or three times as long.

Differences in Symptoms, Health-Related Behaviors, and Urine Dipstick by Job Category

Cane cutters, compared with all other jobs, reported higher proportions of the urinary symptoms, except *chistata* (Table 2.2). Agrichemical applicators generally reported the lowest proportions of these symptoms. These findings generally remained consistent after adjusting for age and employment duration (Table 2.3).

Seed cutters were more likely to report using antibiotics in the three months prior to the late-harvest data collection, while drivers and seeders/reseeders were more likely to report taking pain medications (Table 2.2). Irrigators were the least likely to seek medical treatment during this period.

Leukocyte esterase and proteinuria at pre-harvest were relatively uncommon (Table 2.2). Urinary nitrites were very uncommon (n=1) (not shown). Leukocyte esterase at late-harvest was more common among cane cutters, seed cutters, and seeders/reseeders (33.3%, 22.2%, and 21.4%, respectively) compared to factory workers (1.8%).

When comparing self-reported symptoms, dipstick parameters, health behaviors, and diagnoses, minimal differences were found between field workers and non-field workers (not shown). Odds of symptoms and dipstick parameters were slightly higher among field workers. Late-harvest leukocyte esterase results were the only substantial difference between the two groups, with field workers being more likely to have positive leukocyte esterase results (OR: 8.34, 95% CI: 1.88, 37.04).

Associations with Late Harvest Kidney Injury and Kidney Function

In unadjusted analyses, late-harvest eGFR was 5.3 mL/min/1.73 m^2 (95% CI: -12.4, 1.8) lower for workers who reported taking pain medications for at least three days in the prior three months, 10.6 mL/min/1.73 m^2 (95% CI: -23.0, 1.9) lower for workers with hematuria at late-harvest, and 3.1 mL/min/1.73m^2 (95% CI: -8.3, 2.1) lower for workers reporting abdominal, back, or flank pain (Table 2.4). Late-harvest eGFR was 14.1 mL/min/1.73 m^2 (95% CI: -37.5, 9.3) lower for workers with proteinuria at late-harvest; however, these findings are based on few events (n=8). Large differences in eGFR were also found for workers with positive leukocyte esterase at late-harvest (mean difference: -13.3 mL/min/1.73 m^2, 95% CI: -25.0, -1.6). After adjusting for age, employment duration, and job category, these differences in mean eGFR were largely attenuated, except for leukocyte esterase and hematuria at late-harvest, which were still strongly associated with a lower eGFR (mean differences: -12.9 mL/min/1.73 m^2 and -8.5 mL/min/1.73 m^2; 95% CI: -18.7, -7.0 and -15.0, -2.0, respectively).

In unadjusted analyses, workers who reported having experienced fever or chills (prior 3 months) had >50% greater mean NGAL and NAG values at late-harvest compared to those not experiencing fever or chills (Table 2.4). These were both slightly attenuated after adjustment. Proteinuria at pre-harvest and late-harvest were associated with large relative increases in all four injury biomarkers, in both unadjusted and adjusted analyses (Table 2.4), however, these are based on relatively few events (n=6 for pre-harvest, n=8 for late-harvest). Positive leukocyte esterase at late-harvest, but not pre-harvest, was associated with higher NGAL levels (adjusted relative mean: 2.8, 95% CI: 1.8, 4.3) and lower IL-18 levels (adjusted relative mean: 0.5, 95% CI: 0.3, 0.7). There is some suggestion that self-reported use of antibiotics and pain medications are associated with higher levels of NGAL, NAG, and IL-18, even after adjustment for potential confounders (Table 2.4). Workers who reported experiencing *chistata* and periods of frequent urination had higher relative mean NGAL and IL-18 levels at late-harvest, respectively, even after adjustment. Workers reporting periods of frequent urination or abdominal, back, or flank pain had higher mean ACR at late-harvest.

Medications Recommended and Used

Of the workers who reported seeking and receiving treatment recommendations from medical care providers (n=38), over a third (36.8%) reported that the recommended treatment was an analgesic. Fewer reported that their medical care provider recommended an antibiotic (13.1%). Four workers said their care provider recommended a change in diet and hydration. Eleven workers (28.9%) said they did not remember the name of the treatment. Among the workers who reported taking an antibiotic in the three months prior to late-harvest (n=31), the most commonly reported antibiotic taken was amoxicillin (n=5; 16.1%) followed by azithromycin (n=3; 9.7%). Cephalexin and tetracycline were reported by one worker each. Five workers did not remember the name of the antibiotic they took, and fifteen (48.4%) did not answer the question. Not all antibiotics were taken for UTI symptoms. A few workers reported that antibiotics were taken for other ailments (e.g., foot infection, influenza, sore throat.) Of workers who reported taking a pain medication for at least three days in the three months prior to the late-harvest (n=34), 23.5% reported taking ibuprofen and 11.8% reported taking acetaminophen. One worker did not remember the name of the pain medication and twenty (58.8%) did not answer the question.

Urine Cultures

Of the 70 workers who gave urine samples for culturing (demographics provided in Table 2.S1), 27.1% reported urinary symptoms in the prior 24 hours (n=19) and 31.4% had a positive leukocyte esterase test result from the dipstick analyses (n=22). A small portion of these were both positive for leukocyte esterase and reported symptoms in the last 24 hours (n=7). Approximately half of the subset (48.6%) were neither positive for leukocyte esterase nor reported experiencing recent symptoms (n=34) and therefore represented a negative control. Finally, 5 participants (7.1%) had missing symptom data for the prior 24 hours—3 were positive for leukocyte esterase and 2 were negative. Of these 70 workers, none had positive urine culture results.

Discussion

In a cohort of male Nicaraguan sugarcane workers followed across one harvest season, we identified differences in self-reported urinary symptoms, urine dipstick results, and health-related behaviors by job category. Cane cutters reported higher proportions of urinary-related symptoms, while agrichemical applicators and irrigators generally reported lower proportions. Leukocyte esterase was relatively common, particularly at late-harvest and among cane cutters, seeders/reseeders, and seed cutters (range: 21.4%-33.3% at late-harvest). Late-harvest leukocyte esterase was associated with lower eGFR and higher NGAL. Late-harvest hematuria was associated with lower eGFR and higher NAG and ACR, which could suggest that crystalluria is present and may be contributing to symptoms. In general, workers who reported experiencing urinary-related symptoms had higher mean kidney injury biomarker levels at late-harvest.

This study logically follows our prior study—which demonstrated an association between certain job tasks and higher levels of kidney injury biomarkers and lower late-harvest eGFR—by correlating these jobs with a higher symptom burden and more frequent findings of positive leukocyte esterase on urine dipstick. This is important because the disease is currently believed to be asymptomatic at early stages, with no established early indicators of disease. Chistata and other urinary symptoms may be related to crystalluria caused by dehydration and not UTIs. We found urinary nitrites to be uncommon (0.4%) and all 70 urine cultures to be negative, indicating that dipstick leukocyte esterase in our current study was not a marker of a urinary tract infection in men, which suggests a different source of kidney pathology than infection.

This finding appears to be supported by other research: all twelve urine cultures conducted in a study by Fischer et al. were negative, and all CKD patients were negative for nitrites [111]. Sterile pyuria can be attributed to a number of different causes, including sexually-transmitted infections, viral or fungal infections, parasitic diseases, and interstitial nephritis from medications or other causes of cystitis [112,113]. In this population, reported use of pain medications and antibiotics were weakly associated with leukocyte esterase, in both crude and adjusted analyses. While analgesics were commonly recommended to workers who sought medical treatment, relatively few male workers sought treatment in the first place (15.6%). It is worth noting that during the duration of this study participants were able to access the employer-provided healthcare clinic at no cost, as it is funded by the country's social security fund. Ibuprofen, an NSAID, was the most common pain medication reported amongst workers who used pain medications, but this represents very few workers in total. Similarly, few workers reported taking an antibiotic, and among those who did, there was a wide variety of antibiotics used. These findings seem to indicate that NSAIDs and antibiotics may not be the cause of sterile pyuria in this population, but better data on medication history are needed for a more robust analysis of this hypothesis. Given the limited biopsy series in MeN/CKDu, we suspect that NSAID and antibiotic use are not causing interstitial nephritis, but rather are being used to treat symptoms that are associated with increased MeN/CKDu risk.

There are aspects of our study that limit the interpretation and generalizability of our findings. Regarding data collection, the data are potentially subject to both non-differential and differential misclassification, as well as differential loss-to-follow-up, as workers who were not available for late-harvest data collection may have differed from the entire study population at pre-harvest. Workers had trouble recalling specific names of medications they were prescribed or took during the study period, which limited data analysis. The study design was limited to changes across a single harvest season, limiting ability to draw conclusions about longer-term kidney function decline. The categorization of some of the symptoms, including *chistata*, is somewhat subjective and therefore, may differ between workers and introduce misclassification. The sample sizes in a few of the job categories were relatively small and may have reduced our power to detect differences. Lastly, it is possible that the criteria we used for assessing positive urine cultures was too strict [114,115].

The prediction of late-harvest eGFR and tubular injury biomarkers using late-harvest urine dipstick parameters (late harvest dipstick leukocyte esterase, protein, and blood) should be interpreted with caution, as these are cross-sectional analyses, which prevents us from determining whether proteinuria and dipstick leukocyte esterase are precursors or sequelae of kidney damage. The results presented in Table 2.4 should generally be considered as exploratory and require confirmation. However, our findings regarding the prevalence of symptoms, leukocyte esterase, and proteinuria are generally supported by other studies [4,5,7,23,27,101,107]. We also suspect that there may be some uncontrolled confounding in our models related to occupational exposures or willingness to seek medical care.

In conclusion, we found that leukocyte esterase and *chistata* were common among workers involved strenuous field work, like cane cutting, generally correlating with workers who were found to have increased tubular injury biomarkers and larger cross-harvest eGFR declines in previous studies. We found no evidence of UTIs in male workers, despite the urinary symptoms and positive leukocyte esterase results. These findings support the hypothesis that MeN/CKDu in this region is related to occupation, perhaps through recurrent exposure to heat stress and volume depletion, and that UTIs are likely not involved in the disease etiology. Our urine culture findings also indicate that urine dipstick analyses and symptom reporting alone are not adequate for diagnosing UTIs among males in this population. These findings were communicated with the participants' employer and the physicians at the employer healthcare clinic. Patient education on hydration and healthy urine color is now provided.

Table 2.1. Participant characteristics, late-harvest eGFR, and late-harvest kidney injury biomarker results by job category (n=251)

		Non-Field Workers		Field Workers				
	Overall	Drivers	Factory Workers	Cane Cutters	Irrigators	Agrichemical Applicators	Seed Cutters	Seeders/ Reseeders
Total, N (%)	251	41 (16.3%)	57 (22.7%)	51 (20.3%)	49 (19.5%)	29 (11.6%)	9 (3.6%)	15 (6.0%)
Age, Years								
Mean	33.9	40.9	36.3	30.5	30.3	34.7	27.1	31.7
Median	31	41.0	36	28.0	28.0	35.0	27	29
SD	10.5	11.2	9.9	10.6	8.8	8.2	3.7	10.0
Range	18-63.5	24.0-60.0	20-57	18.0-63.5	19.0-48.0	21.0-51.0	23-34	19-56
Employment duration, Years								
Mean	10.1	14.4	14.2	3.6	9.7	12	2	7.3
Median	7	13.0	13	1.0	7.0	12.0	1	5
SD	9.2	8.9	10.7	5.3	8.0	8.0	2.3	6.2
Range	0-40	1.0-35.0	0.08-40	0-27.0	0-32.0	0-30.0	0-6	2-22
eGFR (mL/min/1.73m^2)								
Mean	112.4	110.2	114.8	108.2	115.5	114.4	112.3	110.38
SD	19.0	14.2	16.2	26.3	16.9	13.7	20.9	24.1
Range	28.9-180.9	78.3-139.3	76.2-180.9	28.9-150.2	45.7-142.7	81.8-138.8	63.3-128.6	56.8-143.7
NAG (U/g)								
GM	0.90	0.68	0.60	1.5	.90	0.93	0.63	1.6
GSD	2.8	2.3	2.8	2.1	3.5	2.3	3.5	3.0
NGAL (µg/g)								
GM	10.4	7.5	6.8	19.3	14.7	6.9	6.2	15.3
GSD	3.4	2.2	4.3	2.9	3.3	3.2	1.7	2.9
IL-18 (ng/g)								
GM	8.8	9.9	6.4	12.6	7.5	8.5	5.4	14.8
GSD	3.1	2.9	3.1	3.5	2.8	3.0	2.7	3.1
ACR (mg/g)								
GM	2.4	2.0	1.6	2.0	4.2	2.7	1.0	6.6
GSD	3.6	4.4	3.1	2.8	3.6	3.2	2.9	3.3

Note: Urine analytes are normalized to the urine creatinine concentration. SD = standard deviation; eGFR = estimated glomerular filtration rate; NAG = N-acetyl-β-D glucosaminidase; GM = geometric mean; GSD = geometric standard deviation; NGAL = neutrophil gelatinase-associated lipocalin; IL-18 = interleukin-18; ACR = albumin-to-creatinine ratio

Table 2.2. Self-reported urinary symptoms and health-related behaviors in the previous 3 months by job category

	Non-Field Workers		**Field Workers**				
	Factory workers (ref)	**Drivers**	**Cane cutters**	**Irrigators**	**Agri-chemical applicators**	**Seed cutters**	**Seeders/ reseeders**
Total, N	57	41	51	49	29	9	15
Symptoms, Prior 3 Months [a]							
% Abdominal, Back, Flank Pain	28.1%	29.3%	38.6%	20.4%	17.2%	0.0%	20.0%
% Burning/Pain during Urination	12.3%	7.3%	22.7%	8.2%	3.5%	22.2%	6.7%
% Frequent Urination	8.8%	9.8%	18.2%	6.1%	0.0%	0.0%	6.7%
% Fever or Chills	10.5%	7.3%	31.8%	4.1%	0.0%	11.1%	13.3%
% Chistata [b]	28.1%	26.8%	54.6%	26.5%	24.1%	66.7%	26.7%
Health-Related Behaviors and Diagnoses, Prior 3 Months [a]							
% Taken Antibiotics	19.3%	12.2%	9.1%	10.2%	6.9%	22.2%	13.3%
% Taken Pain Medication ≥ 3 days	10.5%	26.8%	15.9%	8.2%	3.5%	11.1%	26.7%
% Sought Medical Treatment	17.5%	12.2%	20.5%	6.1%	13.8%	22.2%	33.3%
% Diagnosed with a UTI	5.3%	2.5%	2.3%	2.0%	3.5%	0.0%	6.7%
Dipstick Parameters, Pre-harvest							
% Positive Leukocyte Esterase	5.4%	2.5%	9.8%	2.1%	3.5%	0.0%	0.0%
% Positive Proteinuria	1.8%	0.0%	5.9%	4.3%	0.0%	0.0%	0.0%
Dipstick Parameters, Late-harvest							
% Positive Leukocyte Esterase	1.8%	2.5%	33.3%	8.2%	3.5%	22.2%	21.4%
% Positive Proteinuria	3.6%	2.5%	2.0%	6.1%	0.0%	0.0%	7.1%
% Positive Blood	8.9%	5.0%	6.0%	10.4%	6.9%	11.1%	21.4%

a – Workers reported symptoms, health-related behaviors, and recent UTI diagnoses during late-harvest data collection

b – Chistata is a colloquial term that describes a set of symptoms including painful urination, burning during urination, and back pain.

Table 2.3. Adjusted association of symptoms and clinical findings with job task

	Non-Field Workers			Field Workers			
	Factory Workers	Drivers	Cane Cutters	Irrigators	Agrichemical Applicators	Seed Cutters	Seeders/ Reseeders
	Odds Ratios (95% Confidence Intervals)						
Symptoms, Prior 3 Months [a]							
Abdominal, Black, Flank Pain	***Reference***	1.22 (0.49, 3.02)	2.28 (0.89, 5.82)	0.69 (0.28, 1.73)	0.60 (0.20, 1.82)	0.18 (0.01, 3.94)	0.84 (0.21, 3.36)
Burning or Pain During Urination	***Reference***	0.67 (0.16, 2.81)	2.68 (0.79, 9.07)	0.60 (0.16, 2.27)	0.27 (0.03, 2.28)	2.46 (0.37, 16.3)	0.55 (0.06, 5.08)
Periods of Frequent Urination	***Reference***	1.23 (0.33, 4.62)	2.62 (0.72, 9.61)	0.72 (0.17, 3.00)	0.17 (0.01, 3.12)	0.57 (0.02, 13.8)	1.07 (0.15, 7.67)
Any Fever or Chills	***Reference***	0.75 (0.19, 2.99)	4.69 * (1.42, 15.5)	0.44 (0.10, 2.05)	0.14 (0.01, 2.60)	1.73 (0.21, 14.4)	1.68 (0.32, 8.78)
Chistata	***Reference***	1.13 (0.45, 2.84)	3.24 * (1.29, 8.17)	0.79 (0.33, 1.91)	0.80 (0.29, 2.27)	4.67 (0.98, 22.3)	0.88 (0.24, 3.28)
Health-Related Behaviors and Diagnoses, Prior 3 Months [a]							
Taken Antibiotics	***Reference***	0.68 (0.21, 2.18)	0.54 (0.14, 2.02)	0.46 (0.14, 1.48)	0.32 (0.07, 1.58)	1.49 (0.25, 9.10)	0.72 (0.14, 3.83)
Taken Pain Medications > 3 Days	***Reference***	2.93 (0.96, 8.96)	1.96 (0.54, 7.16)	0.87 (0.23, 3.38)	0.32 (0.04, 2.81)	1.43 (0.14, 14.7)	3.63 (0.83, 15.9)
Sought Medical Treatment	***Reference***	0.58 (0.18, 1.90)	1.56 (0.50, 4.87)	0.37 (0.09, 1.48)	0.81 (0.23, 2.89)	2.01 (0.33, 12.4)	2.93 (0.77, 11.1)
Diagnosed with a UTI	***Reference***	0.40 (0.06, 2.95)	0.41 (0.05, 3.39)	0.57 (0.08, 4.03)	0.81 (0.11, 5.75)	0.87 (0.03, 23.4)	1.41 (0.17, 11.9)
Dipstick Parameters, Pre-harvest							
Leukocyte Esterase	***Reference***	0.70 (0.10, 4.82)	2.85 (0.56, 14.7)	0.53 (0.07, 3.80)	0.88 (0.12, 6.33)	1.24 (0.04, 34.7)	0.63 (0.03, 14.0)
Proteinuria	***Reference***	0.43 (0.02, 9.68)	4.32 (0.48, 39.1)	2.69 (0.34, 21.4)	0.70 (0.03, 17.0)	3.41 (0.09, 126)	1.71 (0.06, 46.2)

Dipstick Parameters, Late-harvest							
Leukocyte Esterase	***Reference***	1.45 (0.15, 14.0)	15.4 * (2.55, 92.4)	3.25 (0.50, 21.3)	1.83 (0.19, 18.1)	9.59 (0.97, 95.1)	9.86 * (1.28, 76.0)
Proteinuria	***Reference***	1.00 (0.12, 8.28)	2.38 (0.23, 24.4)	2.82 (0.48, 16.8)	0.53 (0.02, 11.8)	4.73 (0.14, 164)	5.78 (0.56, 59.6)
Blood	***Reference***	0.44 (0.08, 2.49)	0.75 (0.14, 3.91)	1.49 (0.38, 5.76)	0.81 (0.15, 4.50)	1.80 (0.16, 19.9)	3.16 (0.60, 16.6)

Odds ratios (95% CI) for self-reported symptoms and dipstick parameters (pre-harvest and late-harvest) compared to reference group (factory workers), adjusting for age and employment duration (n=244 for symptoms and behaviors/treatments, n=247 for dipstick except blood, n=246 for blood at late-harvest, n=243 for UTI diagnoses).

* = $p < 0.05$

a - Workers reported symptoms, health-related behaviors, and recent UTI diagnoses during late-harvest data collection

Table 2.4. Associations between symptoms and positive urine dipstick results and kidney function and kidney injury

	Events (N)	eGFR		NGAL[b]		NAG[b]		IL-18[b]		ACR	
		Mean difference (95% CI)					Relative Means (95% CI)				
		Crude	Adjusted[a]	Crude	Adjusted[a]	Crude	Adjusted[a]	Crude	Adjusted[a]	Crude	Adjusted[a]
Symptoms, Prior 3 Months [c, d]											
Abdominal, Back, Flank Pain	63	-3.1 (-8.3, 2.1)	-2.3 (-6.5, 1.9)	1.2 (0.9, 1.7)	1.2 (0.8, 1.6)	1.3 (0.9, 1.7)	1.2 (0.9, 1.6)	1.0 (0.7, 1.3)	0.9 (0.7, 1.2)	1.3 (0.9, 1.8)	1.3 (0.9, 1.8)
Burning or Pain During Urination	28	3.0 (-1.6, 7.6)	3.7 (-2.1, 9.4)	1.5 (1, 2.3)	1.4 (0.9,2.1)	1.1 (0.7, 1.7)	1.0 (0.7, 1.6)	1.3 (0.9, 1.9)	1.2 (0.8, 1.8)	1.0 (0.6, 1.7)	1.1 (0.7, 1.8)
Periods of Frequent Urination	21	-1.4 (-8.5, 5.7)	0.2 (-6.3, 6.7)	1.3 (0.8, 2.1)	1.1 (0.7, 1.8)	0.8 (0.5,1.3)	0.7 (0.5,1.2)	1.4 (0.9, 2.2)	1.3 (0.8, 2)	1.3 (0.7, 2.3)	1.3 (0.8, 2.3)
Any Fever or Chills	28	-2.1 (-8.3, 4.1)	1.2 (-4.8, 7.1)	1.5 (1, 2.4)	1.2 (0.8, 1.9)	1.8 * (1.2, 2.8)	1.5 (1, 2.2)	1.2 (0.8, 1.8)	1.0 (0.7, 1.5)	1.0 (0.6, 1.6)	1.0 (0.6, 1.7)
Chistata	81	1.7 (-2.6, 5.9)	1.4 (-2.5, 5.4)	1.4 * (1, 1.8)	1.3 (1, 1.7)	1.2 (0.9, 1.6)	1.1 (0.9, 1.5)	1.2 (0.9, 1.5)	1.1 (0.9, 1.5)	0.8 (0.6, 1.2)	0.9 (0.6, 1.2)
Health-Related Behaviors and Diagnoses, Prior 3 Months [c, d]											
Taken Antibiotics	31	-0.4 (-6.4, 5.6)	-1.3 (-6.8, 4.1)	1.1 (0.7, 1.7)	1.2 (0.8, 1.9)	1.3 (0.9, 2)	1.5 * (1, 2.2)	1.1 (0.8, 1.6)	1.2 (0.8, 1.7)	1.1 (0.7, 1.8)	1.2 (0.7, 1.8)
Taken Pain Medications > 3 Days	34	-5.3 (-12.4, 1.8)	-2.8 (-8.2, 2.5)	1.4 (0.9, 2)	1.3 (0.9, 1.9)	1.5 * (1, 2.3)	1.4 (1, 2.1)	1.5 * (1.1, 2.2)	1.4 (1, 2)	0.7 (0.5, 1.2)	0.7 (0.5, 1.1)
Dipstick Parameters, Pre-harvest [e]											
Leukocyte Esterase	11	-2.7 (-12.2, 6.9)	-1.8 (-10.6, 7.0)	1.6 (0.8, 3.3)	1.5 (0.8, 2.9)	1.5 (0.8, 2.9)	1.3 (0.7, 2.5)	1.3 (0.7, 2.5)	1.3 (0.7, 2.3)	1.0 (0.5, 2.2)	1.1 (0.5, 2.3)
Proteinuria	6	-0.9 (-13.7, 11.9)	2.8 (-8.9, 14.5)	6.7 * (2.7, 16.2)	4.9 * (2.1, 11.6)	3.8 * (1.6, 9.1)	3.0 * (1.3, 6.7)	3.0 * (1.3, 6.6)	3.1 * (1.4, 6.9)	5.7 * (2.1, 15.7)	5.5 * (2.1, 14.4)

Dipstick Parameters, Late-harvest $^{e, f}$											
Leukocyte Esterase	29	-13.3 * (-25.0, -1.6)	-12.9 * (-18.7, -7.0)	3.4 * (2.2, 5.1)	2.8 * (1.8, 4.3)	1.5 (1, 2.2)	1.0 (0.7, 1.6)	0.6 * (0.4, 0.9)	0.5 * (0.3, 0.7)	1.0 (0.6, 1.6)	0.9 (0.6, 1.5)
Proteinuria	8	-14.1 (-37.5, 9.3)	-10.9 * (-21.1, -0.7)	3.7 * (1.7, 8.1)	3.0 * (1.4, 6.6)	2.5 * (1.2, 5.5)	2.1 (1, 4.3)	2.5 * (1.3, 5.1)	3.1 * (1.5, 6.3)	12.4 * (5.3, 28.9)	9.4 * (4.2, 21.4)
Blood	21	-10.6 (-23.0, 1.9)	-8.5 * (-15.0, -2.0)	0.9 (0.5, 1.4)	0.8 (0.5, 1.3)	1.6 * (1, 2.7)	1.5 (0.9, 2.3)	1.0 (0.6, 1.5)	1.0 (0.7, 1.6)	1.7 (1, 3)	1.6 (0.9, 2.7)

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

* = p-value < 0.05

a – Adjusted for age, employment duration, and job category

b – All crude and adjusted models predicting NGAL, NAG, and IL-18 were additionally adjusted for urinary creatinine

c – Reference = workers not reporting symptoms or health-related behaviors

d – Workers reported symptoms and health-related behaviors during late-harvest data collection

e – Reference = workers with negative dipstick results

f – Cross-sectional analyses

Supplemental Material

Table of Contents

1. Selection Criteria for Urine Culture Analyses

Workers were selected for urine culture analyses based on the following criteria:

- All workers who answered ‘yes’ to experiencing any of the urinary symptoms in the prior 24 hours, excluding ‘fever and chills’
- All workers who tested positive (1+, 2+, or 3+) for leukocyte esterase on the urine dipstick tests

But with the following additional restrictions:

- Culture a maximum of 5 workers who were negative for leukocyte esterase but positive for symptoms per day
- Culture a maximum of 25 workers who were negative for leukocyte esterase but positive for symptoms per week
- Do not culture any workers who have taken antibiotics within the prior week

Finally, the original protocol excluded workers with both negative leukocyte esterase results and reporting no symptoms, but the final analyses included 34 of these workers.

The demographic characteristics of the subset of workers receiving a urine culture are summarized in Table 2.S1. Workers included in the urine culture subset had slightly shorter employment durations, on average. Drivers, irrigators, seed cutters, and seeders/reseeders were slightly underrepresented while cane cutters were substantially overrepresented.

2. Analyses of Female Workers (n=33)

We calculated summary statistics for the women in the cohort (n=33) (Tables 2.S2 and 2.S3). We found that the injury biomarker averages were much higher for women compared to their male counterparts, with the exception of ACR and NAG among seeders/reseeders (Table 2.S2). Female seeders/reseeders had higher proportions of urine leukocyte esterase at pre-zafra. Both female seeders/reseeders and seed cutters had higher proportions of urine leukocyte esterase at late zafra than their male counterparts (Table 2.S3). We also found differences in the symptoms reported by sex: female seed cutters more often reported back/flank pain, frequent urination, and fever/chills, and chistata than male seed cutters (Table 2.S3). Female seeders/reseeders were more likely to report fever or chills. Occupations with a greater proportion of women (seeders/reseeders and seed cutters) had higher proportions of health-related behaviors. Females were largely driving this difference for seed cutters, reporting consistently higher proportions of the health-related behaviors (Table 2.S3).

Table 2.S1. Participant characteristics for the urine culture subset compared to all participants

	Full Cohort	Urine Culture Subset
Total, N	251	70
Age (Years)		
Mean	33.9	34.3
Median	31	32.0
SD	10.5	11.1
Range	18-63.5	19.8-60.0
Employment Duration (Years)		
Mean	10.1	8.7
Median	7	4.5
SD	9.2	9.7
Range	0-40.0	0-40.0
eGFR (mL/min/1.73m^2)		
Mean	112.4	106.6
SD	19.0	24.1
Range	28.9-180.9	28.9-139.3
Job Task		
Drivers	41 (16.3%)	9 (12.9%)
Factory Workers	57 (22.7%)	14 (20.0%)
Cane Cutters	51 (20.3%)	28 (40.0%)
Irrigators	49 (19.5%)	7 (10.0%)
Agrichemical Applicators	29 (11.6%)	10 (14.3%)
Seed Cutters	9 (3.6%)	1 (1.43%)
Seeders/Reseeders	15 (6.0%)	1 (1.43%)

Table 2.S2. Participant characteristics and late-zafra biomarker results by job category among females (n=33)

	Overall	Non-Field Workers	Field Workers	
		Factory Workers	Seed Cutters	Seeders/ Reseeders
Total, N	33	2	10	21
Age (Years)				
Mean	31.1	42.0	33.3	29.1
Median	29.0	42.0	33.5	27.0
SD	9.5	7.0	11.8	7.7
Range	19.0-56.0	37.0-47.0	19.0-56.0	19.0-51.0
Employment Duration (Years)				
Mean	4.6	14.5	5.3	3.3
Median	3.0	14.5	3.5	3.0
SD	4.5	2.1	5.4	2.6
Range	0-20.0	13.0-16.0	1.0-20.0	0-10.0
eGFR (mL/min/1.73m^2)				
Mean	118.3	113.1	111.7	122.0
SD	21.8	10.0	26.4	20.1
Range	50.0-147.7	106.1-120.2	52.2-134.9	50.0-147.7
NAG (U/g)				
GM	1.3	0.8	1.4	1.3
GSD	3.6	1.3	2.9	4.2
NGAL (µg/g)				
GM	25.0	28.4	25.1	24.7
GSD	3.8	2.5	2.8	4.6
IL-18 (ng/g)				
GM	25.1	51.4	15.1	29.8
GSD	3.0	2.0	3.1	2.9
ACR (mg/g)				
GM	4.7	2.4	3.7	5.6
GSD	3.6	2.4	3.2	3.9

Table 2.S3. Self-reported urinary symptoms and health-related behaviors in the previous 3 months by job category among females (n=33)

	Factory workers (ref)	**Seed cutters**	**Seeders/ reseeders**
Total, N	2	10	21
Symptoms, Prior 3 Months			
% Abdominal, Back, Flank Pain	100%	50%	28.6%
% Burning/Pain during Urination	0%	10%	9.5%
% Frequent Urination	50%	20%	9.5%
% Fever or Chills	50%	40%	19.1%
% Chistata	50%	40%	23.8%
Dipstick Parameters, Pre-zafra			
% Positive Leukocyte Esterase	0%	4%	33.3%
% Positive Proteinuria	0%	0%	0%
Dipstick Parameters, Late zafra[a]			
% Positive Leukocyte Esterase	0%	55.6%	57.1%
% Positive Proteinuria	0%	0%	4.8%
% Positive Blood	0%	11.1%	14.3%
Health-Related Behaviors and Diagnoses, Prior 3 Months			
% Taken Antibiotics	0%	80%	19.1%
% Taken Pain Medication ≥ 3 days	100%	40%	14.3%
% Sought Medical Treatment	100%	40%	19.1%
% Diagnosed with a UTI	50%	10%	19.1%

[a] n=9 for seed cutter

CHAPTER THREE: ASSESSMENT OF HEAT STRESS AND HEAT STRAIN AMONG OUTDOOR WORKERS IN EL SALVADOR AND NICARAGUA

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Abstract

There is growing attention being placed on occupational heat stress in Central America, as workers in this region are being disproportionately affected by a unique form of chronic kidney disease first described in the early 2000s, with workers in the sugarcane industry gaining much of the focus. Workers in other outdoor jobs, however, such construction and brickmaking, also experience this disease and growing evidence supports the hypothesis that occupational heat stress plays a role in the disease's etiology. We used data from the MesoAmerican Nephropathy Occupational Study (MANOS), a cohort of 569 workers, who underwent extensive workplace exposure monitoring, which included continuous measurement of core body temperature (T_c), heart rate (HR), physical activity, and wet bulb globe temperature (WBGT), over the course of three days in January 2018 - May 2018. Participants represented five industries, three agricultural and two non-agricultural, in El Salvador and Nicaragua: sugarcane, corn, plantain, brickmaking, and construction. The primary aims were to compare measures of heat stress and heat strain, as well as shift characteristics such as break duration, shift duration, and self-reported hydration practices, between outdoor workers in various job tasks and industries in El Salvador and Nicaragua. We also examined whether break duration, hydration practices, and kidney function were associated with measures of heat strain—elevated T_c and HR. Sugarcane workers, especially cane cutters and Nicaraguan agrichemical applicators, had the highest estimated work rates. Median WBGTs were high (> 26°C) at all sites, but particularly so among workers whose shift spanned the afternoon hours (e.g., 29.2°C among plantain workers). Workers in most industries spent

little time on break (<10% of the shift), as determined from accelerometer data. Overall, sugarcane workers, particularly those in Nicaragua, experienced higher T_c and HR values than other workers. However, we also found evidence that workers in other industries occasionally reach high core temperatures (> 39°C) as well. Finally, we found that workers with impaired kidney function (estimated glomerular filtration rate <90 mL/min/1.73 m^2) have higher average T_c and HR values and that spending more time on break was associated with lower average HR.

Introduction

Occupational heat stress is a growing concern for outdoor workers in physically demanding jobs under climate change projections that predict increasingly dangerous ambient conditions [116,117]. Physical exertion in high heat can lead to heat-related illnesses (such as heat stroke, heat exhaustion, and heat rash), death, decline in cognitive function, and increased risk of being injured on the job [118–122]. Yet there has been relatively little research on characterizing heat stress for different jobs in different regions and the health effects of chronic occupational heat stress [123–125].

There is growing attention being placed on occupational heat stress in Central America, as workers in this region experience alarming rates of a unique form of chronic kidney disease first described in the early 2000s, especially among agricultural workers, with workers in the sugarcane industry gaining much of the focus [3–6,22,42,43]. However, workers in other outdoor jobs, such construction and brickmaking, also experience this disease [3,8,9] and growing evidence supports the hypothesis that chronic exposure to occupational heat stress plays a role in the disease's etiology [40,126].

Occupational heat stress describes the combined heat load that a worker experiences from metabolic heat (i.e., the body's own heat production as a byproduct of maintaining normal bodily functions and performing physical work), environmental conditions (e.g., ambient temperature and humidity), and the clothing or protective equipment worn on the job that may impair the body's ability to lose heat to the environment. Occupational heat stress is often characterized using wet bulb globe thermometers, which measure wet bulb globe temperatures (WBGT), an index value combining ambient dry temperature, humidity, and solar radiation. To protect workers from heat strain, WBGT values are typically combined with information about workers' estimated metabolic heat load and clothing, are then compared against established thresholds meant to protect workers from exceeding dangerous core body temperatures (T_c) [124].

Heat strain is the physiological response resulting from exposure to this heat load, and when measured, can determine if exposure to heat stress should be reduced or discontinued to prevent adverse health effects [124,127]. Measuring heat strain can be more expensive, invasive, and labor-intensive, as it requires use of personal monitoring devices to measure T_c, heart rate (HR), and/or change in hydration status/body weight [124]. Relatively new technologies, however, have made measurements of continuous T_c in field settings more feasible.

To date, personal monitoring of heat strain among outdoor workers in Central America has been limited and many studies have relied on a combination of WBGT and work productivity as the primary measures of heat stress. However, these studies have consistently demonstrated exceedances of health-protective heat stress thresholds while observing workers at their worksite [71,128]. For example, a 2018 study among sugarcane workers in El Salvador at different locations and time periods during the 2015 harvest consistently recorded maximum WBGTs above 28°C, the U.S. Occupational Safety and Health Administration (OSHA) recommended threshold for workers performing continuous work at a moderate workload (which is likely under-protective for sugarcane workers) [83,129]. A 2019 study among sugarcane workers in Guatemala during the 2017 harvest recorded maximum WBGTs above 35°C and mean WBGTs above 30°C [70]. Historical data from Nicaragua demonstrates that heat index values (a combination of ambient temperature and relative humidity) at one sugarcane company met OSHA's high or very high risk criteria on at least 19.6% of harvest days between 2000-2014 [79]. A 2015 study among Salvadoran sugarcane cutters found workers spent the majority of a typical workday above 50% of their estimated maximum heart rate while being exposed to WBGTs above 26°C for 79% of the day (maximum WBGT: 32.1°C) [77]. To our knowledge, no studies have measured core body temperature or focused more broadly on characterizing heat strain among workers at risk of MeN.

A small handful of studies have monitored core body temperature among agricultural workers in the United States and Mexico using ingestible core temperature sensors [125]. They have found that core body temperatures occasionally or regularly exceed 38°C during the shift (depending on the population) and regularly increase ≥ 1°C over the course of a work-shift [130–133]. However, differences in job activities, workplace protections [134], hydration practices, co-morbidities, medication use, age, body size, and climate make these findings difficult to extrapolate to other working populations, like those developing kidney disease in Central America.

This chapter attempts to address some of these gaps in the literature by presenting an analysis of heat stress and heat strain among workers in a variety of industries participating in the MesoAmerican Nephropathy Occupational Study (MANOS). MANOS is a longitudinal, occupational cohort study in El Salvador and Nicaragua designed to assess occupational risk factors for kidney injury and kidney disease among workers in five industries—sugarcane (five different companies), corn, plantain, brickmaking, and construction (Chapter 1). Our primary goal was to characterize heat stress (WBGT and estimated work rate) and the physiological effects of heat stress (elevations in HR and T_c) among these workers during a subset of work shifts and assess differences by country, industry, company (sugar only), and job task. We also sought to understand the potential protective effects of increased hydration and longer breaks on reducing heat strain among these workers, and explore whether baseline kidney function, pain medication use, and genetics (assessed via self-report of family history of CKD) were important factors in predicting these outcomes.

Methods

Study Population

The study design for the MANOS cohort has been previously described (Chapter 1). In brief, MANOS participants (n=569) underwent extensive workplace exposure monitoring at baseline, including continuous core body temperature, heart rate, physical activity, and wet bulb globe temperature over the course of three days (mostly consecutive) in January 2018 - May 2018. Participants represented five industries, three agricultural and two non-agricultural, in El Salvador and Nicaragua: sugarcane, corn, plantain, brickmaking, and construction. These have been abbreviated in tables as the following: CORN (corn), PLAN (plantain), BRICK (brickmaking), CONS (construction), and SUGAR (sugarcane). As we recruited workers from two companies in El Salvador and three in Nicaragua, we used the following codes to indicate each: SUGAR-ES1, SUGAR-ES2, SUGAR-NI1, SUGAR-NI2, and SUGAR-NI3. The inclusion criteria for participation were: 1) males aged 18-45 and 2) employment in current occupation for at least one year and/or harvest season. The exclusion criteria were: 1) prior physician diagnosis of CKD, diabetes, hepatitis B, hepatitis C, HIV, or polycystic kidney disease, 2) medical treatment for hypertension or last reported blood pressure > 160/95, and 3) any diseases or medical procedures and devices that would prevent the use of an ingestible temperature sensor.

The MANOS study protocol was approved by the Boston University Medical Campus Institutional Review Board, the Salvadoran National Ethics Committee for Health Research (Comité Nacional de Ética de las Investigaciones en Salud), and the National Ethics Committee (Comité Institutional de Revisión Etica) and the Office of Teaching and Research (Dirección General de Docencia e Investigaciones), both of the Nicaraguan Ministry of Health.

Environmental Monitoring

Wet bulb globe temperature was measured every minute during all three work shifts using TSI QUESTemp 46 Waterless Wet Bulb Globe Thermometers (TSI Incorporated, Shoreview, MN). Wind speed was also measured, using a TSI air velocity sensor attachment (TSI, Shoreview, MN). Thermometers were mounted on tripods one meter above the ground as close as possible to the participants. If participants moved locations during the work shift, the thermometers were moved to maintain proximity.

Personal Monitoring

Core body temperature (Tc) during the work shift was assessed using wireless ingestible CorTemp® Disposable Temperature Sensors (HQ Inc., Palmetto, FL). Participants were randomly divided into two groups at baseline to determine the timing and frequency of Tc monitoring—half of participants were monitored during work shifts on Days 1 and 3, and half on Day 2. The CorTemp Data Recorder was worn in a pouch strapped to the small of participants' backs and recorded Tc readings every 10 seconds.

Physical activity was characterized using an ActiGraph wGT3X BT (ActiGraph, LLC, Pensacola, FL) accelerometer, which captures measured movement at 30Hz or higher, worn on a belt around the participants' hips during the work shift on all three days. Polar H7 heart rate (HR) monitors (Polar Electro Oy, Kempele, Finland), attached to a strap around the chest below the pectoral muscle, were worn during the work shift on all three days at baseline. Data were collected at a beat-to-beat resolution and transmitted via Bluetooth to the ActiGraph wGT3X BT devices.

Tympanic temperature was measured before and after each work shift using a Braun PRO 6000 tympanic thermometer (Welch Allyn, Skaneateles Falls, NY). Height and weight were measured with a Seca 769 column scale (Seca GmbH, Hamburg, Germany)—before and after each shift for weight, while only once for height. Weight was averaged across all six measurements to determine the participant's average weight at baseline for Recommended Exposure Limit (REL) calculations and estimated energy expenditure calculations. Differences between pre- and post-shift weight measurements were not used to assess water loss via sweating as the protocol used at each measurement (e.g., clothing and equipment worn) varied drastically enough to impair their quality and invalidate their use for that purpose.

Biological Samples

Blood samples were collected before and after the shift on the third day only, except for several brick workers (n=29) for whom blood was collected on the first or second day due to unpredictable work schedules. Serum samples from Nicaragua were analyzed at the National Laboratory in Nicaragua and samples from El Salvador were shipped to Boston University to be sent to Quest Diagnostics. Serum creatinine (IDMS-traceable) was analyzed at each laboratory and used to estimate glomerular filtration rate (eGFR) using the CKD-EPI equation [109]. Subsequent serum testing of a random subset of baseline samples (n=50 for each country), conducted at Quest Diagnostics in 2021, confirmed minimal-to-no effect from the use of two separate laboratories. Pre-shift eGFR < 60 mL/min/1.73 m^2 was used as a dichotomous variable characterizing whether participants had impaired kidney function during baseline exposure monitoring.

Q*uestionnaires*

Questionnaires were administered to participants by trained field team members. A baseline questionnaire was administered upon enrollment to capture basic demographic information, work history, medical history, diet, hydration, alcohol and smoking habits, and family history of CKD. A questionnaire was also administered at the end of the work shift on each day to capture characteristics of the workday (start and stop time, breaks, comparisons to the previous day), hydration practices that day, medications taken, personal protective equipment worn, and symptoms experienced.

Statistical Analyses

WBGT, Tc, HR, and physical activity data were all cropped for each participant based on when their work shift began and ended. Implausible values for each device (e.g., <30 for HR, < 32°C for Tc) were marked as missing and are therefore not captured in the reported summary statistics. WBGT, Tc, HR, or physical activity data with more than 50% of the work shift missing were excluded from relevant analyses.

All MANOS participants were outdoor workers, so the WBGT formula for outdoor settings was used. The first 10 minutes of each WBGT dataset was removed, prior to cropping at the work shift, to account for the stabilization period defined by the manufacturer [135]. For some participants, two wet bulb globe thermometers were used simultaneously because extra devices were available. In these cases, the values from each device were averaged at each time point. Effective WBGT ($WBGT_{eff}$) was calculated by adding a clothing adjustment factor of 0.5°C to the WBGT for agrichemical applicators in Nicaragua, who were the only participants who wore polypropylene and plastic coveralls [124,127]. Data were smoothed using a 20-minute rolling average. The REL—the recommended heat stress exposure threshold defined by the National Institute of Occupational Safety and Health (NIOSH)—was calculated for each participant using their average body weight and estimated average work rate (kcalories/hour) derived from physical activity data (described below). Time above REL and percent of the work shift above REL were calculated for each participant on each day. Heat index was derived from dry temperature and humidity using the National Weather Service formula through the *weathermetrics* package in R [136,137].

For accurate Tc readings, CorTemp® sensors need to have passed through the digestive system to the small intestine, which requires swallowing the sensor several hours before monitoring and ideally eating/drinking something with the sensor. If the sensor is too high in the digestive tract, the Tc data can be influenced by the consumption of liquids and foods. The swings in Tc this can produce have been referred to as the "bouncing ball" effect [138]. Despite study protocol stating that sensors should be swallowed the night before the monitoring workday, it was often difficult to put this into practice. Workers and investigators were concerned about bowel movements prior to the work shift and protocol compliance with unsupervised consumption of the sensors. The number of hours before the work shift that the sensor was swallowed varied widely, and there are distinct patterns by country, industry, and work site due to logistics. For this reason, the number of hours before the shift the sensor was swallowed was estimated and examined in sensitivity analyses. In addition to the sensor being higher in the digestive tract than desired, other issues can cause nonsensical Tc data. For instance, workers standing close to one another may cause interference in the transmission of Tc data to the correct data recorder and workers who unintentionally have two sensors in their body at once may produce unusable data. For these reasons, the Tc data for each participant were examined to identify files that appeared to be affected by these interferences and therefore were determined to be unusable (i.e., cases where most of the data was deemed unrealistic based on absolute Tc values as well as sharp fluctuations in the values). A script in R was applied to Tc data afflicted by the “bouncing ball” effect—but otherwise deemed usable—that identified and removed portions of the Tc data that were unrealistic based on the magnitude of the slope between neighboring points 1, 2, and 3 points away. The criteria for removal were as follows:

1) The average slope between a given point and its neighboring points on either side was > 2*SD away from the mean of all slopes of that window size (i.e., 1 point away, 2 points away, etc.) *and* the value of the temperature at that point was > 2*SD away from the mean of all temperature values for that individual, or

2) The absolute slope between a given point and its neighboring points was greater than the equivalent of a 2°C change over 15 minutes.

All Tc data were then smoothed using local regression (LOESS) using a 25% smoothing span.

Vector magnitude—defined as the square root of the sum of the squares of the counts for each of the three axes measured by the accelerometers—was used to estimate energy expenditure in kilocalories at each minute interval using the 2011 Freedson VM3 equation [139] combined with the 1998 Williams Work-Energy Equation [140]:

$$\textbf{if CPM > 1951 then kcals/min} = 0.00094 \times \text{CPM} + (0.1346 \times \text{BM} - 7.37418))$$

$$\textbf{else kcals/min} = \text{CPM} \times 0.0000191 \times \text{BM}$$

where BM is body mass in kilograms and CPM is the counts per minute (i.e., vector magnitude at a minute interval). Vector magnitude was also used to determine when participants were on break or otherwise performing limited physical activity, using the threshold of VM < 150 CPM [141].

HR data were smoothed using LOESS regression using a 10% smoothing span. Maximum HR (HR_{max}) was calculated using the formula 220-age and percent of HR_{max} at each minute interval was calculated using the smoothed HR at that interval. The percent of the shift spent above 75% and 85% of HR_{max} was determined for each person-day.

Multivariable linear regression models were used to estimate the effects of hydration practices, break duration, baseline kidney function, family history of CKD and recent use of non-steroidal anti-inflammatory drugs (NSAIDs) on maximum Tc experienced during the work shift, controlling for confounders which were selected using a literature review of relevant research [124] and a directed acyclic graph. These included shift duration, median WBGT, estimated work rate (as hourly energy expenditure), industry of employment, and job task. A mixed effects model with a random intercept and random slope for day was used to model the median percent of HR_{max} experienced during each work shift for each participant. Data for overnight shifts (n=48 person-days; 2.8%) were removed from these models. Participants with pre-shift eGFR < 60 mL/min/1.73 m^2 (n=53) were removed from all models, except for those examining the effects of kidney function on measures of heat strain.

Analyses were performed using SAS Version 9.4 and R Version 3.6.1 (The R Foundation for Statistical Computing, www.r-project.org)[142].

Results

Participants were recruited across five industries in the two countries--with 41% of total participants in sugar, 30% in non-sugar agriculture (corn and plantain), and 29% in non-agriculture (brickmaking and construction) (Table 3.1). On average, construction and plantain workers had the longest shifts, with median shift durations of 9.5–10 hours, however, some oven burners in brickmaking had shifts as long as 24–30 hours and some plantain workers had much shorter shifts (2–4 hours). Sugar and corn workers had much shorter shifts, with median durations of 2–4 hours, except for SUGAR-NI3. The study team was able to capture HR and accelerometer data for the entirety of most participants' work shifts (Table 3.S1). This was also true for WBGT monitoring in El Salvador, but less so in Nicaragua (median percent of shift captured: 61-84%). T_c monitoring covered at least 80% of the work shift for most participants on their respective T_c monitoring day(s), even after data cleaning procedures removed unrealistic T_c values. WBGT monitoring was absent during a majority of brick workers' shifts, due to only having two thermometers and multiple, concurrent work sites. Other physiological monitoring (e.g., tympanic temperature) and self-reported hydration variables were captured on most every work shift for every participant. Agrichemical applicators in Nicaragua (sugar and plantain) wore plastic coveralls, so a clothing adjustment factor (CAF) of 0.5°C was applied to their respective WBGT measurements.

The mean age for participants in most industries was 28-31 years; mean age was slightly higher for SUGAR-ES2, construction, and brickmaking (Table 3.1). Participants across industries were largely the same height and weight, with the exceptions of

SUGAR-NI1 and construction (heavier weights).

Higher WBGT values were observed at construction, plantain, and two sugar work sites (SUGAR-ES2 and SUGAR-NI1), but this can partially be explained by the more frequent afternoon work hours for construction and plantain workers, when the temperatures are higher. The highest T_c values were observed, on average, at SUGAR-NI2, although some extreme maximum T_c values were observed at other sites. All industries except corn had a least one worker with a Tc reading above 39°C. When participants who swallowed the core temperature sensor too close to the shift were removed, SUGAR-ES1, SUGAR-NI1, and SUGAR-NI2 had the most extreme maximum T_c readings, with SUGAR-ES1 at 40.1°C being the highest. Based on estimated work rate, Nicaraguan sugarcane workers were doing the most strenuous work, while workers in corn, construction, and plantain had much lower estimated work rates. Similarly, the sugarcane workers in Nicaragua also experienced higher percentages of their HR_{max} during the work shift.

Based on RELs derived from estimated work rates, it was predominantly workers at SUGAR-NI1, SUGAR-ES2, and SUGAR-NI3 who experienced temperatures above their REL for at least a quarter of the work shift (Table 3.1). Approximately 4 of every 5 workers at SUGAR-NI1 experienced effective WBGTs above their REL for at least a quarter of their shift. Nicaraguan sugar companies had the highest rates of workers experiencing $T_c > 38°C$ and $T_c > 38.5°C$ for ≥ 5 minutes during their work shift, with the majority of person-days captured at those three companies and SUGAR-ES2 having $T_c >$ 38°C for ≥ 5 minutes. Workers at the Nicaraguan sugar companies were also more likely to spend a significant percentage of their work shift with Tc > 38°C, with the medians ranging from 16%-29% of the work shift. Construction workers and workers at SUGAR-NI1 and SUGAR-NI3 regularly spent a quarter of the work shift above 75% of their estimated HR_{max} (16%, 35%, and 16% of person-days, respectively).

Self-reported water consumption was mostly consistent across industries and companies despite different shift durations, with workers reporting 3–4 L during the work shift, except for SUGAR-ES2 (median: 5.5 L) and SUGAR-NI3 (median: 8.0 L) (Table 3.1). Overall, workers in Nicaragua reported drinking more non-water drinks than in El Salvador. Most participants did not report consuming electrolyte solution during the work shift, except for Nicaraguan sugarcane workers, who reported consuming, on average, 0.6-2.0 L. Plantain and construction workers spent the greatest percent of their work shift on break (~20%), according to their accelerometer data, followed by SUGAR-ES2 (12%). Plantain and construction workers also had the longest shifts, on average (7.2 hours and 9.3 hours, respectively). The average percent of shift on break at other sugar companies and other industries was much lower, between 4-7%.

Due to pre-harvest serum creatinine screening at the Nicaraguan sugarcane companies, there were far fewer participants with a baseline eGFR < 60 mL/min/1.73 m^2 (range: 0–2%) compared to other sites (range: 3–23%). However, there were participants at these companies with eGFR between 60 and 90 ml/min/1.73 m^2, with an especially large percentage at SUGAR-NI1 (41%, n=9). Reporting a father with diagnosed CKD was relatively common across the different industries and sugarcane companies, with workers at SUGAR-NI2 reporting the highest percentage (25%) and SUGAR-ES2 and construction workers reporting the lowest percentage (7%). Reporting a brother with diagnosed CKD was more common in Nicaragua (range: 5%–23%) than in El Salvador (range: 0%-9%).

Sugarcane workers in Nicaragua were predominantly harvesters (e.g., cane cutters) or agrichemical workers (i.e., applicators or mixers), while in El Salvador, participants had more diverse jobs including drivers/machine operators, supervisors, irrigators, sowers, and crop maintenance (Table 3.2). Plantain and corn workers performed similar tasks, with harvesting the most common job in corn and crop maintenance the most common in plantain. Brick workers performed more specialized tasks, including clay work (e.g., molding bricks and shingles), oven work, and carrying loads of bricks/shingles. Construction workers were primarily manual workers performing various tasks, but some workers had more specialized job tasks, such as driver/machine operator and machinery assistant.

Across the different job tasks within the sugar industry, participants generally started working at similar times, between 6:30–7:30 am, however some workers such as drivers/machine operators and supervisors/irrigators worked longer hours on average, which meant working into the afternoon and early evening (Table 3.3). Nicaraguan harvesters worked longer hours than their harvester counterparts in El Salvador, which meant more frequently working in the midday when ambient temperatures increase. Harvesters and Nicaraguan agrichemical workers had the highest median T_c. These groups also had the highest median work rates, with Nicaraguan agrichemical applicators having the highest. Interestingly, agrichemical workers in El Salvador had a much lower median work rate than those in Nicaragua. Harvesters in both countries and Nicaraguan agrichemical workers also experienced the highest HRs. Nicaraguan harvesters consumed the most water per hour (median: 1.3 L/hr), but most sugarcane workers in both countries were at or above 1 L/hr, except for among the lower-intensity jobs of driver, machine operator, and supervisor/irrigator where the median was around 0.5L/hr. Nicaraguan agrichemical applicators did not report consumption of non-water liquids, except electrolyte solution. Nicaraguan harvesters reported consuming slightly more electrolyte solution than agrichemical applicators (median: 1.6 L vs. 1.2 L) and Salvadoran workers largely reported consuming none.

Some important differences were observed between the two non-agricultural industries (construction and brick) (Table 3.4). Brick workers had more years of experience in their jobs and performed, on average, more physically intense work. WBGT temperatures were much higher at construction sites and slightly higher for oven workers and brick carriers, which is largely explained by the more frequent afternoon working hours for these workers. T_c were not very different between the jobs in both industries and were lower than sugar workers. Oven workers had lower average Tc because they often worked overnight shifts, capturing a time when Tc naturally lowers. HR levels were also largely consistent. Oven workers had higher pre-shift tympanic temperature, which is a result of a later shift start time than most workers and the natural diurnal variation in body temperature. However, brick workers having higher post-shift tympanic temperatures across the board did not appear to be a function of time of day. Finally, workers in these industries reported much lower water consumption rates than sugarcane workers (median rates: 0.3–0.5 L/hr).

The intensities of the job tasks within the non-sugar agricultural industries (corn and plantain) were highly divergent from one another. Among corn workers, the agrichemical workers had higher estimated work rates, yet among the plantain workers, it was those working in crop maintenance and harvesting who had higher estimates (Table 3.5). WBGT temperatures were higher at plantain, but this is again a function of working more hours in the afternoon. Tc was highest among plantain crop maintenance and harvesters and corn harvesters, but these were on average lower than the levels observed for sugar harvesters. HRs were higher among corn workers, but these were also slightly lower than those experienced by workers in the same job in sugar. Corn workers also had higher cross-shift increases in tympanic temperature and higher water consumption rates (although self-reported rates were somewhat more variable).

Greater consumption of water during the shift, greater time spent at a low vector magnitude (“on break”), and use of NSAIDs in the past 7 days all appeared to have no effect on maximum T_c during the shift (Table 3.6). Counterintuitively, greater consumption of electrolyte solution was positively associated maximum T_c (increase of 0.15°C per L consumed; 95% CI: 0.04, 0.26). Workers with lower eGFRs (<60, 60–90) experienced higher maximum T_c when compared to workers with eGFR > 90 (0.20°C and 0.11°C, respectively). The findings for having a family member with diagnosed CKD suggest a null-to-minimally-protective effect on T_c.

Spending a greater portion of the work shift on break appears to be protective for heart rate, as an increase of 10% of the work shift spent on break was associated with an absolute decrease in median % HR_{max} of 1.5% (95% CI: -2.1%, -0.85%) (Table 3.7). Contrary to expected, consumption of water and electrolyte solution were associated with greater median % HR_{max} (+0.46% and +1.2%, respectively). NSAID use in the past 7 days, low eGFR, and reporting a father/brother with CKD were all positively associated with median % HR_{max}, however, with relatively larger confidence intervals.

Discussion

This is the largest study to-date measuring physical activity and markers of heat strain among outdoor workers in various industries in Central America at risk of developing MeN. We found that workers in the sugarcane industry, especially the Nicaraguan companies, seem to be performing the most physically intense work and experiencing the greatest levels of heat strain, as measured by T_c and HR. However, precautions should be taken at all worksites as workers in other industries were shown to be exposed ambient temperatures above their RELs for ≥ 25% of the work shift on 6-10% of the shifts we monitored.

Despite typically avoiding work during the afternoon hours, high median WBGTs were observed at SUGAR-ES2 and SUGAR-NI1 (28.9°C and 28.3°C, respectively), comparable to the median WBGTs observed at construction and plantain (28.9°C and 29.2°C), the industries with the longest average shift durations (7–9 hours) and most likely to work in the afternoon heat. Plantain and construction workers also spent more time on break according to their accelerometer data (21% and 19% of the shift with a vector magnitude < 150 cpm, respectively).

The median estimated work rates were much higher among sugarcane workers (range: 195-402 kcal/hour) than the other industries (range: 83–175 kcal/hour), with the highest at SUGAR-NI1, which was comprised entirely by agrichemical applicators (whose median work rate was >3x that of Salvadoran agrichemical applicators). The high heat and work rates observed at SUGAR-ES2 and SUGAR-NI1 contributed to the high percentages of monitored work shifts during which the $WBGT_{eff}$ exceeded the estimated REL for at least 25% of the shift (35% and 79% of shifts respectively). At SUGAR-NI1, this is also in part due to the clothing adjustment factor of 0.5°C added to the WBGT to account for the plastic coveralls the workers there wore.

The relatively intense work rates translated into consistent findings for the heat strain measures—with workers at the Nicaraguan sugarcane companies experiencing the highest levels of T_c and HR. Despite working in relatively cooler WBGTs, workers at SUGAR-NI2 had the highest median T_c at 37.9°C. Interestingly, the median T_c values observed at sugarcane companies were consistently higher than those observed in a small pilot study of migrant agricultural workers in northern Mexico in June/August [133], which were more closely aligned with the values we observed for corn and plantain workers. Extreme maximum T_c values (>39°C) were observed at all work sites, with the highest in each country being observed at the sugarcane sites. A third of SUGAR-NI1 workers exceeded 38.5°C for at least 5 minutes during the shift, while only a quarter of crop workers in a Florida study reached the same threshold [132]. The median percent of HR_{max}

experienced during the work shift by Nicaraguan sugarcane workers ranged from 61-66% with SUGAR-NI1 the highest. This was comparable among Salvadoran cane cutters (median: 60%). These are higher than the median value reported by Lucas et al. 2015 [77] among Salvadoran cane cutters—54%. While that study did employ a different approach for estimating HR_{max}, that would only over-estimate % HR_{max} for individuals > 40 years old, so is an unlikely explanation for the difference.

Likely resulting from the demanding work and heat strain experienced, sugarcane workers also reported consuming the most water, with most workers consuming more than 1 L/hour. Only workers in less strenuous jobs reported lower consumption rates. We have been told by the leadership at the Nicaraguan sugar companies that they have instituted workplace protections to reduce heat strain, which includes obligatory hydration with water (at least 1L/hour) and electrolyte serum, which is supported by the self-reported hydration data from workers. However, despite any policies currently in place at the sugarcane sites, we still observed relatively infrequent periods of low movement (VM<150cpm), with an average of only 4.8-6.9% of the shift spent on break at the Nicaraguan sugarcane companies.

Reporting a father or brother with diagnosed CKD was unfortunately common at some worksites (e.g., 25% reporting their father has CKD at SUGAR-NI2 and 23% reporting a brother with CKD at SUGAR-NI1), while it was rare at others (e.g., 0 workers reporting a brother with CKD at construction). Salvadoran sugarcane workers were more likely to have an eGFR < 60 (16% at SUGAR-ES1 and 23% at SUGAR-ES2), while these numbers were around 10% for corn, brick, and plantain workers, and virtually

0 at construction and Nicaraguan sugarcane.

Surprisingly, greater time spent on break and greater consumption of water did not have the protective effect on elevated T_c that we had expected. This may be due in part to exposure misclassification and incomplete control of confounding but may also indicate that hydration levels are adequate for the workers performing the most intense activities (sugarcane harvesters and Nicaraguan agrichemical applicators). However, we want to stress that we do not advocate for sugar companies to relax any hydration policies, especially since intervention studies provide some evidence that increased hydration during the work shift may slow or prevent kidney function decline [83,143]. It may be that the causal mechanism explaining these interventions' effectiveness is not through reduced T_c. The finding that electrolyte solution consumption was positively associated with T_c may be a result of incomplete control for confounding by work rate. The most interesting finding for the T_c and % HR_{max} models, was the evidence that workers with low eGFR have higher average T_c and HR values. If heat strain is causally involved in the progression of MeN, these findings suggest that there could be a cycle of impaired kidney function leading to impaired thermoregulation (potentially via impaired water retention) during the work shift, which could increase an individual's risk of acute kidney injury.

Building a causal model for HR is difficult as HR increases both when an individual is working strenuously (to carry oxygen to muscles) and when an individual is experiencing heat stress (because sweating leads to a loss of blood volume/low BP, which results in a higher HR to maintain cardiac output). However, we found that spending more time on break was associated with a lower average $\%HR_{max}$, which is consistent with both mechanisms. However, increased consumption of water and electrolyte solution were positively associated with %HR_{max}, which is not consistent with the hypothesis that rehydration during the work shift helps maintain blood volume lost from sweating, which should in turn lower the HR. This unexpected finding may be a result of incomplete control for confounding by work intensity or urine concentrating ability.

There are a few limitations with these data, that require consideration before extrapolating to other workers and other time periods. For one, we only conducted three days of monitoring (fewer for T_c) and ambient conditions likely varied by industry and month of year because of our monitoring schedule (i.e., each industry and sugar company was visited sequentially and not concurrently). Work activities and conditions may not have been representative of typical workdays for a few reasons: 1) some workers perform varied tasks, which change day-to-day, and 2) the employers may have had an incentive to alter work conditions on observation days to make the working conditions appear less intense. This latter point may have been true at the sugarcane companies, as prior research and anecdotal knowledge suggests that sugarcane cutters' shift durations are typically longer than what we observed for MANOS. However, if true, it's worth noting that we still found evidence of increased heat strain even on relatively "short" days.

Accelerometer data was used to estimate work rate, which definitely introduced some misclassification as efficiency varies between individuals. We also potentially underestimated work rate among sugarcane cutters because of placement of the accelerometers on the hip instead of the arm and cane cutting is a very arm-intensive activity. T_c sensors were not always swallowed early enough for high quality data and varied by industry/company, however, precautions were taken to avoid using inaccurate data.

We also relied on some self-reported data (e.g., hydration, breaks, and medication use), which means we should expect some misclassification and a potential bias in our model results for these variables towards the null.

One important next step is to determine whether these measures of heat strain correlate with outcomes of interest, such as acute kidney injury, dehydration, and muscle damage. These data can also be used to determine which WBGT thresholds/screening guidance should be used for preventing adverse health outcomes (e.g., kidney injury) in these workers once exposure-response estimates are better established. Conveniently, serum data were collected for one of the three days of baseline exposure monitoring and the MANOS cohort has been followed every 6 months since baseline for 5 rounds, so these data can be used to address some of these other outstanding questions about MeN etiology.

Conclusion

This the largest to-date study examining heat stress and heat strain among outdoor workers in Central America. We found evidence that sugarcane workers, particularly those in Nicaragua, perform physically intense work and experience concerning levels of heat strain, as captured by core body temperature and heart rate, despite consuming more than 1L water per hour and additional electrolyte solution. We found that workers in other industries also experience elevated core body temperatures and that most workers in the study spend relatively low percentages of their work shift on break. Regardless of whether heat strain is causally involved in MeN, we identified levels of heat strain that warrant interventions to reduce work load for workers in the most strenuous job tasks, especially on days with higher ambient temperatures.

Table 3.1. Summary statistics for participant and work shift characteristics and monitoring and biomarker data, by industry/work site.

	El Salvador				**Nicaragua**				
	SUGAR-ES1	**SUGAR-ES2**	**CORN**	**CONS**	**SUGAR-NI1**	**SUGAR-NI2**	**SUGAR-NI3**	**BRICK**	**PLAN**
Total number of participants	55	56	110	58	22	52	50	107	59
Total person-days	165	168	330	174	66	156	150	321	177
Participant Characteristics									
Mean Age (SD)	27 (7.5)	31 (8.2)	28 (7.7)	31 (7.0)	29 (6.5)	28 (6.1)	29 (5.0)	31 (7.7)	30 (7.4)
Mean Weight (SD) (kg)	67.6 (10.7)	64.7 (9.6)	67.3 (12.0)	73.5 (11.8)	72.4 (12.5)	68.2 (12.2)	66.1 (8.7)	69.6 (13.9)	68.7 (9.7)
Mean Height (SD) (meters)	1.66 (0.06)	1.66 (0.06)	1.68 (0.05)	1.68 (0.06)	1.69 (0.05)	1.69 (0.06)	1.68 (0.07)	1.68 (0.06)	1.67 (0.06)
Work Shift Characteristics									
Mean Work Shift Duration (SD) (hours)	4.6 (3.3)	5.0 (2.2)	3.2 (1.5)	9.3 (1.1)	3.7 (0.3)	4.2 (1.4)	5.1 (1.9)	6.6 (2.9)	7.2 (3.2)
Typical Shift Start Time (1st quartile-3rd quartile)	6:15-7:15	6:50-7:30	6:00-6:45	7:00-7:20	6:30-7:00	6:30-7:00	7:10-7:55	2:17-6:49	6:20-6:40
Typical Shift Stop Time (1st quartile-3rd quartile)	8:00-16:00	10:00-14:20	8:40-10:00	15:38-17:00	10:15-10:35	9:40-12:00	10:57-14:15	9:08-12:00	11:30-17:00
Ambient Heat									
Median WBGT (MAD) (°C)	26.0 (2.1)	28.9 (1.1)	27.1 (1.5)	28.9 (0.52)	28.3 (0.23)	26.4 (0.98)	27.1 (0.55)	27.0 (1.7)	29.2 (0.83)
Median Heat Index (MAD) (°C)	28.5 (3.5)	32.5 (1.3)	29.8 (2.7)	33.0 (1.1)	31.0 (0.53)	30.7 (0.89)	31.7 (0.98)	31.1 (2.7)	36.1 (1.5)
Number of person-days with $\mathrm{WBGT}_{\mathrm{eff}}$ exceeding $\mathrm{REL}_{\mathrm{accel}}$ at least 5 minutes (% of person-days)	13 (8%)	69 (41%)	51 (16%)	32 (18%)	54 (82%)	34 (22%)	58 (39%)	4 (4%)	28 (20%)

Number of person-days with $WBGT_{eff}$ exceeding REL_{accel} at least 25% of shift (% of person-days)	7 (4.3%)	58 (35%)	20 (6.3%)	11 (6.3%)	52 (79%)	23 (15%)	42 (28%)	0 (0%)	15 (10%)
Core Body Temperature									
Maximum T_c (MAD) (°C)	40.1 (0.30)	39.1 (0.23)	38.9 (0.37)	40.2 (0.25)	39.8 (0.53)	39.3 (0.26)	39.3 (0.27)	39.1 (0.22)	39.2 (0.40)
Median T_c (MAD) (°C)	37.7 (0.31)	37.7 (0.31)	37.4 (0.35)	37.5 (0.26)	37.7 (0.66)	37.9 (0.31)	37.8 (0.37)	37.5 (0.36)	37.6 (0.30)
(Sensor swallowed > 3 hours before shift only) Maximum T_c (MAD) (°C)	40.1 (0.24)	38.8 (0.21)	38.9 (0.36)	38.6 (0.20)	39.5 (0.15)	39.3 (0.27)	38.8 (0.21)	39.1 (0.25)	39.2 (0.42)
(Sensor swallowed > 3 hours before shift only) Median T_c (MAD) (°C)	37.7 (0.32)	37.7 (0.30)	37.8 (0.52)	37.6 (0.17)	37.8 (0.75)	37.9 (0.31)	37.8 (0.35)	37.5 (0.34)	37.5 (0.32)
Number of person-days with T_c > 38°C at least 5 minutes (% of person-days)	29 (41%)	37 (52%)	37 (22%)	23 (28%)	16 (73%)	54 (81%)	30 (73%)	25 (21%)	12 (35%)
Number of person-days with T_c > 38.5°C at least 5 minutes (% of person-days)	4 (6%)	3 (4%)	9 (5%)	4 (5%)	7 (32%)	7 (10%)	6 (15%)	4 (3%)	2 (6%)
Median percent of shift T_c > 38°C	0%	4%	0%	0%	21%	29%	16%	0%	0%
Work Rate and Time on Break									
Median Work Rate (MAD) (kcals/hour)	224.5 (207.7)	195.0 (115.4)	101.5 (54.7)	82.5 (52.0)	402.1 (56.8)	291.3 (80.0)	291.6 (74.3)	174.9 (82.8)	102.8 (93.1)
Median percent shift VM < 150 CPM (MAD)	4.8% (7.1%)	12% (7.2%)	4.1% (6.0%)	19% (10%)	5.3% (4.5%)	4.8% (3.7%)	6.9% (3.7%)	6.1% (6.1%)	21% (12%)
Heart Rate									
Median % HR_{max} (MAD)	56% (12%)	54% (8.5%)	54% (6.4%)	50% (8.1%)	66% (7.2%)	61% (6.6%)	62% (5.6%)	51% (7.3%)	47% (7.7%)

Number of participants with HR > 85% HR_{max} at least 5 minutes (% of participants)	2 (3.6%)	0 (0%)	4 (3.6%)	2 (3.4%)	2 (9.1%)	2 (3.8%)	9 (18%)	1 (0.9%)	2 (3.4%)
Number of person-days with HR > 75% $HR_{max} \geq$ 25% shift (% of person-days)	11 (6.8%)	3 (1.9%)	12 (3.7%)	28 (16%)	20 (35%)	8 (6.0%)	23 (16%)	6 (2.0%)	5 (3.5%)
Tympanic Temperature									
Median Pre-Shift T_{tymp} (MAD) (°C)	35.8 (0.5)	36.3 (0.3)	36.1 (0.4)	36.2 (0.2)	36.1 (0.3)	36.4 (0.4)	36.3 (0.3)	36.4 (0.4)	36.2 (0.4)
Median Post-Shift T_{tymp} (MAD) (°C)	36.9 (0.3)	37.2 (0.3)	37.1 (0.2)	36.6 (0.3)	37.1 (0.2)	37.2 (0.3)	37.1 (0.3)	37.0 (0.3)	36.9 (0.3)
Hydration Practices									
Median water consumption during shift (MAD) (L)	3.0 (0.79)	5.5 (2.0)	3.0 (0.79)	3.8 (0.79)	4.1 (0.45)	4.0 (2.0)	8.0 (1.8)	3.0 (1.0)	3.8 (1.2)
Median electrolyte solution consumption during shift (MAD) (L)	0 (0)	0 (0)	0 (0)	0 (0)	1.2 (0)	0.6 (0.9)	2.0 (0.7)	0 (0)	0 (0)
Median fructose-sweetened beverage consumption during shift (MAD) (L)	0 (0)	0 (0)	0 (0)	0.3 (0)	0 (0)	0 (0)	0.3 (0.5)	0.5 (0.7)	0.3 (0.4)
Median consumption of non-water, non-electrolyte liquids during shift (MAD) (L)	0.3 (0.3)	0.3 (0.3)	0.3 (0.3)	0.3 (0.0)	0 (0.0)	0.8 (0.8)	1 (0.6)	0.6 (0.4)	0.5 (0.5)
Baseline Kidney Function and Family History of CKD									
Participants with pre-shift eGFR < 60 (%)	9 (16%)	13 (23%)	12 (11%)	2 (3%)	0 (0%)	1 (2%)	0 (0%)	11 (10%)	5 (9%)
Participants with pre-shift eGFR 60-90 (%)	1 (1.8%)	8 (14%)	8 (7.2%)	3 (5.2%)	9 (41%)	7 (14%)	1 (2%)	13 (12%)	10 (17%)
Participants reporting a father with diagnosed CKD (%)	8 (15%)	4 (7%)	19 (17%)	4 (7%)	3 (14%)	13 (25%)	4 (8%)	20 (19%)	9 (15%)
Participants reporting a brother with diagnosed CKD (%)	5 (9%)	2 (4%)	5 (5%)	0 (0%)	5 (23%)	8 (15%)	7 (14%)	12 (11%)	3 (5%)

MAD = Median absolute deviation; REL_{accel} = Recommended Exposure Limit for wet bulb globe temperatures determined by accelerometer-based estimates of metabolic workload

Table 3.2. Summary of main job tasks on the three days of monitoring at baseline, by industry/company and country.

	El Salvador				Nicaragua				
	SUGAR-ES1	**SUGAR-ES2**	**CORN**	**CONS**	**SUGAR-NI1**	**SUGAR-NI2**	**SUGAR-NI3**	**BRICK**	**PLAN**
Breakdown of main job tasks and % of person-days	54% harvester, 21% driver/ machine operator, 10% supervisor/ irrigator, 5% crop maintenance	49% harvester, 32% sower, 8% agrichemical worker, 5% supervisor/ irrigator	77% harvester, 18% agrichemical worker	64% manual assistant, 8% machinery assistant, 8% road safety, 7% driver/ machine operator	100% agrichemical worker	60% harvester, 38% agrichemical worker	60% harvester, 39% agrichemical worker	48% clay worker, 26% oven worker, 13% carrier, 10% clay worker and carrier	32% crop maintenance, 22% agrichemical worker, 16% harvester and crop maintenance, 14% supervisor/ irrigator

Table 3.3. Summary statistics for monitoring and biomarker data, by job task within sugar industry

	El Salvador				Nicaragua			
	Harvester	Sower	Driver/ Machine Operator	Supervisor / Irrigator	Agrichemical Worker	Crop Maintenance	Harvester	Agrichemical Worker
Number of person-days	168	66	34	24	13	12	171	176
Mean Age (SD)	30 (7.4)	30 (8.8)	26 (5.8)	30 (9.5)	29 (9.8)	26 (11)	30 (5.2)	28 (6.1)
Median Time in Current Job (MAD) (Years)	7.0 (5.9)	5.5 (5.2)	5.0 (1.5)	2.0 (1.5)	6.0 (5.9)	2.5 (0.7)	5.0 (4.4)	2.2 (1.8)
Mean Work Shift Duration (SD) (hours)	4.1 (2.6)	4.1 (2.3)	8.5 (1.3)	8.2 (1.7)	3.5 (0.4)	2.7 (0.3)	5.8 (1.2)	3.0 (0.7)
Typical Shift Start Time (1st quartile-3rd quartile)	6:20-7:16	6:40-7:00	6:41-7:30	7:00-7:35	6:49-6:51	6:53-7:08	6:51-7:30	6:41-7:42
Typical Shift Stop Time (1st quartile-3rd quartile)	8:00-13:52	9:56-10:29	15:48-16:00	16:00-16:05	10:00-10:30	9:35-10:00	11:45-14:15	9:11-10:45
Median WBGT (MAD) (°C)	26.9 (4.3)	27.4 (1.5)	27.8 (0.6)	28.2 (0.8)	25.5 (0.4)	26.3 (1.0)	27.1 (1.3)	27.3 (1.3)
Median Heat Index (MAD) (°C)	29.4 (6.0)	31.5 (1.4)	32.2 (1.0)	32.4 (0.9)	30.4 (0.4)	29.1 (1.2)	31.8 (1.5)	31.0 (0.9)
Median T_c (MAD) (°C)	37.8 (0.27)	37.7 (0.23)	37.5 (0.27)	37.6 (0.27)	37.5 (0.06)	37.8 (0.21)	37.9 (0.22)	37.8 (0.37)
(Sensor swallowed > 3 hours before shift only) Median T_c (MAD) (°C)	37.8 (0.28)	37.7 (0.23)	37.6 (0.37)	37.7 (0.26)	37.5 (0.06)	N/A	37.9 (0.22)	37.8 (0.27)
Median Work Rate (MAD) (kcals/hour)	299.2 (77.7)	131.3 (60.4)	57.5 (31.4)	57.9 (16.9)	111.4 (77.2)	159.2 (66.6)	297.7 (52.1)	317.5 (145.3)
Median % HR_{max} (MAD)	60% (7%)	50% (7%)	46% (6%)	45% (4%)	48% (7%)	51% (8%)	62% (4%)	62% (9%)

Median Pre-Shift T_{tymp} (MAD) (°C)	36.0 (0.74)	36.1 (0.74)	35.8 (0.82)	36.1 (0.82)	36.5 (0.30)	36.3 (0.22)	36.3 (0.44)	36.3 (0.44)
Median Post-Shift T_{tymp} (MAD) (°C)	37.0 (0.59)	37.1 (0.3)	37.1 (0.22)	37.0 (0.37)	37.0 (0.44)	36.8 (0.37)	37.2 (0.44)	37.0 (0.36)
Median water consumption rate (MAD) (L/hour)	1.2 (0.4)	1.1 (0.6)	0.5 (0.2)	0.5 (0.2)	1.2 (0.5)	1.1 (0.3)	1.3 (0.4)	1.2 (0.4)
Median consumption of other liquids during shift (MAD) (L)	0.3 (0.4)	0.3 (0.4)	0.3 (0.4)	0.3 (0.4)	0.3 (0)	0.3 (0.2)	1.3 (0.7)	0.0 (0)
Median electrolyte consumption during shift (MAD) (L)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.6 (0.6)	1.2 (0.3)

MAD = Median Absolute Deviation

Table 3.4. Summary statistics for monitoring and biomarker data, by job task within non-agricultural industries.

	El Salvador				Nicaragua			
	Construction				Brick			
	Driver/ Machine Operator	Machinery Assistant	Manual Assistant	Road Safety Worker	Carrier	Clay Worker	Clay Worker/ Carrier	Oven Worker
Number of person-days	13	15	113	15	42	150	31	74
Mean Age (SD)	34 (5.7)	31 (8.1)	31 (7.1)	26 (3.6)	30 (6.1)	31 (8.0)	31 (7.8)	32 (7.7)
Median Time in Current Job (MAD) (Years)	2.0 (2.0)	5.0 (5.9)	1.0 (0.0)	4.0 (1.5)	8.0 (5.9)	8.0 (7.4)	7.0 (7.4)	8.0 (7.4)
Mean Work Shift Duration (SD) (hours)	9.2 (0.7)	9.9 (0.3)	9.1 (1.2)	9.4 (1.4)	7.5 (4.2)	6.2 (2.0)	7.5 (2.7)	10.4 (6.7)
Typical Shift Start Time (1st quartile-3rd quartile)	7:00-7:04	7:00-7:05	7:00-7:20	7:12-7:30	3:33-6:48	2:00-4:00	2:03-4:03	3:40-9:40
Typical Shift Stop Time (1st quartile-3rd quartile)	16:00-16:53	16:45-17:00	15:00-17:00	16:45-17:38	10:40-16:39	8:35-10:40	9:50-11:07	7:25-17:35
Median WBGT (MAD) (°C)	28.9 (0.7)	28.9 (1.1)	29.0 (0.7)	29.0 (1.4)	27.9 (1.0)	24.9 (1.3)	27.0 (1.9)	28.1 (1.7)
Median Heat Index (MAD) (°C)	32.8 (0.7)	33.5 (2.2)	33.2 (1.7)	34.1 (2.0)	32.5 (2.0)	29.2 (1.7)	29.9 (0.8)	34.8 (2.7)
Median T_c (MAD) (°C)	37.5 (0.09)	37.5 (0.09)	37.6 (0.17)	37.4 (0.19)	37.5 (0.27)	37.6 (0.19)	37.5 (0.21)	37.3 (0.29)
(Sensor swallowed > 3 hours before shift only) Median T_c (MAD) (°C)	N/A	N/A	37.6 (0.08)	37.6 (0.01)	37.7 (0.37)	37.6 (0.15)	37.5 (0.13)	37.4 (0.39)
Median Work Rate (MAD) (kcals/hour)	58.9 (30.9)	87.7 (75.8)	97.4 (46.5)	36.8 (18.0)	157.9 (58.4)	209.8 (61.9)	209.9 (64.7)	58.2 (76.9)

Median % HR_{max} (MAD)	50% (7%)	51% (2%)	51% (9%)	48% (4%)	51% (8%)	53% (8%)	51% (4%)	49% (6%)
Median Pre-Shift T_{tymp} (MAD) (°C)	36.2 (0.30)	36.0 (0.44)	36.2 (0.30)	36.3 (0.44)	36.5 (0.59)	36.3 (0.52)	36.0 (0.59)	36.7 (0.59)
Median Post-Shift T_{tymp} (MAD) (°C)	36.5 (0.30)	36.2 (0.59)	36.7 (0.44)	36.6 (0.30)	37.1 (0.44)	37.0 (0.30)	37.1 (0..30)	37.0 (0.44)
Median Water consumption rate (MAD) (L/hour)	0.4 (0.1)	0.4 (0.0)	0.4 (0.1)	0.4 (0.1)	0.5 (0.3)	0.4 (0.2)	0.3 (0.1)	0.4 (0.3)
Median consumption of other liquids during shift (MAD) (L)	0.3 (0)	0.3 (0)	0.3 (0)	0.6 (0.4)	0.8 (0.8)	0.6 (0.6)	0.9 (0.9)	0.6 (0.4)

MAD = Median Absolute Deviation

Table 3.5. Summary statistics for monitoring and biomarker data, by job task within non-sugar agricultural industries.

	El Salvador		Nicaragua			
	Corn		Plantain			
	Agrichemical Worker	Harvester	Agrichemical Worker	Crop Maintenance	Harvester/ Crop Maintenance	Supervisor/ Irrigator
Number of person-days	59	252	35	43	25	17
Mean Age (SD)	24 (6.5)	28 (7.6)	30 (6.3)	29 (7.0)	29 (6.5)	29 (6.5)
Median Time in Current Job (MAD) (Years)	4.0 (3.0)	4.0 (3.0)	2.0 (0.7)	2.0 (1.0)	2.0 (0.9)	3.0 (2.9)
Mean Work Shift Duration (SD) (hours)	1.6 (0.7)	3.5 (1.3)	9.6 (2.6)	6.2 (2.5)	8.9 (2.5)	10.5 (7.1)
Typical Shift Start Time (1st quartile-3rd quartile)	6:56-7:55	5:50-6:10	6:10-6:25	6:25-6:38	6:24-6:45	6:30-6:44
Typical Shift Stop Time (1st quartile-3rd quartile)	8:41-9:20	8:30-10:00	17:00-17:00	11:07-12:00	13:00-17:00	10:41-16:50
Median WBGT (MAD) (°C)	26.0 (2.5)	27.1 (2.2)	29.3 (1.8)	29.0 (1.8)	29.2 (1.3)	29.9 (0.9)
Median Heat Index (MAD) (°C)	27.1 (3.9)	30.3 (3.6)	35.0 (0.2)	34.8 (2.9)	35.4 (1.0)	36.3 (1.2)
Median T_c (MAD) (°C)	37.4 (0.25)	37.4 (0.28)	37.4 (0.32)	37.6 (0.30)	37.7 (0.10)	37.4 (0.24)
(Sensor swallowed > 3 hours before shift only) Median T_c (MAD) (°C)	37.5 (0.21)	37.6 (0.34)	37.4 (0.32)	37.7 (0.52)	37.7 (0.10)	37.4 (0.24)
Median Work Rate (MAD) (kcals/hour)	179.2 (77.8)	88.2 (41.4)	47.6 (47.2)	173.1 (97.8)	124.8 (80.6)	39.8 (38.0)

Median % HR_{max} (MAD)	55% (6%)	54% (6%)	47% (9%)	50% (10%)	45% (5%)	42% (4%)
Median Pre-Shift T_{tymp} (MAD) (°C)	35.9 (0.44)	36.1 (0.59)	36.2 (0.44)	36.3 (0.59)	36.2 (0.59)	36.4 (0.59)
Median Post-Shift T_{tymp} (MAD) (°C)	36.9 (0.30)	37.1 (0.30)	36.9 (0.30)	36.9 (0.44)	36.9 (0.44)	36.9 (0.44)
Median water consumption rate (MAD) (L/hour)	1.9 (1.2)	0.9 (0.4)	0.4 (0.1)	0.6 (0.2)	0.6 (0.2)	0.4 (0.1)
Median consumption of other liquids during shift (MAD) (L)	0.3 (0)	0.2 (0.2)	0.6 (0.4)	0.3 (0.4)	0.5 (0.7)	0.5 (0.3)

MAD = Median Absolute Deviation

Table 3.6. Summary of multivariable linear regression models examining risk/protective factors associated with maximum Tc among participants with monitoring data capturing ≥ 50% of shift

	Crude Models		**Adjusted Model 1**[a]		**Adjusted Model 2**[b]	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Water Consumption Rate (per 0.6 L/hr)	0.02 (-0.01, 0.05)	0.25	0.00 (-0.03, 0.04)	0.82	-0.02 (-0.06, 0.01)	0.22
a – Adjusted for industry/company, job task, shift duration, and consumption of other beverages b – Additionally adjusted for median WBGT, mean work rate, and percent shift VM<150 cpm						
Total Electrolyte Solution Consumption (per 1 L)	0.06 (-0.02, 0.14)	0.14	0.16 (0.06, 0.27)	0.002	0.15 (0.04, 0.26)	0.01
a – Adjusted for industry/company, job task, shift duration, and consumption of other beverages b – Additionally adjusted for median WBGT, mean work rate, and percent shift VM<150 cpm						
Percent Shift Vector Magnitude <150 cpm (per 10% increase)	-0.03 (-0.01, 0.00)	0.07	-0.02 (-0.06, 0.02)	0.24	0.00 (-0.04, 0.04)	0.93
a – Adjusted for industry/company, job task, and shift duration b – Additionally adjusted for total liquid consumption, median WBGT, and mean work rate						
NSAID Use in Past 7 Days (yes vs. no)	0.10 (0.00, 0.20)	0.05	0.01 (-0.08, 0.11)	0.83	0.02 (-0.07, 0.11)	0.69
a – Adjusted for industry/company and job task b – Additionally adjusted for mean work rate						
eGFR < 60 ml/min/1.73m^2 (vs > 90)	0.23 (0.11, 0.34)	0.0002	0.20 (0.09, 0.31)	0.001		

eGFR 60-90 ml/min/1.73m^2 (vs > 90)	0.14 (0.01, 0.27)	0.03	0.11 (-0.01, 0.22)	0.07	
a – Adjusted for industry/company, job task, and age					
Father Diagnosed with CKD (yes vs. no)	0.00 (-0.11, 0.10)	0.94	-0.03 (-0.12, 0.06)	0.53	
Brother Diagnosed with CKD (yes vs. no)	0.12 (-0.03, 0.27)	0.11	-0.08 (-0.22, 0.05)	0.24	
a – Adjusted for industry/company, job task, and age					

WBGT=wet bulb globe temperature, VM=vector magnitude, cpm=counts per minute, L/hr =liters per hour

Table 3.7. Summary of mixed effects models examining risk/protective factors associated with median % HR_{max} among participants with monitoring data capturing ≥ 50% of shift

	Crude Models		Adjusted Model 1[a]		Adjusted Model 2[b]	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Water Consumption Rate (per 0.6 L/hr)	1.6 (1.1, 2.0)	<.0001	1.2 (0.65, 1.7)	<.0001	0.46 (-0.04, 0.97)	0.07
a – Adjusted for industry/company, job task, shift duration, and consumption of other beverages b – Additionally adjusted for median WBGT, mean work rate, and percent shift VM<150 cpm						
Total Electrolyte Solution Consumption (per 1 L)	1.6 (0.67, 2.6)	0.001	1.4 (0.42, 2.5)	0.007	1.2 (0.34, 2.1)	0.009
a – Adjusted for industry/company, job task, shift duration, and consumption of other beverages b – Additionally adjusted for median WBGT, mean work rate, and percent shift VM<150 cpm						
Percent Shift Vector Magnitude <150 cpm (per 10% increase)	-3.2 (-3.7, -2.7)	<0.0001	-2.8 (-3.4, -2.2)	<0.0001	-1.5 (-2.1, -0.85)	<0.0001
a – Adjusted for industry/company, job task, and shift duration b – Additionally adjusted for total liquid consumption, median WBGT, and mean work rate						
NSAID Use in Past 7 Days (yes vs. no)	2.1 (-0.26, 4.4)	0.08	1.7 (-0.21, 3.6)	0.08	1.3 (-0.44, 3.0)	0.15
a – Adjusted for industry/company and job task b – Additionally adjusted for mean work rate						
eGFR < 60 ml/min/1.73m^2 (vs > 90)	2.2 (-0.42, 4.9)	0.10	1.7 (-0.40, 3.9)	0.11		

eGFR 60-90 ml/min/1.73m^2 (vs > 90)	4.2 (1.5, 6.8)	0.002	2.9 (0.87, 5.0)	0.005	
a – Adjusted for industry/company, job task, age, and mean work rate					
Father Diagnosed with CKD (yes vs. no)	3.0 (0.54, 5.4)	0.02	1.4 (-0.63, 3.4)	0.18	
Brother Diagnosed with CKD (yes vs. no)	5.1 (2.0, 8.2)	0.002	0.59 (-2.1, 3.2)	0.66	
a – Adjusted for industry/company, job task, and age					

Parameter estimates are reported as absolute increase/decrease in HR_{max} percentage points, not relative change in percentage of HR_{max}.

WBGT=wet bulb globe temperature, VM=vector magnitude, cpm=counts per minute, L/hr =liters per hour

Supplemental Material

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Table 3.S1. Summary of collected monitoring data, by industry/company

	El Salvador				**Nicaragua**				
	SUGAR-ES1	**SUGAR-ES2**	**CORN**	**CONS**	**SUGAR-NI1**	**SUGAR-NI2**	**SUGAR-NI3**	**BRICK**	**PLAN**
Total number of participants	55	56	110	58	22	52	50	107	59
Total person-days	165	168	330	174	66	156	150	321	177
Person-days of WBGT monitoring	164	168	318	174	66	155	149	106	143
Median % of Work Shift with WBGT data	100%	98%	100%	93%	68%	84%	70%	79%	61%
Person-days with coverall use	0	0	0	0	66	60	59	0	39
Person-days of T_c monitoring	71	71	172	81	22	67	41	119	34
Median % of Work Shift with Tc data	80%	87%	85%	69%	74%	89%	85%	72%	84%
Person-days of accelerometer monitoring	162	162	324	172	58	141	148	299	142
Median % of Work Shift with accelerometer data	100%	100%	100%	100%	100%	100%	100%	100%	100%
Person-days of HR monitoring	162	162	324	172	58	141	148	299	142
Median % of Work Shift with HR data	100%	100%	100%	97%	99%	100%	100%	93%	94%
Person-days with pre- and post-shift T_{tymp} measurements	165	164	327	171	64	154	148	298	172
Person-days with self-reported hydration practices	164	168	330	174	66	156	150	321	177

WBGT = wet bulb globe temperature; T_c = core body temperature; HR = heart rate; T_{tymp} = tympanic temperature

CHAPTER FOUR: HEAT EXPOSURE AND CROSS-SHIFT KIDNEY INJURY AMONG OUTDOOR WORKERS IN EL SALVADOR AND NICARAGUA

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Abstract

An epidemic of chronic kidney disease of unknown etiology has afflicted young, primarily male workers in Central America for the past several decades. Previous research has demonstrated that sugarcane workers in this region experience cross-shift increases in serum creatinine (a measure of acute kidney injury, or AKI) and that ambient temperatures, physical exertion, and dehydration are risk factors. Workers in other industries are affected by this disease, but little is known about whether they experience AKI at work. No studies to-date have measured core body temperature as a risk factor for AKI among workers in Central America. The primary aims were to: 1) compare markers of kidney injury, dehydration, and muscle damage across industries and sugarcane companies, and 2) identify risk factors for and preventative factors against cross-shift AKI. We used the data from the MesoAmerican Nephropathy Occupational Study (MANOS), an occupational cohort of 569 workers in El Salvador and Central America. We found that 23% of workers at one Nicaraguan sugarcane company met the KDIGO criteria for cross-shift AKI, while a less conservative estimate of serum creatinine-derived AKI identified relatively high rates at two Nicaraguan sugarcane companies (42.3% and 22.0%) and among Salvadoran construction workers (13.8%). Job task was an important predictor of AKI, with cane cutters experiencing disproportionately higher rates than other sugarcane workers. Maximum core temperature and average work rate were strong predictors of cross-shift increases in serum creatinine. We found moderate protective effects for time spent on break and increased liquid consumption (water,

electrolyte, and fructose-containing beverages). We found mixed evidence for serum uric acid as a risk factor for AKI.

Introduction

An epidemic of chronic kidney disease (CKD) of unknown etiology, often referred to as CKDu or Mesoamerican Nephropathy (MeN), has afflicted young, primarily male workers in Central America for the past several decades [2,11,13,14,22,144]. The disease is not associated with traditional risk factors for CKD (e.g., hypertension, diabetes) and generally presents with little/no proteinuria, hypokalemia, hyponatremia, hypomagnesemia, and hyperuricemia [2,27,31]. In recent years, much attention has been given to the hypothesis of chronic exposure to heat stress and dehydration as important causal factors in the disease's etiology [32,40,65–68], as many of these workers regularly perform physically demanding jobs in hot and humid surroundings, sometimes with limited break periods and access to water [65,73,75–78].

These work conditions are suspected to contribute to volume depletion, breakdown of muscle tissue, and elevated core body temperature, which can contribute to kidney injury through several possible mechanisms, including reduced renal blood flow and ischemia, increased production of vasopressin, activation of the aldose reductase/fructokinase pathway leading to oxidative stress, hyperuricemia and crystalluria, rhabdomyolysis, and increased tubular reabsorption of toxicants, like heavy metals and agrichemicals [85,145,146]. Despite accumulating evidence in support of the heat stress hypothesis, there remain questions as to whether heat stress and dehydration are sufficient causes [11,147], which other exposures may be causally involved (e.g., non-steroidal anti-inflammatory drugs, fructose-sweetened beverages, agrochemicals, and heavy metals) [40,46], the role of genetics and early life exposures [96,148], and whether recurrent, subclinical kidney injury in otherwise healthy individuals is a cause of CKDu [146,147].

Previous research has demonstrated that sugarcane cutters in Central America experience cross-shift increases in serum creatinine (a measure of acute kidney injury, or AKI) and that ambient temperatures, physical exertion, dehydration, and elevated uric acid levels appear to be risk factors, while access to water and longer breaks appear to be protective [33,63,70,83,128,149]. Cross-harvest intervention studies suggest that increased access to water, rest, and shade reduce cross-shift and cross-harvest drops in eGFR, supporting the hypothesis that heat strain and/or dehydration are associated with this kidney disease [82,83]. Similar patterns have been observed for agricultural workers in Brazil and the United States—countries not currently considered to have epidemics of this kidney disease—however with somewhat mixed findings about the specific risk factors [21,131,150].

These findings are supported by studies conducted in animal models and among humans in controlled laboratory settings. Human adults exercising in hot conditions with restricted water intake experienced greater increases in biomarkers of kidney injury than when they were provided water, cooling, or both [84]. The role of the aldose reductase/fructokinase pathway was studied in wild-type and fructokinase deficient mice exposed to recurrent heat-induced dehydration [88]. Delayed rehydration in severely dehydrated mice activated the aldose reductase pathway and led to kidney injury. Mice that were adequately hydrating and fructokinase deficient mice, even when dehydrated, did not experience kidney injury. In another study by Roncal-Jimenez et al., rats exposed to heat stress and dehydration experienced tubulointerstitial fibrosis and inflammation, which was exacerbated by the administration of desmopressin (a form of the antidiuretic vasopressin) [86]. Similar studies have found that heat-exposed mice have greater levels of renal injury when combined with fructose rehydration [151] and lower levels of renal injury when given allopurinol, which reduces serum uric acid [152].

A recent study conducted in mice found that an increase in core body temperature of 1°C was associated with greater tubular injury and interstitial inflammation [153]. A study of agricultural workers in California found that physiological strain index (as defined by a combination of core body temperature and heart rate), and not volume depletion, was associated with risk of AKI [131]. This population differed from workers in Central America, however, in that they had a higher prevalence of underlying comorbidities (e.g., obesity, hypertension, and diabetes) that are known risk factors for CKD. No studies to-date have measured core body temperature as a risk factor for AKI among workers in Central America. However, a recent study among migrant farm workers in northern Mexico has found evidence that heat strain (core body temperature and heart rate) and dehydration are linked to declines in kidney function observed across a harvest season—declines that were not observed among office workers [154].

While the causal link between recurrent AKI and MeN is not established, there is growing evidence that recurrent fluctuations in serum creatinine predict longer term declines in kidney function in this population [69,100,149,155], however the direction of effect is not entirely established. There is research, however, among non-CKDu populations that demonstrates a strong association between reversible AKI and development of chronic kidney disease among patients with previously normal kidney function[156].

While much of the research focus has been on sugarcane workers, and cane cutters in particular, workers in other industries are affected by MeN, such as brickmaking and construction [7,9]. Understanding whether and to what extent these workers experience cross-shift AKI may help inform where interventions are needed and why discrepancies in MeN rates are observed. Furthermore, within the sugar industry there are different workplace practices between companies and different levels of intensity between job tasks (Chapter 3), yet little research has been conducted to examine whether incidence of AKI differs between companies and job tasks.

The MesoAmerican Nephropathy Occupational Study (MANOS) is a longitudinal occupational cohort study in El Salvador and Nicaragua designed to fill some of these gaps in understanding differences between industries, sugar companies, and job tasks using cross-shift data from workers in sugarcane, plantain, corn, construction, and brickmaking. The objectives of this research were to: 1) Examine cross-shift AKI as measured by increases in serum creatinine in workers across industries, sugar companies, and job tasks, 2) Compare biomarkers of dehydration among workers in different industries and sugar companies, and 3) Identify risk factors for and protective factors against cross-shift serum creatinine increases in the MANOS cohort.

Methods

Study Population

The MANOS study population has been described previously (Chapter 1, Chapter 3). In summary, 569 male workers from five industries in El Salvador and Nicaragua were recruited and observed for three workdays between January 2018 – May 2018. Workers at Nicaraguan sugar companies were screened at the beginning of the harvest for kidney function; individuals with high serum creatinine values (≥ 1.3 or 1.5 mg/dL, depending on the company) were not hired and were therefore not recruited into the MANOS study.

The MANOS study protocol was approved by the Boston University Medical Campus Institutional Review Board, the Salvadoran National Ethics Committee for Health Research (Comité Nacional de Ética de las Investigaciones en Salud), and the National Ethics Committee (Comité Institutional de Revisión Etica) and the Office of Teaching and Research (Dirección General de Docencia e Investigaciones), both of the Nicaraguan Ministry of Health.

Similar to Chapter 3, we abbreviated the industry names and sugar companies as follows: CORN (corn), PLAN (plantain), BRICK (brickmaking), CONS (construction), SUGAR-ES1, SUGAR-ES2, SUGAR-NI1, SUGAR-NI2, and SUGAR-NI3 (sugarcane companies).

Heat Monitoring

The heat stress and heat strain monitoring protocol have been previously described (Chapter 3). In brief, wet bulb globe monitoring was conducted at every work shift using TSI QUESTemp 46 Waterless wet bulb globe thermometers (TSI Incorporated, Shoreview, MN) placed near workers. ActiGraph wGT3X BT (ActiGraph, LLC, Pensacola, FL) accelerometers (worn on the hip) and Polar H7 heart rate monitors (Polar Electro Oy, Kempele, Finland) were worn by workers on all three workdays. Finally, core body temperature (T_c) was measured continuously using wireless, ingestible CorTemp® Disposable Temperature Sensors (HQ Inc., Palmetto, FL) on either 1 or 2 workdays (workers were randomly assigned to specific days of T_c monitoring). Data processing methods and summary statistics for these monitoring data can be found in Chapter 3.

Physical Monitoring

Weight and blood pressure were measured each day before and after the work shift using a Seca 769 medical grade column scale (Seca GmbH, Hamburg, Germany) and an Omron automatic blood pressure cuff (Omron Healthcare, Kyoto, Japan). Differences between pre- and post-shift weight measurements were not used to assess water loss via sweating as the protocol used at each measurement (e.g., clothing and equipment worn) were not consistent and the data there for not comparable. However, all six weight measurements were averaged in order to produce a best guess as to a worker’s typical weight for estimating energy expenditure; summary statistics for weight can be found in Chapter 3.

Biological samples

Clean catch urine samples were collected before and after each of the three work shifts, while blood samples were collected before and after the shift on the third day only, except for several brick workers (n=29) for whom blood was collected on the first or second day due to unpredictable work schedules. Trained lab technicians estimated urine osmolality using the PAL-mOsm handheld digital refractometer (ATAGO, Minato-ku, Tokyo, Japan) and specific gravity, pH, leukocytes, nitrites, protein, glucose, ketones, urobilinogen, bilirubin, and blood using Multistix®10 SG Reagent Strips (Siemens Healthineers AG, Erlangen, Germany) in El Salvador and Combur Test UX strips (Roche Diagnostics, Basel, Switzerland) in Nicaragua. Optical dipstick readers—CLINITEK Status (Siemens Healthineers AG, Erlangen, Germany) in El Salvador and Urisys 1100® (Roche Diagnostics, Basel, Switzerland) in Nicaragua—were also used. Samples were stored in -80°C freezers prior to shipment.

Serum samples from Nicaragua were analyzed at the National Laboratory in Nicaragua and samples from El Salvador were analyzed at Quest Diagnostics in the United States. Serum creatinine (SCr) (IDMS-traceable), calcium, chloride, glucose, phosphate (as phosphorus), potassium, sodium, urea nitrogen (BUN), and uric acid were analyzed at both laboratories. Total creatinine phosphokinase (CPK) was analyzed for Nicaraguan samples only at the National Laboratory in Nicaragua. Albumin and carbon dioxide were analyzed at Quest Diagnostics for Salvadoran samples only. Subsequent testing of a random sample of the pre-shift serum samples from each country (n=50 for each), conducted at Quest Diagnostics in 2021, confirmed minimal-to-no effect from the use of two separate laboratories in 2018.

A summary of the physiological significance captured by various urine and serum biomarkers can be found in Figure 4.1.

Questionnaires

A baseline questionnaire was administered upon enrollment to capture basic demographic information, work history, agrichemical use, medical history, medication use, smoking, alcohol use, diet, and family history of CKD. A post-shift questionnaire was also administered on each day to capture characteristics of that workday (start and stop time, breaks, comparisons to the previous day), hydration practices, medications taken, personal protective equipment worn, and symptoms experienced. All questionnaires were administered to participants by trained field team members.

Statistical Analyses

Pre-shift and post-shift estimated glomerular filtration rates (eGFR) were calculated from the SCr values using the CKD-EPI equation [109]. Cross-shift acute kidney injury was assessed using two definitions—the KDIGO definition of a cross-shift increase in SCr of ≥ 0.3 mg/dL *or* an increase of ≥ 50% [157] and a less conservative definition involving a cross-shift SCr increase of ≥ 0.2 mg/dL *or* an increase of ≥ 20% (“AKI – 20”).

Pre- and post-shift serum osmolality was calculated using the formula:

$$2 * Na^{+} + \frac{Glucose}{18} + \frac{BUN}{2.8}$$

To capture potentially large declines in circulating blood volume across the shift, participants were defined as having large reductions in blood pressure if they met the following criteria: a cross-shift decline in systolic blood pressure of ≥10 mmHg with a post-shift systolic blood pressure < 110 *or* a cross-shift decline in diastolic blood pressure of ≥10 mmHg with a post-shift diastolic blood pressure < 80.

Hydration practices were characterized with three self-reported variables capturing total amounts of different beverages consumed during the shift: water, electrolyte solution, and sweet beverages (soda, juice, and energy drink). The percent of the shift workers spent on break was defined using the amount of time the vector magnitude < 150 counts per minute, as described in Chapter 3. Summary statistics for hydration and time spent on break can also be found in Chapter 3.

Multivariate linear regression models were used to assess risk factors for cross-shift increases in SCr. Covariates for the models were determined using directed acyclic graphs, and increasingly adjusted models were run to examine the potential influence and importance of different confounders. For models including T_c, wet bulb globe temperature, or percent shift on break, workers with monitoring data capturing < 50% of the work shift were excluded. For all models, monitoring data from day 3 (same day as serum collection) were used, except for instances when T_c data were not collected on day 3 and data from day 2 were used instead. Workers whose shifts spanned more than 12 hours on day 3 (n=14) were removed from all models, as well as workers whose serum measurements were taken on day 1 or 2 (n=29). Models were run using three different subsets of the cohort: all participants (except for those excluded due to reasons described above), participants with pre-shift eGFR < 60 mL/min/1.73 m^2, and Nicaraguan sugarcane workers only. These latter two subsets were examined to remove potential confounding by impaired kidney function and to assess associations among workers exposed to greatest levels of heat strain and with the most workplace screening (Chapter 3), respectively. Effect estimates reported reflect an increase in the predictor variable of approximately one standard deviation of the respective variable.

Results

Biological Monitoring

Indicators of urine concentration (urine osmolality and specific gravity) were largely consistent across the non-sugar industries (Table 4.1). Urine osmolality dropped dramatically cross-shift among SUGAR-NI1 and plantain workers, whereas workers at SUGAR-NI3 started with low urine osmolality, but experienced some of the biggest cross-shift increases (along with SUGAR-NI2), suggesting within- and between-industry differences in dehydration before and during the work shift or potentially differences in urine concentrating ability. Interestingly, there were no workers at SUGAR-NI3 that had a urine specific gravity reading of ≥ 1.02. Urine pH findings were also largely consistent, except for workers at SUGAR-NI3, who were less likely to have a post-shift urine pH < 6. SUGAR-NI2 had the highest percentage of workers with post-shift urine < 6 (67.3%), followed by construction (53.4%) and SUGAR-NI1 (50%). Nicaraguan sugarcane workers had lower blood pressure readings, on average, than other workers. They were also much less likely to have hypertension (>140/90 mmHg) than workers at other industries or sugar companies, where the rate of hypertension ranged from 12.1-22.0%. Large declines in cross-shift blood pressure readings on day 3 were most common at SUGAR-NI1 (22.7%) and among brick workers (20.6%).

Post-shift hyponatremia (serum sodium ≤ 135 mmol/L) was most commonly observed at SUGAR-NI1 and SUGAR-ES2 (50% and 37.5%, respectively) (Table 4.2). The percent of workers with hyponatremia at all companies and industries increased from pre-shift to post-shift. Hypokalemia (serum potassium ≤ 3.5 mmol/L) was relatively common at some worksites, with the highest post-shift rates reported at SUGAR-NI2 (42.3%) and SUGAR-ES2 (35.7%). Pre-shift hyperuricemia (serum uric acid ≥ 7.0 mg/dL) was relatively common, especially among SUGAR-NI2 (31.8%), SUGAR-ES2 (26.8%), and plantain (25.4%) workers. Almost a third of workers at SUGAR-NI3 and brick (30% and 29%, respectively) and about a quarter of corn and plantain workers (23.6% and 25.4%, respectively) had a post-shift BUN:SCr ratio < 10. Few participants had a calculated post-shift serum osmolality ≥ 295 mOsm/kg, with the highest being four at SUGAR-ES1 and five among brick workers. Much higher percentages were seen for serum osmolality < 280 mOsm/kg, most notably at SUGAR-NI1 (n=8; 36.4%), SUGAR-ES2 (n=16; 28.6%), and corn (n=30; 27.3%). Post-shift serum CPK levels were largely consistent across the Nicaraguan industries and sugar companies. The highest CPK values measured were slightly above 1,500 U/L but were only observed in 2 workers (1 among brick and 1 at SUGAR-NI3).

Kidney Injury and Kidney Function

Workers at SUGAR-NI2 had the highest median pre-shift eGFR (122 mL/min/1.73 m^2) (Table 4.3). The median pre-shift eGFR was lowest at SUGAR-ES2 (101 mL/min/1.73 m^2) and SUGAR-NI1 (108 mL/min/1.73 m^2). The effect of the pre-harvest kidney function screening at the Nicaraguan sugar companies was apparent, as the percent of workers with pre-shift eGFR < 60 mL/min/1.73 m^2 was much lower at these companies— SUGAR-NI1 and SUGAR-NI3 had 0 workers meeting this criterion and SUGAR-NI2 only had 1. Rates of eGFR < 60 mL/min/1.73 m^2 at the other industries and sugar companies range from 3.4% to 23.2%, with SUGAR-ES2 being the highest followed by SUGAR-ES1.

The mean cross-shift difference in SCr was highest at SUGAR-NI2 (0.17 mg/dL), construction (0.10 mg/dL), and SUGAR-NI3 (0.09 mg/dL) (Table 4.3). This is mostly consistent with which companies and industries had the greatest percentage of workers meeting the two AKI criteria. Very few MANOS participants met the KDIGO AKI criteria on the day of workplace monitoring (n=15; 2.6%), except for SUGAR-NI2, where 23.1% of workers met the criteria (the next highest, SUGAR-ES2, only had 3.6%). Fifty-five participants (9.7%) met the less stringent AKI criteria of SCr increase of ≥ 0.20 mg/dL or ≥ 20%. The percentage was again greatest at SUGAR-NI2 (n=22; 42.3%), but then followed by SUGAR-NI3 (n=11; 22.0%) and construction (n=8; 13.8%).

Participants with pre-shift eGFR < 60 mL/min/1.73 m^2 had substantial differences in serum and urine measurements compared to participants with higher eGFRs (Table

4.4). For one, their urine was on average much more dilute, as evidenced by their lower median urine osmolality levels (post-shift urine osmolality: 355 mOsm/kg versus 500 mOsm/kg) and urine specific gravity values (post shift urine specific gravity ≥ 1.02: 18.9% versus 34.3%). Their post-shift urine was also more likely to be < 6 (56.6% versus 34.1%). These participants also had higher rates of hyponatremia, hypokalemia, hyperuricemia, and BUN:SCr < 10. They were also more likely to have extreme serum osmolality values and lower hemoglobin and hematocrit values.

Urine and serum biomarkers among participants with a cross-shift SCr increase of 0.2 mg/dL or 20% did not differ as drastically from participants not meeting that AKI criteria as the differences described above for eGFR </> 60 (Table 4.4). Their pre-shift urine was slightly more dilute (median pre-shift urine osmolality: 440 mOsm/kg versus 490 mOsm/kg), but interestingly their post-shift urine had similar osmolality readings, but much different dipstick specific gravity readings (post-shift urine specific gravity ≥ 1.02: 49.1% versus 31.0%). Their pre-shift urine was slightly more basic (pH ≥ 7: 40.0% versus 21.8%), but post-shift urine was more acidic (pH < 6: 45.5% versus 35.3%). Participants not meeting the AKI criteria were more likely to have hyponatremia post-shift (22.0% versus 9.1%). Pre-shift hyperuricemia was more common among those not meeting the AKI criteria (18.2% versus 9.1%), while rates of post-shift hyperuricemia were comparable (19.8% versus 21.8%). This is reflected in the mean pre- and post-shift uric acid values, which were slightly higher pre-shift for those without AKI, but slightly higher post-shift for those with AKI. Serum potassium and BUN values were largely consistent, although participants meeting the AKI criteria had slightly higher post-shift

BUN values (14.4 mg/dL versus 12.8 mg/dL). Participants not meeting the AKI criteria were much more likely to have a post-shift serum osmolality < 280 mOsm/kg (21.4% versus 3.6%) and had slightly higher mean hematocrit and hemoglobin values. Median CPK values were slightly higher for participants with AKI, but the participants with the highest CPK values (> 1,000 U/L) were among those not meeting the AKI criteria.

Predictors of Cross-Shift Difference in Serum Creatinine

Maximum core body temperature measured during the work shift was positively associated with a cross-shift increase in serum creatinine in the entire population and in the subset with pre-shift eGFR > 60 mL/min/1.73 m^2—0.023 mg/dL (95% CI: 0.008, 0.039) and 0.015 mg/dL (95% CI: -0.001, 0.030) increase per 0.5°C, respectively (Table 4.5). Post-shift urine osmolality was associated with a smaller increase in serum creatinine in these groups—0.009 (95% CI: -0.001, 0.020) and 0.007 (95% CI: -0.003, 0.017). When restricted to only Nicaraguan sugarcane workers, the effect for maximum Tc diminished (0.001 mg/dL, 95% CI: -0.044, 0.045) while the effect estimate for post-shift urine osmolality increased (0.021 mg/dL, 95% CI: -0.014, 0.056).

Mean work rate was also positively associated with increases in serum creatinine, a finding that remained consistent even after sub-setting to workers with eGFR>60 and Nicaraguan sugarcane workers only (effect estimate across entire cohort: 0.031 mg/dL per 100 kcal/hour, 95% CI: 0.017, 0.044) (Table 4.6). The results for time spent at a low vector magnitude were consistent with either a slightly protective effect or no effect (effect estimate across entire cohort: -0.007 mg/dL per 10% of shift at low vector magnitude, 95% CI: -0.021, 0.008).

There was mixed evidence on the relationship between serum uric acid and serum creatinine, depending on whether pre-shift or post-shift values were used (Table 4.7). When post-shift values for serum uric acid were used, there was a consistent, positive relationship with cross-shift increase in serum creatinine (effect estimate among Nicaraguan workers with eGFR > 60: 0.021 mg/dL per 1.5 mg/dL of uric acid, 95% CI: -0.006, 0.048), however, no evidence of an association was seen for pre-shift uric acid values (-0.004 mg/dL, 95% CI: -0.032, 0.023). The findings for serum CPK were more consistent, with small, positive effect estimates observed for both pre- and post-shift CPK.

Consumption of the three types of beverages examined (water, electrolyte serum, and fructose-containing beverages) appeared protective against cross-shift increases in serum creatinine, with moderate effect estimates ranging from -0.013 to -0.016 mg/dL (Table 4.8). These analyses were restricted to Nicaraguan sugarcane workers only, as they were the only workers consistently reporting consumption of electrolyte serum. However, in models examining water and fructose-containing beverage consumption amongst the other industries and Salvadoran sugar companies, the effect estimates were much closer to the null (water: -0.001 mg/dL per 2.5L, 95% CI: -0.016, 0.015; fructose-containing beverages: -0.002 mg/dL per 0.25L, 95% CI: -0.019, 0.014; model output not shown).

Self-reported medication use on the day of serum collection was very rare—two workers reporting use of any NSAID/analgesic, six workers reporting use of acetaminophen, one reporting use of ibuprofen, and seven reporting use of home remedies or herbal medication. Therefore, self-reported use of medications in the prior week was used to examine potential effects on cross-shift increases in serum creatinine. Sample sizes were still rather small for medication use, so the models were much less stable (Table 4.9). Mixed findings were observed for acetaminophen, while consistently positive associations were seen for NSAID use and consistently negative associations were seen for ibuprofen use (however, with large confidence intervals).

Workers who reported having a father diagnosed with CKD unexpectedly had lower cross-shift changes in serum creatinine (effect estimate among Nicaraguan sugarcane workers: -0.023 mg/dL, 95% CI: -0.076, 0.029) while workers who reported having a brother with CKD appeared to have very little difference in cross-shift serum creatinine, except when sub-set among only Nicaraguan sugarcane workers (effect estimate: 0.021, 95% CI: -0.035, 0.077) (Table 4.10). It should be noted that the sample sizes for self-reported family history of CKD were also relatively small (14.8% reported a father with CKD; 8.3% reported a brother with CKD) and varied by industry and company (range for father with CKD: 6.9% - 25.0%; range for brother with CKD: 0.0% - 22.7%).

Discussion

In this large observational study of cross-shift measures of kidney injury among outdoor workers in various industries and job tasks in El Salvador and Nicaragua, we found high cross-shift incidence of AKI (SCr increase of 0.3 mg/dL or 50%) among workers at one Nicaraguan sugarcane company (23.1% of workers), but virtually none among workers in other sugar companies and industries (overall incidence: 2.6%). When using a less conservative definition (SCr increase of ≥ 0.2 mg/dL or 20%), we found rates to be relatively high at two sugarcane companies (42.3% and 22.0%) and among construction workers (13.8%). These latter rates were consistent with what has been observed in previous studies of cross-shift kidney injury among outdoor workers in Central America [83]. Job task was an important predictor of AKI among Nicaraguan sugarcane workers in this study; cane cutters represented ~60% of the workers recruited at SUGAR-NI2 and SUGAR-NI3, but 86% and 91% of the workers with AKI at these companies, respectively. Cross-shift decline in eGFR was greatest among workers at Nicaraguan sugarcane companies and construction workers in El Salvador (range: -5.4 to -14.0 mL/min/1.73 m^2). The findings for construction workers are somewhat unexpected as they had relatively low average work rates and spent a greater portion of the work shift on break, but could potentially be explained by the longer work shifts and exposure to higher average wet bulb globe temperatures (Chapter 3).

Interestingly, higher pre-shift eGFR was not associated with smaller cross-shift changes in SCr after controlling for industry/company of employment and job task, except among Salvadoran sugarcane workers (effect estimate: -0.012 mg/dL per 20 mL/min increase in eGFR). Among brick and plantain workers, higher pre-shift eGFR was associated with larger cross-shift changes in SCr. This is in contrast with other studies, which have found low baseline eGFR to be a strong risk factor for cross-shift AKI [128] and may suggest some misclassification in these variables in our study (e.g., estimation of eGFR in non-steady state conditions) or could indicate that cross-shift serum creatinine is being influenced by factors unrelated to kidney function (e.g., creatinine production).

The different findings between Nicaraguan sugarcane companies are interesting from a qualitative perspective, as we know from speaking with company personnel that there are differences in hydration practices and break schedules between the three companies. SUGAR-NI1 and SUGAR-NI3 had certain policies in place at the time of MANOS data collection, including required breaks and obligatory hydration with both water and rehydration serum. During the MANOS data collection, SUGAR-NI2 was still in the process of developing similar policies. Interestingly though, we observed similar break patterns between the companies (Chapter 3). Workers at SUGAR-NI3 reported greater water intake (L/hr) and total electrolyte solution intake. Direct comparisons between the companies should be done with caution, however, as we did not recruit any cane cutters at SUGAR-NI1. However, the average cross-shift increases in SCr were comparable for the agrichemical applicators across all three companies. The high rates of AKI at SUGAR-NI2 were driven by cane cutters (86% of AKI cases) and when cane cutters were compared between SUGAR-NI2 and SUGAR-NI3, we saw higher cross-shift SCr increases at SUGAR-NI2, which is also consistent with the anecdotal information about hydration and break practices, if these interventions are causally protective.

Markers of concentrated urine (e.g., urine osmolality > 700 mOsm/kg and urine specific gravity > 1.02) were common among MANOS participants, particularly among workers of specific sugarcane companies (SUGAR-NI2 and SUGAR-ES1) and construction workers. High BUN:SCr ratios (>20) were very uncommon, suggesting that increases in SCr were not entirely due to reduced renal blood flow. Hyperuricemia (serum uric acid > 7.0 mg/dL) was not uncommon across the industries, even among individuals with eGFR > 60 mL/min/1.73 m^2 (14.3%). Extreme serum CPK values were uncommon (maximum: 1,591.89 U/L); CPK > 1,000 U/L was only observed in three individuals, all of whom had eGFR>60 and did not experience AKI cross-shift.

Participants with baseline eGFR < 60 had similar urine and serum findings as other studies on CKDu—higher rates of hypokalemia, hyperuricemia, and extreme serum sodium values [2,27,33]. These participants also had less concentrated urine pre- and post-shift, even after controlling for industry/company of employment, suggesting potential impaired urine concentrating ability. If true, this impaired urine concentrating ability possibly has a detrimental effect on these workers during their work shifts, as increased water loss through sweating necessitates water retention by the kidneys to maintain blood volume, organ perfusion, and continued heat loss through sweating. If this is happening, we might expect a cyclical effect by which impaired concentrating ability induces recurrent AKI, which induces the progression of kidney disease. A recent study conducted on French CKD patients found that lower fasting urine osmolality (suggestive of impaired concentrating ability) was associated with greater declines in measured GFR over time and a 2-fold increase in the risk of end stage renal disease, even when controlling for baseline GFR [158]. This may indicate greater tubular damage in certain patients than would be expected based solely on the GFR.

Core body temperature was one of the strongest predictors of cross-shift SCr increases after industry/company and job task. Interestingly, urine osmolality, which had a relatively small effect estimate in the full sample of workers, had a greater effect estimate among Nicaraguan sugarcane workers, while Tc had a near-null effect among this subset. This is perhaps indicative of greater T_c misclassification among these workers, acclimatization to greater T_c levels, or noise in the data. However, these findings may also be the result of the pre-harvest SCr screening conducted at these companies, if these workers were less likely to have kidney disease at the time of sampling and therefore were less susceptible to the effects of T_c on kidney injury. These models should be interpreted with some caution, however, as it is difficult to adequately capture the elevations in core body temperature as well as dehydration experienced throughout a work shift with single values, which is made more difficult since they would be expected to continuously influence each other (e.g., rises in core body temperature necessitates increased sweat rate and reduced blood volume impairs thermoregulatory capacity).

Greater metabolic heat load, as captured by the mean estimated work rate, was also a relatively strong risk factor for cross-shift increases in SCr, while the percent of the shift spent at a low vector magnitude (<150 cpm), which is a proxy for being on break, was only moderately protective. These findings can be interpreted as further support for calls to reduce the physical workload for workers in the most demanding jobs (e.g., cane cutters) through administrative and engineering controls and increase the duration of time spent on break. It is very possible that the effect estimate for the percent of shift on break is an underestimate due to misclassification, as VM < 150 cpm has not been validated in this population.

Consumption of water, electrolyte serum, and fructose-containing beverages appeared protective against cross-shift increases in serum creatinine, but with moderate effect estimates. These findings provide further support for the importance of adequately hydrating during the work shift, as well as evidence against a detrimental effect of fructose-containing beverages (at least on an acute time scale). Given that these variables were not associated with decreases in maximum T_c, it appears that any real protective effect may be through another pathway (perhaps adequate renal perfusion).

The findings for serum uric acid and cross-shift SCr increases simultaneously support and contradict the prior research on this causal question [128]. While we also found that post-shift serum uric acid is associated with cross-shift increases in SCr, when we examined the relationship between these biomarkers using pre-shift uric acid values, we found a null-to-marginally-protective effect estimate. These findings support the possibility that uric acid is not a risk factor for AKI in this population, but rather that previous studies have found an association because reductions in glomerular filtration during the shift would cause SCr and uric acid to rise concurrently, which is impossible to disentangle with cross-sectional data. More research is needed on this topic, but future studies will need to be designed to avoid this potential for confounding. Interestingly, we found that CPK was consistently associated with small increases in SCr, whether pre-shift or post-shift values are used, a finding which is also not consistent with previous research on this question [128].

The findings for medications and family history should be interpreted with caution as sample sizes are small, confidence intervals are wide, and misclassification is expected. The medication findings are difficult to believe—especially for ibuprofen, which appeared protective—especially given that very few workers reported taking any medications on the day of serum sampling. The findings for family history of CKD—namely having a father with CKD appearing protective—may be explained by misclassification (e.g., perhaps some cases of classic CKD and not just CKDu were included). Self-report of having a brother with CKD might be less misclassified as brothers are more likely to be younger than fathers and therefore a CKD diagnosis is more likely to represent CKDu. We might also expect Nicaraguan sugarcane workers to have less misclassification, as the self-reported occupation of brothers with a CKD diagnosis indicates that almost all of them were also sugarcane workers and therefore received screening for their serum creatinine values, which have been reported back to them.

There are certainly limitations with these analyses that warrant consideration when putting the findings into the context of disease etiology. For one, the observed increases in SCr may not indicate kidney injury or reduced glomerular filtration at all, but rather increased creatinine production through protein or muscle metabolism (a critique that has been raised by other researchers). In fact, it would take around 5 hours to observe an SCr increase of 0.5 mg/dL in extreme cases when creatinine clearance has reduced by 100% and closer to 10 hours if creatinine clearance is reduced by 60% [159]. Given that many of the sugarcane workers in this study worked 5 hours or less and we wouldn't expect a reduction in creatinine clearance of 100%, it is unreasonable to assume that the observed cross-shift increases in SCr are entirely due to reduced creatinine clearance.

However, in lab-controlled settings, researchers have demonstrated serum creatinine increases of comparable or greater magnitudes, as well as concurrent increases in kidney injury biomarkers, among participants exercising in hyperthermic and/or dehydrated states after only two hours [84]. Similarly, Hansson et al. 2021 found that kidney injury biomarkers were higher among Nicaraguan sugarcane workers with cross-harvest serum creatinine increases [160]. These studies support the idea that fluctuations in SCr are not due to muscle or protein metabolism alone and do correlate with established kidney injury biomarkers. There is also evidence that fluctuations in SCr are predictive of eGFR declines cross-harvest in populations at risk for MeN [69,155], however, we cannot exclude the possibility that these findings may represent reverse causation.

There are also limitations with using urine concentration metrics (urine osmolality, urine specific gravity) as proxy measures for dehydration, as these are both influenced by urine concentrating ability and may not fully capture whether workers are adequately hydrated. Cross-shift change in body weight is a more ideal measure, but is very difficult to measure accurately in a field setting. High serum osmolality (>295 mOsm/kg), while estimated and not directly measured, was not common at post-shift, suggesting workers were not severely dehydrated at the time of blood collection. The best ways to accurately assess dehydration is an ongoing topic of debate, even among clinicians [161], and this task is made even more complicated by the potential confounding of kidney injury/disease.

Another possible limitation is the potential that employers and workers may have altered their work behaviors while they knew they were being observed for the study, to give the appearance that the work is not as intense as it is. We suspect this may have been the case at some of the sugarcane companies, in part because the short work shift durations we observed during the study are not consistent with what we have observed previously or has been reported in other studies. This might potentially explain the lower AKI rates we observed compared to other studies conducted among sugarcane workers in the region [83].

We are also limited by the fact that we only collected serum samples on one day and are therefore limited in the conclusions we can draw, as this day of monitoring may not be reflective of every participant's typical workday. We also had to use spot urines for the urine biomarkers, which may influence many of the biomarkers. We only had CPK for Nicaragua, limiting the analyses for rhabdomyolysis. Finally, workers were monitored during different months, purely because of the logistics of enrolling and monitoring hundreds of workers across three workdays each, which could introduce some healthy worker selection bias, if workers became sick as the harvest season went on and left their job before we had a chance to enroll them in the study. Finally, for some analyses, we found quite large confidence intervals, suggesting limited precision in our effect estimates. However, our findings are largely in agreement with previous studies and directions of effect are consistent with *a priori* hypotheses (excluding family history and medication use).

Conclusion

Sugarcane workers, especially in Nicaragua, and construction workers were more likely to have cross-shift increases in serum creatinine. Among the three Nicaraguan sugarcane companies, the company with less developed workplace interventions to reduce heat stress had higher cross-shift increases in serum creatinine and had the highest percentage of workers experiencing AKI. Among sugarcane workers, cane cutters had higher increases in serum creatinine, despite having similar accelerometer-based work rates as agrichemical applicators in Nicaragua. This study provides further evidence that elevated core body temperatures, work rate, and potential dehydration are involved in the incidence of AKI experienced among outdoor workers in Central America, which may increase the risk of MeN.

Kidney injury biomarkers will be needed to better assess kidney injury. Kidney injury biomarker analyses that corroborate these findings would strengthen the evidence in this chapter and in prior research on the topic. This would further support the hypothesis that intense physical work in hot environments leads to elevated core body temperature and dehydration, causing reduced renal blood flow, direct injury to kidney tissue, and/or indirect injury through the release of vasopressin/activation of aldose reductase pathway. Other important avenues for future research include examining the relationship between recurrent AKI and CKD onset and progression, as well as exploring potential interactive effects between heat strain, dehydration, and exposure to nephrotoxic chemicals.

Biomarker	Definition	Physiological Significance
Urine osmolality	• Concentration of particles in the urine (e.g., sodium, chloride, proteins, glucose) per kg	• Clinically used to assess urine concentrating ability • In healthy individual, fluid restriction leads to the release of antidiuretic hormone (ADH), prompting the kidneys to concentrate the urine, which results in urine with high osmolality • Used in prior research on MeN as a surrogate for dehydration from inadequate hydration/excessive sweating (>700)
Urine specific gravity	• A ratio of urine density relative to water density • A less accurate measure of urine concentration than urine osmolality	• Clinically used to assess urine concentrating ability • Used in prior research on MeN as a surrogate for dehydration (>1.02)
Urine pH	• A measure on the logarithmic scale assessing the acidity/alkalinity of urine	• High urine pH can be caused by kidney disease while a low pH can be caused by dehydration • Influences the potential for crystallization of various urine solutes and risk of kidney stones • Can be used clinically to assess metabolic disorders
Serum sodium	• Electrolyte/mineral in the blood involved in vital body functions (e.g., muscle and nerve activity, fluid regulation)	• Indicative of total body water; higher serum sodium indicates less water in the body • High serum sodium can be caused by loss of urine concentrating ability or dehydration
Serum potassium	• Electrolyte/mineral in the blood involved in vital body functions (e.g., muscle and nerve activity, protein synthesis)	• Consumed in the diet and released by muscles during physical activity • Typically increases with lower eGFR, as kidneys are unable to excrete excess amounts into the urine • Hypokalemia (<3.5) unlikely in healthy individuals but common among MeN patients (may be an early indicator)
Serum osmolality	• Concentration of particles in the blood (e.g., sodium, chloride, proteins, glucose) per kg • Mainly determined by serum sodium concentration	• Increases with dehydration as blood is more concentrated • High serum osmolality leads to the release of ADH, prompting the kidneys to concentrate the urine/retain water • When directly measured, is considered a good measure of dehydration clinically

Serum uric acid	• Compound released during the breakdown of purines in cells or certain foods	• Increases with lower eGFR and dehydration; found to be higher than expected in MeN patients (compared to CKD patients in U.S.) • High levels can cause kidney stones and kidney disease • Crystallizes in acidic urine; made worse by dehydration • Hyperuricemia may be an early indicator of MeN
Blood urea nitrogen (BUN)	• Concentration of urea in the blood, a waste product of protein breakdown in the body	• Increases with lower eGFR and dehydration; decreases with malnutrition and overhydration • Clinically, the ratio of BUN to SCr is often calculated; values > 20 suggest impaired renal blood flow (severe dehydration)

Figure 4.1. Definitions and physiological significance of various urine and serum biomarkers

Table 4.1. Summary of pre- and post-shift urine biomarker and blood pressure data on Day 3, by industry/company

	El Salvador				Nicaragua				
	SUGAR-ES1	**SUGAR-ES2**	**CORN**	**CONS**	**SUGAR-NI1**	**SUGAR-NI2**	**SUGAR-NI3**	**BRICK**	**PLAN**
Total participants	55	56	110	58	22	52	50	107	59
Urine Osmolality (mOsm/kg)									
Pre-Shift, Mean (SD)	491 (233)	421 (212)	468 (232)	521 (245)	502 (207)	511 (227)	341 (202)	556 (251)	617 (200)
Post-Shift, Mean (SD)	539 (283)	459 (251)	456 (246)	508 (220)	380 (259)	628 (188)	414 (229)	547 (258)	500 (225)
Pre-Shift ≥ 700, n (%)	14 (25.5%)	6 (10.7%)	14 (12.7%)	12 (20.7%)	3 (13.6%)	11 (21.2%)	3 (6.0%)	34 (31.8%)	23 (39.0%)
Post-Shift ≥ 700, n (%)	19 (34.5%)	9 (16.1%)	21 (19.1%)	15 (25.9%)	4 (18.2%)	22 (42.3%)	6 (12.0%)	31 (29.0%)	14 (23.7%)
Urine Specific Gravity									
Pre-Shift, Median (Min, Max)	1.015 [1.005, 1.030]	1.013 [1.005, 1.030]	1.020 [1.005, 1.030]	1.015 [1.005, 1.030]	1.010 [1.000, 1.025]	1.010 [1.000, 1.025]	1.005 [1.000, 1.015]	1.015 [1.000, 1.030]	1.010 [1.000, 1.030]
Post-Shift, Median (Min, Max)	1.020 [1.005, 1.030]	1.010 [1.000, 1.030]	1.015 [1.005, 1.030]	1.020 [1.005, 1.030]	1.010 [1.005, 1.020]	1.015 [1.000, 1.030]	1.010 [1.000, 1.015]	1.010 [1.000, 1.030]	1.010 [1.000, 1.030]
Pre-Shift ≥ 1.02, n (%)	26 (47.3%)	13 (23.2%)	55 (50.0%)	27 (46.6%)	2 (9.1%)	7 (13.5%)	0 (0%)	33 (30.8%)	10 (16.9%)
Post-Shift ≥ 1.02, n (%)	26 (47.3%)	17 (30.4%)	49 (44.5%)	36 (62.1%)	6 (27.3%)	19 (36.5%)	0 (0%)	25 (23.4%)	9 (15.3%)
Urine pH									
Pre-Shift, Median (Min, Max)	6.00 [5.00, 8.50]	6.00 [5.00, 7.50]	6.00 [5.00, 8.50]	6.00 [5.00, 7.50]	6.00 [5.00, 8.00]	6.00 [5.00, 8.00]	7.00 [5.00, 9.00]	6.00 [5.00, 8.00]	6.00 [5.00, 8.00]

Post-Shift, Median (Min, Max)	6.00 [5.00, 8.50]	6.00 [5.00, 7.50]	6.00 [5.00, 8.50]	5.50 [5.00, 7.00]	5.50 [5.00, 7.00]	5.00 [5.00, 8.00]	7.00 [5.00, 9.00]	6.00 [5.00, 9.00]	7.00 [5.00, 9.00]
Pre-Shift < 6.0, n (%)	24 (43.6%)	25 (44.6%)	26 (23.6%)	23 (39.7%)	7 (31.8%)	23 (44.2%)	5 (10.0%)	35 (32.7%)	17 (28.8%)
Post-Shift < 6.0, n (%)	24 (43.6%)	22 (39.3%)	32 (29.1%)	31 (53.4%)	11 (50.0%)	35 (67.3%)	3 (6.0%)	32 (29.9%)	16 (27.1%)
Blood Pressure (mmHg)									
Pre-Shift Systolic BP, Mean (SD)	127 (13.2)	125 (12.7)	124 (12.6)	125 (12.0)	125 (13.2)	120 (10.7)	119 (10.9)	129 (14.7)	128 (12.3)
Post-Shift Systolic BP, Mean (SD)	124 (12.9)	124 (12.3)	122 (11.5)	127 (12.0)	121 (9.31)	118 (10.3)	118 (11.7)	124 (12.3)	128 (13.6)
Pre-Shift Diastolic BP, Mean (SD)	73.6 (8.73)	71.9 (11.0)	74.0 (9.39)	75.2 (9.07)	66.3 (11.5)	67.6 (10.3)	66.8 (10.8)	79.0 (10.2)	75.7 (9.17)
Post-Shift Diastolic BP, Mean (SD)	73.1 (8.05)	71.8 (11.6)	71.9 (9.05)	77.7 (8.90)	70.7 (11.7)	69.0 (9.39)	69.4 (9.19)	75.6 (8.77)	78.9 (9.92)
Pre-Shift Hypertension (>140/90), n (%)	9 (16.4%)	8 (14.3%)	14 (12.7%)	7 (12.1%)	2 (9.1%)	1 (1.9%)	3 (6.0%)	18 (16.8%)	13 (22.0%)
Substantial Cross-Shift Decline in BP, n (%)	7 (12.7%)	6 (10.7%)	20 (18.2%)	3 (5.2%)	5 (22.7%)	8 (15.4%)	8 (16.0%)	22 (20.6%)	2 (3.4%)

BP = blood pressure

Table 4.2. Summary of pre- and post-shift serum biomarker data on Day 3, by industry/company

	El Salvador				**Nicaragua**				
	SUGAR-ES1	**SUGAR-ES2**	**CORN**	**CONS**	**SUGAR-NI1**	**SUGAR-NI2**	**SUGAR-NI3**	**BRICK**	**PLAN**
Total participants	55	56	110	58	22	52	50	107	59
Sodium (mmol/L)									
Pre-Shift, Mean (SD)	138 (2.75)	138 (1.78)	137 (3.34)	137 (2.09)	139 (1.47)	139 (1.70)	138 (1.40)	138 (2.17)	138 (1.79)
Post-Shift, Mean (SD)	138 (4.03)	136 (2.54)	137 (2.88)	137 (2.05)	136 (2.74)	138 (2.37)	137 (2.13)	138 (2.76)	138 (2.65)
Pre-Shift ≤ 135, n (%)	6 (10.9%)	3 (5.4%)	19 (17.3%)	6 (10.3%)	0 (0%)	2 (3.8%)	0 (0%)	9 (8.4%)	3 (5.1%)
Post-Shift ≤ 135, n (%)	12 (21.8%)	21 (37.5%)	25 (22.7%)	10 (17.2%)	11 (50.0%)	7 (13.5%)	10 (20.0%)	12 (11.2%)	9 (15.3%)
Potassium (mmol/L)									
Pre-Shift, Mean (SD)	3.95 (0.47)	3.88 (0.44)	3.91 (0.41)	3.79 (0.26)	4.01 (0.43)	3.76 (0.50)	4.14 (0.29)	4.01 (0.52)	4.07 (0.40)
Post-Shift, Mean (SD)	3.85 (0.49)	3.69 (0.41)	3.74 (0.35)	3.76 (0.28)	3.80 (0.38)	3.72 (0.60)	3.96 (0.47)	3.95 (0.58)	3.96 (0.38)
Pre-Shift ≤ 3.5, n (%)	10 (18.2%)	13 (23.2%)	17 (15.5%)	4 (6.9%)	4 (18.2%)	15 (28.8%)	2 (4.0%)	15 (14.0%)	6 (10.2%)
Post-Shift ≤ 3.5, n (%)	10 (18.2%)	20 (35.7%)	25 (22.7%)	10 (17.2%)	6 (27.3%)	22 (42.3%)	6 (12.0%)	24 (22.4%)	10 (16.9%)
Uric Acid (mg/dL)									
Pre-Shift, Mean (SD)	5.71 (1.93)	6.10 (1.83)	5.68 (1.73)	6.09 (1.32)	6.38 (1.08)	5.65 (1.17)	5.19 (1.06)	5.91 (1.90)	5.96 (1.76)
Post-Shift, Mean (SD)	5.86 (1.93)	6.22 (1.96)	5.83 (1.73)	6.35 (1.18)	6.39 (1.10)	6.09 (1.23)	5.47 (1.26)	5.85 (1.75)	5.92 (1.78)

Pre-Shift ≥ 7.0, n (%)	8 (14.5%)	15 (26.8%)	17 (15.5%)	10 (17.2%)	7 (31.8%)	7 (13.5%)	2 (4.0%)	17 (15.9%)	15 (25.4%)
Post-Shift ≥ 7.0, n (%)	10 (18.2%)	16 (28.6%)	18 (16.4%)	15 (25.9%)	6 (27.3%)	9 (17.3%)	5 (10.0%)	19 (17.8%)	15 (25.4%)
Blood Urea Nitrogen (mg/dL)									
Pre-Shift, Mean (SD)	13.6 (7.80)	14.1 (6.60)	11.7 (6.59)	10.4 (3.36)	12.5 (2.87)	11.7 (4.22)	9.64 (3.27)	13.1 (7.68)	11.4 (6.81)
Post-Shift, Mean (SD)	14.7 (8.16)	15.3 (6.45)	12.5 (6.57)	12.3 (3.82)	12.7 (2.64)	13.1 (4.46)	11.0 (3.74)	13.0 (7.76)	11.9 (6.47)
Post-Shift BUN: SCr < 10, n (%)	8 (14.5%)	8 (14.3%)	26 (23.6%)	10 (17.2%)	3 (13.6%)	9 (17.3%)	15 (30.0%)	31 (29.0%)	15 (25.4%)
Post-Shift BUN: SCr 10-20, n (%)	45 (81.8%)	47 (83.9%)	82 (74.5%)	46 (79.3%)	19 (86.4%)	43 (82.7%)	35 (70.0%)	73 (68.2%)	42 (71.2%)
Post-Shift BUN: SCr ≥ 20, n (%)	2 (3.6%)	1 (1.8%)	0 (0%)	2 (3.4%)	0 (0%)	0 (0%)	0 (0%)	3 (2.8%)	1 (1.7%)
Calculated Serum Osmolality (mOsm/kg)									
Pre-Shift, Mean (SD)	286 (4.07)	286 (3.89)	284 (6.66)	283 (4.55)	288 (3.12)	286 (3.42)	285 (2.75)	286 (4.62)	286 (3.51)
Post-Shift, Mean (SD)	285 (6.93)	282 (5.83)	283 (5.28)	283 (4.34)	282 (5.15)	285 (5.12)	282 (4.66)	285 (5.85)	285 (4.62)
Post-Shift ≥ 295, n (%)	4 (7.3%)	1 (1.8%)	0 (0%)	1 (1.7%)	0 (0%)	1 (1.9%)	0 (0%)	5 (4.7%)	0 (0%)
Post-Shift < 280, n (%)	12 (21.8%)	16 (28.6%)	30 (27.3%)	11 (19.0%)	8 (36.4%)	5 (9.6%)	11 (22.0%)	10 (9.3%)	8 (13.6%)
Creatine Phosphokinase (U/L)									
Pre-Shift, Median (Min, Max)	-	-	-	-	224 [136, 414]	213 [100, 561]	187 [79.2, 1,080]	230 [65.4, 1,310]	176 [92.4, 645]
Post-Shift, Median (Min, Max	-	-	-	-	272 [174, 529]	253 [122, 701]	227 [103, 1,540]	256 [73.8, 1,590]	216 [108, 820]

BUN = blood urea nitrogen

Table 4.3. Summary of pre- and post-shift serum creatinine and eGFR data on Day 3 and participants meeting AKI criteria, by industry/company

	El Salvador				**Nicaragua**				
	SUGAR-ES1	**SUGAR-ES2**	**CORN**	**CONS**	**SUGAR-NI1**	**SUGAR-NI2**	**SUGAR-NI3**	**BRICK**	**PLAN**
Serum Creatinine (mg/dL)									
Pre-Shift, Median (Min, Max)	0.870 [0.620, 4.70]	0.985 [0.690, 4.84]	0.840 [0.550, 5.95]	0.820 [0.600, 2.12]	0.990 [0.670, 1.40]	0.805 [0.610, 2.67]	0.840 [0.680, 1.24]	0.900 [0.580, 8.54]	0.880 [0.650, 10.2]
Post-Shift, Median (Min, Max)	0.890 [0.650, 4.60]	1.02 [0.670, 4.76]	0.875 [0.590, 6.01]	0.920 [0.670, 2.29]	1.02 [0.740, 1.48]	0.995 [0.670, 2.97]	0.945 [0.700, 1.47]	0.880 [0.530, 8.23]	0.860 [0.620, 9.90]
Cross-shift difference, mean (SD)	0.04 (0.06)	0.04 (0.15)	0.03 (0.07)	0.10 (0.09)	0.06 (0.05)	0.17 (0.17)	0.09 (0.07)	-0.03 (0.11)	-0.02 (0.10)
Percent cross-shift change, mean (SD)	4.0% (6.2%)	2.8% (11%)	3.3% (8.8%)	11.6% (9.6%)	6.7% (5.9%)	20.4% (20.5%)	10.9% (8.3%)	-0.90% (8.2%)	-0.57% (8.7%)
Estimated Glomerular Filtration Rate (mL/min/1.73 m^2)									
Pre-Shift, Median (Min, Max)	118 [14.0, 143]	101 [14.3, 135]	120 [10.9, 145]	117 [39.1, 140]	108 [66.7, 137]	122 [31.5, 144]	118 [78.6, 135]	114 [7.1, 149]	117 [6.2, 138]
Post-Shift, Median (Min, Max)	116 [14.4, 140]	100 [14.6, 137]	116 [10.8, 143]	109 [35.7, 132]	102 [60.0, 132]	105 [27.7, 138]	110 [64.0, 130]	113 [7.4, 154]	117 [6.4, 138]
Pre-Shift < 60, n (%)	9 (16.4%)	13 (23.2%)	12 (10.9%)	2 (3.4%)	0 (0%)	1 (1.9%)	0 (0%)	11 (10.3%)	5 (8.5%)
Cross-shift difference, mean (SD)	-2.9 (4.7)	-1.8 (7.7)	-2.2 (7.0)	-8.5 (8.0)	-5.4 (4.6)	-14.0 (14)	-9.0 (7.5)	+0.27 (6.7)	1.0 (7.5)
Percent cross-shift change, mean (SD)	-2.9% (4.6%)	-2.3% (8.5%)	-2.0% (6.5%)	-7.7% (7.4%)	-5.4% (4.7%)	-12% (13%)	-7.9% (6.8%)	+0.97% (7.5%)	1.6% (8.5%)
Acute Kidney Injury									
KDIGO Criteria, n (%)	0 (0%)	2 (3.6%)	0 (0%)	1 (1.7%)	0 (0%)	12 (23.1%)	0 (0%)	0 (0%)	0 (0%)

SCr increase ≥ 0.2 mg/dL or 20%, n (%)	1 (1.8%)	4 (7.1%)	7 (6.4%)	8 (13.8%)	0 (0%)	22 (42.3%)	11 (22.0%)	2 (1.9%)	0 (0%)
Job Tasks Meeting AKI Criteria									
KDIGO Criteria	N/A	Cane Cutter (n=2)	N/A	Traffic Control (n=1)	N/A	Cane Cutter (n=11); Agri-chemical Applicator (n=1)	N/A	N/A	N/A
SCr increase of 0.2 mg/dL or 20%	Irrigator (n=1)	Cane Cutter (n=4)	Harvester (n=6); Carrier (n=7)	Various Tasks (n=5); Supervisor/ Driver (n=2); Traffic Control (n=1)	N/A	Cane Cutter (n=19); Agri-chemical Applicator (n=3)	Cane Cutter (n=10); Agri-chemical Applicator (n=1)	Brick mixer/ molder (n=2)	N/A

SCr = serum creatinine

Table 4.4. Summary of serum and urine biomarkers and blood pressure findings, by pre-shift eGFR </> 60 and AKI-20 criteria

	Pre-Shift eGFR		**SCr increase of 0.2 mg/dL or 20%**	
	> 60	**<60**	**No**	**Yes**
Total participants	516	53	510	55
Urine Osmolality				
Pre-Shift, Median (Min, Max)	500 [40.0, 1280]	350 [150, 910]	490 [40.0, 1280]	440 [100, 1000]
Post-Shift, Median (Min, Max)	500 [40.0, 1130]	355 [130, 790]	490 [40.0, 1130]	500 [150, 1110]
Pre-Shift ≥ 700, n (%)	118 (22.9%)	2 (3.8%)	112 (22.0%)	7 (12.7%)
Post-Shift ≥ 700, n (%)	138 (26.7%)	3 (5.7%)	126 (24.7%)	15 (27.3%)
Urine SG				
Pre-Shift ≥ 1.02, n (%)	165 (32.0%)	8 (15.1%)	157 (30.8%)	14 (25.5%)
Post-Shift ≥ 1.02, n (%)	177 (34.3%)	10 (18.9%)	158 (31.0%)	27 (49.1%)
Urine pH				
Pre-Shift < 6.0, n (%)	166 (32.2%)	19 (35.8%)	165 (32.4%)	20 (36.4%)
Pre-Shift ≥ 7.0, n (%)	129 (25.0%)	6 (11.3%)	111 (21.8%)	22 (40.0%)
Post-Shift < 6.0, n (%)	176 (34.1%)	30 (56.6%)	180 (35.3%)	25 (45.5%)
Post-Shift ≥ 7.0, n (%)	143 (27.7%)	6 (11.3%)	131 (25.7%)	17 (30.9%)
Blood Pressure				
Day 2 - Substantial Cross-Shift Decline, n (%)	95 (18.4%)	3 (5.7%)	87 (17.1%)	10 (18.2%)
Day 3 - Substantial Cross-Shift Decline, n (%)	72 (14.0%)	9 (17.0%)	73 (14.3%)	8 (14.5%)
Serum Sodium				
Pre-Shift ≤ 135, n (%)	34 (6.6%)	14 (26.4%)	44 (8.6%)	4 (7.3%)
Post-Shift ≤ 135, n (%)	92 (17.8%)	25 (47.2%)	112 (22.0%)	5 (9.1%)

Serum Potassium				
Pre-Shift ≤ 3.0, n (%)	6 (1.2%)	8 (15.1%)	12 (2.4%)	2 (3.6%)
Post-Shift ≤ 3.0, n (%)	14 (2.7%)	11 (20.8%)	21 (4.1%)	4 (7.3%)
Serum Uric Acid				
Pre-Shift, Mean (SD)	5.51 (1.25)	8.84 (2.10)	5.86 (1.67)	5.50 (1.56)
Post-Shift, Mean (SD)	5.66 (1.29)	8.77 (2.00)	5.92 (1.64)	6.26 (1.67)
Pre-Shift ≥ 7.0, n (%)	59 (11.4%)	39 (73.6%)	93 (18.2%)	5 (9.1%)
Post-Shift ≥ 7.0, n (%)	74 (14.3%)	39 (73.6%)	101 (19.8%)	12 (21.8%)
Blood Urea Nitrogen				
Pre-Shift, Mean (SD)	10.6 (3.28)	25.8 (10.7)	12.1 (6.40)	11.8 (5.56)
Post-Shift, Mean (SD)	11.6 (3.47)	26.2 (10.9)	12.8 (6.37)	14.4 (6.01)
Post-Shift BUN:Cr < 10, n (%)	103 (20.0%)	22 (41.5%)	112 (22.0%)	13 (23.6%)
Post-Shift BUN:Cr 10-20, n (%)	401 (77.7%)	31 (58.5%)	389 (76.3%)	42 (76.4%)
Post-Shift BUN:Cr ≥ 20, n (%)	9 (1.7%)	0 (0%)	9 (1.8%)	0 (0%)
Calculated Serum Osmolality (mOsm/kg)				
Post-Shift ≥ 295, n (%)	8 (1.6%)	4 (7.5%)	9 (1.8%)	3 (5.5%)
Post-Shift < 280, n (%)	96 (18.6%)	15 (28.3%)	109 (21.4%)	2 (3.6%)
Creatine Phosphokinase (U/L)				
Pre-Shift, Median (Min, Max)	210 [65.4, 1310]	225 [75.9, 645]	210 [65.4, 1310]	226 [115, 561]
Post-Shift, Median (Min, Max	251 [73.8, 1590]	225 [95.7, 820]	242 [73.8, 1590]	317 [140, 701]
Hematocrit				
Pre-Shift, Mean (SD)	42.0 (2.77)	39.2 (2.23)	41.9 (2.84)	40.9 (2.42)
Post-Shift, Mean (SD)	40.9 (2.55)	38.9 (2.53)	41.0 (2.61)	39.9 (1.98)
Hemoglobin				
Pre-Shift, Mean (SD)	14.2 (1.12)	12.4 (1.25)	14.0 (1.20)	13.4 (1.43)
Post-Shift, Mean (SD)	13.8 (1.04)	11.8 (1.37)	13.6 (1.20)	13.1 (1.20)

SCr = serum creatinine; eGFR = estimated glomerular filtration rate; SG = specific gravity

Table 4.5. Summary of models examining associations between maximum Tc and post-shift urine osmolality with cross-shift change in serum creatinine (mg/dL)

All MANOS Participants (Tc < 50% shift and Accel < 50% shift excluded) (n=389)								
	Crude Models		**Adjusted Model 1**[a]		**Adjusted Model 2**[b]		**Adjusted Model 3**[c]	
	$R^2 = 0.11; 0.02$		$R^2 = 0.33$		$R^2 = 0.34$		$R^2 = 0.35$	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Maximum Tc (per 0.5°C)	0.049 (0.035, 0.064)	<0.0001	0.031 (0.015, 0.046)	<0.0001	0.027 (0.011, 0.043)	0.001	0.023 (0.008, 0.039)	0.004
Post-Shift Urine Osmolality (per 250 mOsm/kg)	0.017 (0.006, 0.028)	0.004	0.009 (-0.001, 0.020)	0.09	0.009 (-0.001 0.020)	0.07	0.009 (-0.001, 0.020)	0.09
+ eGFR > 60 mL/min/1.73 m^2 (n=336)								
	Crude Models		**Adjusted Model 1**[a]		**Adjusted Model 2**[b]		**Adjusted Model 3**[c]	
	$R^2 = 0.10; 0.03$		$R^2 = 0.37$		$R^2 = 0.39$		$R^2 =0.42$	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Maximum Tc (per 0.5°C)	0.047 (0.032, 0.062)	<0.0001	0.025 (0.010, 0.040)	0.002	0.019 (0.003, 0.034)	0.02	0.015 (-0.001, 0.030)	0.06
Post-Shift Urine Osmolality (per 250 mOsm/kg)	0.017 (0.006, 0.028)	0.003	0.008 (-0.002, 0.018)	0.14	0.009 (-0.001, 0.019)	0.09	0.007 (-0.003, 0.017)	0.17

+ Nicaragua Sugarcane Workers Only (n=74)								
	Crude Models		Adjusted Model 1[a]		Adjusted Model 2[b]		Adjusted Model 3[c]	
	R^2 = 0.01; 0.05		R^2 = 0.33		R^2 = 0.36		R^2 =0.39	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Maximum Tc (per 0.5°C)	0.021 (-0.026, 0.068)	0.37	0.016 (-0.026, 0.057)	0.46	0.005 (-0.038, 0.049)	0.80	0.001 (-0.044, 0.045)	0.97
Post-Shift Urine Osmolality (per 250 mOsm/kg)	0.034 (0.000, 0.068)	0.05	0.016 (-0.019, 0.051)	0.37	0.017 (-0.018, 0.052)	0.33	0.021 (-0.014, 0.056)	0.23

Tc = core body temperature; eGFR = estimated glomerular filtration rate

a – Adjusted for industry/company, job task, and pre-shift uric acid

b – Adjusted for industry/company, job task, pre-shift uric acid, mean work rate, and percent shift VM<150 cpm

c – Adjusted for industry/company, job task, pre-shift uric acid, mean work rate, percent shift VM<150 cpm, shift duration, and age

Table 4.6. Summary of models examining associations between mean work rate and breaks during the shift with cross-shift change in serum creatinine (mg/dL).

All MANOS Participants (WBGT < 50% shift and Accel < 50% shift excluded) (n=434)								
	Crude Models		**Adjusted Model 1[a]**		**Adjusted Model 2[b]**		**Adjusted Model 3[c]**	
	$R^2 = 0.05; 0.01$		$R^2 = 0.33$		$R^2 = 0.38$		$R^2 = 0.38$	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Work Rate (per 100 kcal/hour)	0.026 (0.016, 0.037)	<0.0001	0.023 (0.009, 0.037)	0.001	0.030 (0.017, 0.044)	<0.0001	0.031 (0.017, 0.044)	<0.0001
Percent Shift VM < 150 cpm (per 10%)	-0.012 (-0.023, -0.002)	0.02	0.002 (-0.013, 0.017)	0.78	-0.007 (-0.021, 0.008)	0.35	-0.007 (-0.021, 0.008)	0.35
+ eGFR > 60 mL/min/1.73 m^2 (n=396)								
	Crude Models		**Adjusted Model 1[a]**		**Adjusted Model 2[b]**		**Adjusted Model 3[c]**	
	$R^2 = 0.06; 0.01$		$R^2 = 0.36$		$R^2 = 0.43$		$R^2 = 0.43$	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Work Rate (per 100 kcal/hour)	0.027 (0.016, 0.037)	<0.0001	0.026 (0.013, 0.040)	0.0002	0.031 (0.018, 0.045)	<0.0001	0.032 (0.019, 0.045)	<0.0001
Percent Shift VM < 150 cpm (per 10%)	-0.012 (-0.023, -0.002)	0.02	0.004 (-0.010, 0.018)	0.58	-0.006 (-0.020, 0.008)	0.43	-0.006 (-0.020, 0.008)	0.43

+ Nicaragua Sugarcane Workers Only (n=118)								
	Crude Models		**Adjusted Model 1[a]**		**Adjusted Model 2[b]**		**Adjusted Model 3[c]**	
	$R^2 = 0.06; 0.08$		$R^2 = 0.36$		$R^2 = 0.37$		$R^2 = 0.39$	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Work Rate (per 100 kcal/hour)	0.039 (0.010, 0.068)	0.01	0.036 (0.005, 0.068)	0.02	0.036 (0.005, 0.068)	0.03	0.035 (0.004, 0.067)	0.03
Percent Shift VM < 150 cpm (per 10%)	-0.094 (-0.153, -0.036)	0.002	0.001 (-0.063, 0.065)	0.97	-0.005 (-0.071, 0.062)	0.89	-0.007 (-0.074, 0.059)	0.83

WBGT = wet bulb globe temperature; VM = vector magnitude; cpm = counts per minute; eGFR = estimated glomerular filtration rate

a – Adjusted for industry/company and job task

b – Adjusted for industry/company, job task, shift duration, and median WBGT

c – Adjusted for industry/company, job task, shift duration, median WBGT, and age

Table 4.7. Summary of models examining associations between uric acid and creatine phosphokinase with cross-shift change in serum creatinine (mg/dL) among participants in Nicaragua.

All Nicaragua Participants (Tc < 50% shift and eGFR < 60 mL/min/1.73 m^2 excluded) (n=133)								
Pre-Shift Serum Biomarkers								
	Crude Models		**Adjusted Model 1[a]**		**Adjusted Model 2[b]**		**Adjusted Model 3[c]**	
	$R^2 = 0.01; 0.03$		$R^2 = 0.51$		$R^2 = 0.51$		$R^2 = 0.52$	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Pre-shift uric acid (per 1.5 mg/dL)	0.017 (-0.013, 0.046)	0.26	-0.001 (-0.027, 0.024)	0.92	-0.002 (-0.028, 0.024)	0.90	-0.004 (-0.032, 0.023)	0.76
Pre-shift CPK (per 100 units/L)	0.024 (0.002, 0.046)	0.04	0.017 (-0.002, 0.036)	0.09	0.018 (-0.002, 0.037)	0.07	0.016 (-0.003, 0.036)	0.10
Post-Shift Serum Biomarkers								
	Crude Models		**Adjusted Model 1[a]**		**Adjusted Model 2[b]**		**Adjusted Model 3[c]**	
	$R^2 = 0.12; 0.05$		$R^2 = 0.51$		$R^2 = 0.52$		$R^2 = 0.52$	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Post-shift uric acid (per 1.5 mg/dL)	0.056 (0.031, 0.082)	<0.0001	0.021 (-0.004, 0.046)	0.09	0.021 (-0.004, 0.046)	0.10	0.021 (-0.006, 0.048)	0.13
Post-shift CPK (per 100 units/L)	0.026 (0.008, 0.044)	0.01	0.008 (-0.008), 0.024)	0.03	0.008 (-0.008, 0.024)	0.33	0.008 (-0.008, 0.024)	0.32

+ Sugarcane Workers Only (n=74)

Pre-Shift Serum Biomarkers

	Crude Models		**Adjusted Model 1[a]**		**Adjusted Model 2[b]**		**Adjusted Model 3[c]**	
	R^2 = 0.00; 0.03		R^2 = 0.35		R^2 = 0.37		R^2 = 0.41	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Pre-shift uric acid (per 1.5 mg/dL)	-0.007 (-0.052, 0.037)	0.74	-0.011 (-0.053, 0.031)	0.61	-0.016 (-0.058, 0.027)	0.46	-0.015 (-0.060, 0.031)	0.52
Pre-shift CPK (per 100 units/L)	0.033 (0.000, 0.066)	0.05	0.026 (-0.004, 0.056)	0.09	0.030 (-0.001, 0.061)	0.06	0.028 (-0.002, 0.059)	0.07

Post-Shift Serum Biomarkers

	Crude Models		**Adjusted Model 1[a]**		**Adjusted Model 2[b]**		**Adjusted Model 3[c]**	
	R^2 = 0.07; 0.09		R^2 = 0.36		R^2 = 0.37		R^2 = 0.41	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Post-shift uric acid (per 1.5 mg/dL)	0.047 (0.007, 0.087)	0.02	0.025 (-0.015, 0.065)	0.22	0.021 (-0.021, 0.063)	0.31	0.027 (-0.019, 0.073)	0.25
Post-shift CPK (per 100 units/L)	0.036 (0.010, 0.061)	0.01	0.012 (-0.013, 0.028)	0.33	0.014 (-0.012, 0.039)	0.30	0.014 (-0.011, 0.040)	0.27

Tc = core body temperature; eGFR = estimated glomerular filtration rate; CPK = creatinine phosphokinase

a – Adjusted for industry/company and job task

b – Adjusted for industry/company, job task, maximum Tc, and post-shift urine osmolality

c – Adjusted for industry/company, job task, maximum Tc, post-shift urine osmolality, age, and pre-shift eGFR

Table 4.8. Summary of models examining associations between consumption of different beverages with cross-shift change in serum creatinine (mg/dL) among participants in Nicaragua.

Nicaragua Sugarcane Workers (WBGT < 50% shift, Accel < 50% shift, and eGFR < 60 mL/min/1.73 m^2 excluded) (n=112)								
	Crude Models		**Adjusted Model 1**[a]		**Adjusted Model 2**[b]		**Adjusted Model 3**[c]	
	R^2 = 0.03, 0.00, 0.00		R^2 = 0.36		R^2 = 0.42		R^2 = 0.43	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Total Water Consumed (per 2.5 L)	0.020 (-0.002, 0.042)	0.08	-0.011 (-0.036, 0.013)	0.35	-0.017 (-0.042, 0.008)	0.18	-0.016 (-0.041, 0.009)	0.20
Total Electrolyte Serum Consumed (per 0.85 L)	0.003 (-0.021, 0.027)	0.78	-0.012 (-0.037, 0.013)	0.34	-0.015 (-0.041, 0.012)	0.28	-0.013 (-0.039, 0.013)	0.33
Total Sweetened Beverage Consumed (per 0.25 L)	-0.002 (-0.028, 0.023)	0.86	-0.017 (-0.042, 0.007)	0.16	-0.018 (-0.044, 0.008)	0.17	-0.016 (-0.042, 0.009)	0.21

WBGT = wet bulb globe temperature; eGFR = estimated glomerular filtration rate

a – Adjusted for industry/company and job task

b – Adjusted for industry/company, job task, shift duration, mean work rate, median WBGT, and percent shift VM<150 cpm

c – Adjusted for industry/company, job task, shift duration, mean work rate, median WBGT, percent shift VM<150 cpm, age, and pre-shift eGFR

Table 4.9. Summary of models examining associations between medication use in the past week with cross-shift change in serum creatinine (mg/dL).

All MANOS Participants (Accel < 50% shift) (n=477)						
	Crude Models		**Adjusted Model 1**[a]		**Adjusted Model 2**[b]	
	R^2 = 0.00, 0.00, 0.00		R^2 = 0.31		R^2 = 0.32	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Acetaminophen Use Past Week (Yes vs. No)	0.019 (-0.006, 0.043)	0.13	0.007 (-0.016, 0.030)	0.55	0.006 (-0.016, 0.029)	0.58
NSAID Use Past Week (Yes vs. No)	0.004 (-0.024, 0.031)	0.79	0.006 (-0.029, 0.040)	0.75	0.010 (-0.029, 0.045)	0.55
Ibuprofen Use Past Week (Yes vs. No)	0.001 (-0.035, 0.036)	0.97	-0.022 (-0.066, 0.022)	0.33	-0.020 (-0.064, 0.024)	0.37
+ eGFR > 60 mL/min/1.73 m^2 (n=432)						
	Crude Models		**Adjusted Model 1**[a]		**Adjusted Model 2**[b]	
	R^2 = 0.01, 0.00, 0.00		R^2 = 0.35		R^2 = 0.37	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Acetaminophen Use Past Week (Yes vs. No)	0.023 (0.000, 0.046)	0.05	0.013 (-0.008, 0.034)	0.23	0.012 (-0.009, 0.033)	0.26
NSAID Use Past Week (Yes vs. No)	0.020 (-0.007, 0.047)	0.14	0.019 (-0.014, 0.053)	0.25	0.024 (-0.009, 0.057)	0.15
Ibuprofen Use Past Week (Yes vs. No)	0.018 (-0.016, 0.053)	0.30	-0.032 (-0.075, 0.011)	0.15	-0.030 (-0.073, 0.012)	0.16

+ Nicaragua Sugarcane Workers Only (n=113)

	Crude Models		**Adjusted Model 1[a]**		**Adjusted Model 2[b]**	
	R^2 = 0.01, 0.00, 0.00		R^2 = 0.33		R^2 = 0.38	
	B (95% CI)	p-val	B (95% CI)	p-val	B (95% CI)	p-val
Acetaminophen Use Past Week (Yes vs. No)	0.028 (-0.035, 0.091)	0.38	-0.007 (-0.064, 0.051)	0.82	-0.002 (-0.058, 0.055)	0.95
NSAID Use Past Week (Yes vs. No)	0.016 (-0.047, 0.079)	0.61	0.067 (-0.048, 0.018)	0.25	0.072 (-0.042, 0.185)	0.21
Ibuprofen Use Past Week (Yes vs. No)	-0.017 (-0.087, 0.052)	0.62	-0.086 (-0.209, 0.037)	0.17	-0.087 (-0.214, 0.040)	0.18

NSAID = non-steroidal anti-inflammatory drug; eGFR = estimated glomerular filtration rate

a – Adjusted for industry/company and job task

b – Adjusted for industry/company, job task, age, and mean work rate

Table 4.10. Summary of models examining associations between family history of CKD with cross-shift change in serum creatinine (mg/dL).

All MANOS Participants (n=528)				
	Crude Models		**Adjusted Model 1[a]**	
	$R^2 = 0.00, 0.00$		$R^2 = 0.32, 0.32$	
	B (95% CI)	p-val	B (95% CI)	p-val
Father with CKD (Yes vs. No)	-0.014 (-0.042, 0.014)	0.03	-0.016 (-0.041, 0.080)	0.19
Brother with CKD (Yes vs. No)*	0.019 (-0.017, 0.056)	0.30	0.003 (-0.030, 0.036)	0.87
+ eGFR > 60 mL/min/1.73 m^2 (n=480)				
	Crude Models		**Adjusted Model 1[a]**	
	$R^2 = 0.00, 0.00$		$R^2 = 0.35, 0.35$	
	B (95% CI)	p-val	B (95% CI)	p-val
Father with CKD (Yes vs. No)	-0.011 (-0.037, 0.016)	0.43	-0.017 (-0.040, 0.006)	0.14
Brother with CKD (Yes vs. No)*	0.026 (-0.008, 0.061)	0.13	0.005 (-0.026, 0.036)	0.77
+ Nicaragua Sugarcane Workers Only (n=118)				
	Crude Models		**Adjusted Model 1[a]**	
	$R^2 = 0.00, 0.00$		$R^2 = 0.34, 0.34$	
	B (95% CI)	p-val	B (95% CI)	p-val
Father with CKD (Yes vs. No)	-0.002 (-0.064, 0.060)	0.95	-0.023 (-0.076, 0.029)	0.38
Brother with CKD (Yes vs. No)*	0.016 (-0.046, 0.078)	0.61	0.021 (-0.035, 0.077)	0.46

eGFR = estimated glomerular filtration rate
a – Adjusted for industry/company and job task
* – Family history of CKD variables were tested independently (i.e., having a brother with CKD was not included in the models examining the effects of having a father with CKD, and vice versa)

CHAPTER FIVE: CONCLUSION

The overall goals of this dissertation were to examine potential early predictors of and risk factors for kidney injury among workers at risk of developing Mesoamerican nephropathy (MeN), as well as to characterize metrics of heat stress and heat strain among outdoor workers in various industries and jobs in El Salvador and Nicaragua. Chapter 2 describes a common pattern of dipstick leukocyte esterase among certain workers that, along with self-reported symptoms, were associated with greater levels of urinary kidney injury biomarkers. Chapter 3 reports that sugarcane workers, especially in Nicaragua, perform more physically intense work and experience higher levels of core body temperatures and heart rates, compared to workers in other industries. Finally, Chapter 4 identified a higher incidence of serum creatinine-defined kidney injury among sugarcane workers, particularly at one Nicaraguan company, and provides evidence that core body temperature and work rate were risk factors for this outcome.

Chapter Summaries

Chapter Two: Kidney Function, Self-Reported Symptoms, and Urine Findings in Nicaraguan Sugarcane Workers Chapter 3

The primary aims of Chapter 2 were to: 1) characterize urine dipstick parameters and self-reported symptom burden among workers in seven sugarcane job tasks in Nicaragua, and 2) examine whether these parameters predict levels of cross-harvest kidney function decline and urinary kidney injury biomarkers. Secondary aims included an examination of self-reported medication use and urine culture findings. We collected urine, serum, and questionnaire data on symptoms from 251 male sugarcane workers at the beginning and end of the 2010-2011 harvest at one Nicaraguan sugarcane company that had been previously reported to have high rates of MeN. We measured urine dipstick parameters and kidney injury biomarkers, and we estimated glomerular filtration rate (eGFR) from serum creatinine. Dipstick leukocyte esterase at the end of the harvest, which was relatively common among cane cutters (33%), seed cutters (22%), and seeders/reseeders (21%), was associated with a 12.9 ml/min per 1.73 m^2 (95% CI: −18.7 to −7.0) lower mean eGFR and 2.8 times (95% CI: 1.8 to 4.3) higher mean neutrophil gelatinase-associated lipocalin (NGAL). We also found that workers who reported experiencing symptoms (e.g., flank pain, fever/chills, and dysuria) in the prior three months had higher mean kidney injury biomarker levels at late harvest. None of the workers had positive urine cultures, suggesting that urinary tract infections were not the cause of the symptoms. The most commonly reported medications were amoxicillin, ibuprofen, and acetaminophen.

Chapter Three: Assessment of Heat Stress and Heat Strain Among Outdoor Workers in El Salvador and Nicaragua

The primary aims of Chapter 3 were to compare measures of heat stress and heat strain, as well as shift characteristics such as break duration, shift duration, and self-reported hydration practices, between outdoor workers in various job tasks and industries in El Salvador and Nicaragua. We also examined whether break duration, hydration practices, and kidney function were associated with measures of heat strain—elevated core body temperatures (T_c) and heart rates (HR). We used data from the MesoAmerican Nephropathy Occupational Study (MANOS), a longitudinal cohort in El Salvador and Nicaragua that includes 569 workers across five industries—sugarcane, corn, plantain, construction, and brickmaking. Exposure monitoring data and self-reported shift characteristics were collected on three days at baseline (January-May 2018) for each worker. We found that sugarcane workers, especially cane cutters and Nicaraguan agrichemical applicators, had the highest estimated work rates. Median wet bulb globe temperatures were high (> 26°C) at all sites, but particularly so among workers whose shift spanned the afternoon hours (e.g., 29.2°C among plantain workers). We also found that workers in most industries spent little time at a low vector magnitude (<10% of the shift), as determined from accelerometer data. Overall, sugarcane workers, particularly those in Nicaragua, experienced higher T_c and HRs than other workers. However, we also found evidence that workers in other industries occasionally reach high core body temperatures (> 39°C) as well. Finally, we found that workers with low eGFR (<60, 60-90) have higher average T_c and HR values and that spending more time on break was associated with lower average HR.

Chapter Four: Heat Exposure and Cross-Shift Kidney Injury Among Outdoor Workers in El Salvador and Nicaragua

The primary aims of Chapter 4 were to: 1) compare markers of kidney injury, dehydration, and muscle damage across industries and sugarcane companies, and 2) identify risk factors for and preventative factors against cross-shift kidney injury. We used the exposure data from Chapter 3 and the serum and urine samples collected on the third day of baseline exposure assessment for MANOS to conduct these analyses. We found that 23% of workers at one Nicaraguan sugarcane company met the KDIGO criteria for cross-shift acute kidney injury (AKI), while a less conservative estimate of serum creatinine-based AKI identified relatively high rates at two Nicaraguan sugarcane companies (42.3% and 22.0%) and the Salvadoran construction workers (13.8%). Job task was an important predictor of AKI, with cane cutters experiencing disproportionately higher rates. Maximum T_c and average work rate were also predictors of cross-shift increases in serum creatinine (SCr), but pre-shift eGFR was not. Similar to previous studies, we found moderate protective effects for time spent on break and increased liquid consumption (water, electrolyte, and fructose-containing beverages). We found mixed evidence for serum uric acid as a risk factor for AKI and suggest different study designs to assess this hypothesis. The measure of muscle damage (creatine phosphokinase) did not indicate any cases of rhabdomyolysis, but was moderately associated with cross-shift SCr increases.

Limitations

The limitations for Chapters 3 and 4 are similar as they describe data and analyses from the same cohort. One of these limitations is the brief period of exposure monitoring (three days for most parameters) and single day for assessment of kidney injury (using SCr). These monitored work shifts may not be reflective of typical workdays and could reflect lighter-than-normal work shifts, as companies may have adjusted workers' shifts on the days of observation, thus some of our estimates would be underestimated. We may have also underestimated work rate for certain workers, such as cane cutters who perform more upper body movements, as the accelerometers were worn on the hips. We would also expect some misclassification for T_c readings, given that the ingestible temperature sensors require specific protocols to improve accuracy, which are logistically difficult to follow consistently in a field setting. Significant data processing was employed to minimize the effect of unreliable T_c readings, but there is no standard practice for how best to perform this type of data cleaning and validation was not performed. Another limitation is the reliance on self-reported data for hydration practices, medication use, and family history of kidney disease, as we would expect these data to have some misclassification and bias our results towards the null.

There were additional limitations specific to Chapter 4, including the biomarkers used to characterize dehydration and kidney injury. Use of urine concentration measures (osmolality, specific gravity), especially in non-fasting spot urine samples, are not unbiased proxies for dehydration, as they may be influenced by kidney physiology in a way that introduces differential misclassification. Use of serum creatinine to characterize cross-shift AKI is also imperfect, as it may be influenced by factors unrelated to kidney injury (e.g., muscle and protein metabolism), and has been criticized by other researchers studying MeN etiology for its potential to overestimate AKI. Complementary use of urinary kidney injury biomarkers to confirm kidney injury would be a better approach.

For Chapter 2, we were again limited by self-reported data (for symptoms and medication use), as well as differential loss-to-follow up given that some workers enrolled at pre-harvest were not available at late harvest data. We also had relatively small sample sizes in a few of the job categories. Models predicting late-harvest eGFR and kidney injury biomarkers using late-harvest dipstick leukocyte esterase should be interpreted with caution as these are cross-sectional analyses, which prevent us from determining whether leukocyte esterase is a precursor or consequence of kidney injury. Finally, the effect estimates for self-reported symptoms and kidney injury biomarkers were relatively small and the 95% confidence intervals suggested that these data would also be consistent with a null association. This may be in part due to the sample size.

A more detailed description of the limitations of this dissertation can be found in the respective chapters.

Public Health Impact

This dissertation contributes evidence in support of a popular hypothesis among MeN researchers that exposure to heat stress and its physiological consequences are involved in the etiology of this kidney disease. We also found evidence that occupation is strongly related to heat strain and kidney injury, and that Nicaraguan sugarcane workers may be the most at risk for both.

Regardless of whether heat strain is causally involved in MeN etiology, we found evidence in Chapter 3 that suggests more work is needed to reduce the occupational heat exposure experienced by many of these workers. There is ample previous research to support the dangers of extreme heat exposure, including increased risk of heat-related illnesses (e.g., heat stroke), workplace injuries, and death. We found elevated core body temperatures (>38.5°C) in some workers—a third of workers at one Nicaraguan sugarcane company—at levels that occupational health guidelines suggest work should be discontinued[127]. We found that industry, job task, work rate, and ambient conditions were the most important factors predicting core body temperature, the latter of which are supported by exercise physiology research. Therefore, the findings in this dissertation suggest a need for greater interventions to reduce the intense workload for certain workers (namely sugarcane cutters and Nicaraguan agrichemical applicators) through mechanization of job tasks and/or elimination of pay incentives, and to eliminate or reduce work on extremely hot days. These suggestions are supported by published occupational health guidelines outlining ways to reduce heat exposure in the workplace [124,127,129]. Enforcement of worker protections will become even more important under predicted climate change scenarios, which is expected to increase ambient temperatures [162]. We also found evidence that workers with impaired kidney function experience greater heat strain and therefore may need greater protections to lower their risk for heat-related illness and injury.

The findings from Chapter 2 indicate that there may be early indicators of kidney injury among these workers, such as leukocyte esterase and symptoms of dysuria. If these findings are confirmed, increased screening could be employed to prevent further kidney injury. Additionally, we found no evidence of urinary tract infections (UTIs), even among workers with positive leukocyte esterase and reporting UTI symptoms, suggesting that these alone are not enough to diagnose UTIs in this population and prescriptions of antibiotics given to these workers to treat UTIs is likely not warranted.

Finally, while not a focus of this dissertation, it is likely that many of these workers are exposed to other insults to their kidneys (e.g., heavy metals, agrichemicals). Regardless of their role in MeN etiology, efforts should be taken to reduce these exposures as well (e.g., water treatment), given the known impacts to other organs.

Directions for Future Research

Future research should validate the findings of kidney injury reported in Chapter 4 using validated kidney injury biomarkers. Additionally, specific biomarkers can indicate where, if any, injury is occurring in the kidneys (e.g., tubules) and could further elucidate mechanisms of disease etiology. Given that urine was collected on all three days of baseline exposure assessment of MANOS, we also can examine incidence of kidney injury across more than one work shift.

More broadly, to inform MeN etiology, future research should involve analyzing trends in kidney function (eGFR) in these workers over time. There is evidence that fluctuations in cross-shift and cross-harvest SCr predict declines in kidney function over time, but these analyses have generally been limited to one or two harvest seasons. MANOS is also well-suited to study the holistic effects of occupational history, as we asked participants about their job history before recruitment into the study and changes in their employment during the study. As the relevant window of exposure for development of MeN is unknown, having detailed data on a broad time scale (i.e., years) for occupational history (which is suspected to be involved in MeN etiology) will allow us to examine the relationship between time in certain jobs and kidney function decline.

In addition to heat stress, the urine samples collected for MANOS have been analyzed for heavy metals and glyphosate, which can be used to study independent and interactive effects between these exposures and heat on risk of kidney injury.

The detailed exposure assessment conducted at baseline for physical activity, wet bulb globe temperatures, T_c, and HR, can be used to build predictive models of heat strain. Furthermore, if a causal effect estimate is established for heat strain and AKI, these data can be used to determine what thresholds (e.g., for heat stress indices) should be used to protect workers from these adverse outcomes. Finally, as new studies are being designed to study the clinical characteristics of and risk factors for MeN, the data collected for MANOS could be used to compare the usefulness of various biomarkers, such as those used for assessing dehydration and heat strain.

LIST OF JOURNAL ABBREVIATIONS

Am J Emerg Med	American Journal of Emergency Medicine
Am J Ind Med	American Journal of Industrial Medicine
Am J Kidney Dis	American Journal of Kidney Diseases
Am J Physiol Regul Integr Comp Physiol	American Journal of Physiology. Regulatory, Integrative and Comparative Physiology
Am J Physiol Renal Physiol	American Journal of Physiology. Renal Physiology
Am J Public Health	American Journal of Public Health
Am J Trop Med Hyg	American Journal of Tropical Medicine and Hygiene
Ann Intern Med	Annals of Internal Medicine
Ann Nutr Metab	Annals of Nutrition & Metabolism
Biotech Histochem	Biotechnic & Histochemistry
BMC Fam Pract	BMC Family Practice
BMC Nephrol	BMC Nephrology
Br J Clin Pharmacol	British Journal of Clinical Pharmacology
Br J Gen Pract	British Journal of General Practice
Can J Kidney Heal Dis	Canadian Journal of Kidney Health and Disease
Clim Change	Climatic Change
Clin J Am Soc Nephrol	Clinical Journal of the American Society of Nephrology
Clin Kidney J	Clinical Kidney Journal
Curr Opin Nephrol Hypertens	Current Opinion in Nephrology and Hypertension
Environ Heal	Environmental Health
Environ Health Perspect	Environmental Health Perspectives
Environ Res	Environmental Research
Environ Res Lett	Environmental Research Letters: ERL
Environ Toxicol	Environmental Toxicology

Extrem Physiol Med	Extreme Physiology & Medicine
Food Chem Toxicol	Food Chemistry and Toxicology
Glob Health Action	Global Health Action
Ind Health	Industrial Health
Int Arch Occup Environ Health	International Archives of Occupational and Environmental Health
Int J Biometeorol	International Journal of Biometeorology
Int J Environ Res Public Health	International Journal of Environmental Research and Public Health
Int J Occup Environ Health	International Journal of Occupational and Environmental Health
J Am Soc Nephrol	Journal of the American Society of Nephrology
J Appl Physiol (1985)	Journal of Applied Physiology
J Clin Pharm Ther	Journal of Clincial Pharmacy and Therapeutics
J Epidemiol Community Heal	Journal of Epidemiology and Community Health
J Geophys Res Atmos	Journal of Geophysical Rearch. Atmospheres
J Occup Environ Med	Journal of Occupational and Environmental Medicine
J Occup Health	Journal of Occupational Health
J Sci Med Sport	Journal of Science and Medicine in Sport
J Sport Heal Sci	Journal of Sport and Health Science
J Toxicol Clin Toxicol	Journal of Toxicology. Clinical toxicology
J Toxicol Environ Heal Part A	Journal of Toxicology and Environmental Health. Part A
Kidney Int	Kidney International
Kidney Int Reports	Kidney International Reports
MEDICC Rev	MEDICC Review
Muscles Ligaments Tendons J	Muscles, Ligaments and Tendons Journal
N Engl J Med	New England Journal of Medicine
Nefrol (English Ed.)	Nefrología (English Edition)

Nephrol Dial Transplant	Nephrology, Dialysis, Transplantation
Nephrourol Mon	Nephro-urology Monthly
Occup Environ Med	Occupational and Environmental Medicine
Pediatr Nephrol	Pediatric Nephrology
PLoS Negl Trop Dis	PLoS Neglected Tropical Diseases
Postgrad Med J	Postgraduate Medical Journal
Ren Fail	Renal Failure
Rev Panam Salud Publica/ Pan Am J Public Heal	Revista panamericana de salud pública = Pan American Journal of Public Health
Saudi J Kidney Dis Transpl	Saudi Journal of Kidney Diseases and Transplantation
Scand J Work Environ Heal	Scandinavian Journal of Work, Environment & Health
Semin Nephrol	Seminars in Nephrology
Sports Med	Sports Medicine
Stat Med	Statistics in Medicine
Toxicol Ind Health	Toxicology and Industrial Health
Toxicol Lett	Toxicology Letters
Toxicol Mech Methods	Toxicology Mechanisms and Methods
West J Nurs Res	Western Journal of Nursing Research

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