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Biomarkers of Kidney Injury Among Nicaraguan Sugarcane Workers

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Supplementary Material

Table S1: Description of sugarcane job categories and likelihood of exposure to putative causal agents.
Table S2: Non-normalized kidney injury biomarkers in sugarcane workers at pre- and late harvest, stratified by sex.
Table S3: Model output for linear mixed effects models evaluating change in kidney injury biomarkers by fieldworker status.
Table S4: Model output for linear mixed effects models evaluating change in kidney injury biomarkers by job.
Table S5: Multivariable analysis of late-harvest kidney injury biomarkers by hydration among sugarcane workers, stratified by job.
Figure S1: Derivation of study population.
Note: The supplementary material accompanying this article (doi:_____) is available at www.ajkd.org

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Abstract

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

Study Design—Longitudinal cohort study.

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

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

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

Results—Mean eGFR was 113 mL/min/1.73 m^2 and less than 5% of workers had albuminuria, field workers had increases in NGAL and IL-18 that were 1.49 (95% CI, 1.06-2.09) and 1.61 (95% CI, 1.12-2.31) times as high, respectively, as in non-field workers. Cane cutters and irrigators had the greatest increase in NGAL during the harvest, while cane cutters and seeders had the greatest increase in IL-18. Consumption of electrolyte solution was associated with lower mean NGAL and NAG among cane cutters and lower mean IL-18 and NAG among seed cutters; however, there was no overall effect of hydration among all workers. On average, workers with the largest increases in NGAL and NAG during the harvest had declines in eGFR of 4.6 (95% CI, −8.2 to −1.0) and 3.1 (95% CI, −6.7 to 0.6) mL/min/1.73 m^2, respectively.

Limitations—Surrogate exposure measure, loss-to-follow-up.

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

Keywords

Chronic kidney disease (CKD); Mesoamerican Nephropathy (MeN); Neutrophil gelatinase-associated lipocalin (NGAL); Interleukin 18 (IL-18); N-acetyl-β-D-glucosaminidase (NAG); estimated glomerular filtration rate (eGFR); Tubulointerstitial; urine tubular injury biomarker; Acute kidney injury (AKI); volume depletion; heat stress; renal disease etiology; occupational safety

There is an epidemic of chronic kidney disease (CKD) in Central America, which is referred to as Mesoamerican nephropathy.[1] This disease disproportionately affects young, male agricultural workers and is not associated with traditional causes of CKD such as diabetes and hypertension.[2-7] The infrequent presence of high levels of proteinuria suggests that the disease is mainly tubulointerstitial in nature.[7-13] Age-adjusted mortality rates from kidney disease in El Salvador and Nicaragua are among the highest in the world.[14] In these countries, the prevalence of reduced estimated glomerular filtration rate (eGFR) (<60 mL/min/1.73m^2) in affected communities is 12%-18% in the general population and 14%-26% in men,[8-12] with age-specific rates among younger men up to 15 times higher than in the United States.[8]

Hypothesized causal agents include agrichemicals, heat stress, heavy metals, nephrotoxic medications, systemic infections like leptospirosis, and genetic factors.[4,5,15,16] The etiology of Mesoamerican nephropathy is presumed to be multifactorial,[2,5,17] and, although specific causes have not yet been identified, there is support for one or more risk factors being occupational.[13] Some studies have suggested that workers in occupations related to sugarcane cultivation, which require heavy manual labor in high ambient temperatures, may be particularly at risk.[10,12,13]

We have previously shown that sugarcane workers performing the most strenuous tasks had the greatest increases in serum creatinine during one harvest.[13] However, serum creatinine is not a sufficiently sensitive marker of early kidney injury, since a measurable change may be evident only after substantial damage has occurred.[18-21] Several novel biomarkers of kidney injury have been identified, including neutrophil gelatinase-associated lipocalin (NGAL), interleukin 18 (IL-18), and *N*-acetyl-β-D-glucosaminidase (NAG). A 22-kDa protein, NGAL is highly upregulated and released into plasma and urine following distal tubule injury.[18,22-24] A pro-inflammatory cytokine, IL-18 is produced by immune and non-immune cells and is excreted in the urine following ischemic proximal tubule injury.[22,25-27] Similarly, NAG, a lysosomal enzyme, is shed into the urine following proximal tubular epithelial cell injury and is indicative of structural damage.[19,22,24] These biomarkers may be useful tools for earlier diagnosis of clinically relevant acute kidney injury (AKI), as well as for localization of injury to a specific site in the nephron.[19,20,22] Furthermore, they may be useful for monitoring disease progression and severity.[18,19,23,28]

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

METHODS

Study Design

The study design and population have been previously described.[13] Briefly, the population included sugarcane workers employed by one company in northwestern Nicaragua. We enrolled participants (n=1249) prior to the harvest season (October 2010–December 2010) at a screening conducted by the company, which aims to identify – so as not to hire -- workers with serum creatinine levels ≥1.4 mg/dL. Four to six months later, near the end of the harvest season (March 2011–May 2011), we re-sampled 506 of these workers (Figure S1, available as online supplementary material). Both assessments included collection of blood and urine and information about personal characteristics, occupational history, and work practices. Information about typical hydration practices during the harvest was collected at late-harvest only; workers were asked to self-report the quantity of water and electrolyte solution packets (100 mL, distributed by the company) consumed during a typical workday.

As described previously[13] and outlined in Figure S1, a subset of the 506 workers sampled at pre- and late-harvest was included in the final study population (n=284). These workers represented seven job categories: cane cutter, seeder, seed cutter, agrichemical applicator, irrigator, driver, and factory worker (Table S1). Our goal was to select jobs that reflected exposures hypothesized to cause Mesoamerican nephropathy. For each job, we determined the likelihood of exposure to putative causal agents, relative to other jobs, based on an industrial hygiene assessment (Table S1).[29]

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

Biomarkers of Kidney Function and Injury

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

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

(Roche Diagnostics, USA) with method intra- and inter-assay CVs of 4.3% and 6.0%, respectively.[30] The detection limits were 1.3 mg/L for urine albumin, 1.6 pg/mL for NGAL, 4 pg/mL for IL-18, and 0.003 U/L for NAG.

Statistical Analyses

For values below the limit of detection, we substituted limit of detection/√2. To account for urine concentration, we normalized biomarkers of kidney damage to urinary creatinine concentration (in g/L) and expressed as follows: albumin-creatinine ratio (ACR) (mg/g), NGAL (μg/g), IL-18 (ng/g), and NAG (U/g). We used the CKD-EPI (CKD Epidemiology Collaboration) creatinine equation to calculate eGFR, considering race as "non-black".[31] We examined the distribution of each biomarker using histograms and other graphical displays as well as summary statistics. To satisfy normality assumptions, biomarkers that exhibited a log-normal distribution were natural log (ln)-transformed prior to analyses.

We evaluated the association between job category and each biomarker of kidney injury using linear mixed effects models with an unstructured covariance matrix. In the first set of models, the primary predictor of interest was 'field worker' (reference: non-field worker). In the second set of models, the 'job category' variable was the independent variable (reference: factory workers). To assess differences by job in the change in each biomarker during the harvest, we included an interaction term between the predictor of interest and time. We also tested whether the change in each biomarker was different by sex. To evaluate differences between biomarkers at pre- and late-harvest, we used paired t-tests stratified by sex and job. We performed Spearman rank correlations to assess relationships between biomarkers of kidney injury and eGFR. We also performed multivariable linear regression models to determine whether change in eGFR (calculated by subtracting each pre-harvest measurement from the corresponding late-harvest measurement) was associated with change in biomarkers of kidney injury (categorized into tertiles).

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

Additional predictors included self-reported daily water and electrolyte solution intake during the harvest. We explored the effect of hydration on kidney injury at late-harvest, overall, and within job categories, using multivariable linear regression models controlling for the pre-harvest biomarker value. We also included sex, age, and years worked at the company in all adjusted models. We analyzed data using Statistical Analysis Software (SAS Institute Inc, version 9.3, Cary, NC).

RESULTS

Description of Study Population

Men constituted the majority of the study population (88%), with women employed as seeders, seed cutters, and factory workers only (Table 1). The mean age of workers was 33.6 (range, 18-63) years, while mean duration of employment at the company was 9.4 (range,

<1-40) years. Workers reported drinking an average 5.1 liters of water and 2.4 electrolyte solution packets while at work each day (Table 1).

Biomarkers of kidney injury and function were normally distributed after natural log transformation, with the exception of eGFR, which was normally distributed. The overall late-harvest geometric means of NGAL, IL-18, and NAG normalized to urine creatinine were, respectively, 10.4 μg/g, 8.8 ng/g, and 0.90 U/g in men and 25.0 μg/g, 25.1 ng/g, and 1.26 U/g in women (Table 2). Comparisons of pre-harvest to late-harvest measurements indicated that, on average, NGAL increased during the harvest in men; this was driven by increases in cane cutters and irrigators. Levels of NAG decreased during the harvest in men, driven primarily by the decrease in factory workers (Table 2). Summary statistics for non-normalized biomarkers, as well as for urine creatinine, are presented in Table S2.

The overall late-harvest mean eGFR was 112 mL/min/1.73 m^2 in men and 118 mL/min/1.73 m^2 in women (Table 2). At pre-harvest, one worker (0.4%) had eGFR <60 mL/min/1.73 m^2, compared to eight at late-harvest (2.8%). Urine ACR was generally low (Table 2); fewer than 5% of workers had levels greater than 30 mg/g at either time point (Table 1).

Job Category and Biomarkers of Kidney Injury

Field workers (cane cutters, seeders, seed cutters, agrichemical applicators, and irrigators) had a mean increase in NGAL concentration during the harvest that was 1.49 times as high as the change among non-field workers (drivers and factory workers) (relative mean [RM], 1.49; 95% confidence interval [CI], 1.06-2.09; Table 3, Model 1). Similarly, the change in IL-18 was 61% higher in field workers than in non-field workers (RM, 1.61; 95% CI, 1.12-2.31; Table 3, Model 1).

When analyzed by individual job category, the mean increase in NGAL during the harvest was more than twice as high in cane cutters (RM, 2.57; 95% CI, 1.54-4.27) and irrigators (RM, 2.07; 95% CI, 1.24-3.47) as the change in factory workers (Table 3, Model 2). Compared to factory workers, cane cutters (RM, 1.89; 95% CI, 1.08-3.29) and seeders (RM, 2.11; 95% CI, 1.14-3.92) had the largest increases in IL-18 during the harvest (Table 3, Model 2).

Among all jobs, factory workers had the highest NAG concentrations pre-harvest and the lowest NAG concentrations late-harvest (Table 2). Changes in NAG during the harvest, compared to factory workers, were significant for all other six jobs (Table 3, Model 2). However, these relative increases were driven by a decrease in NAG among factory workers; therefore, no jobs were associated with increases in NAG during the harvest.

Men had concentrations of NGAL and IL-18 that were roughly one-third those of women at both the pre- and late-harvest time points (Table 3). For both biomarkers, the change during the harvest was not different by sex; this interaction term was therefore excluded from final models. There were no differences in NAG by sex (Table 3). For all models, there was no association between age or years worked and the biomarker of interest.

Differences by job category were maintained in sensitivity analyses restricted to men (Table 3) and to field workers. Results remained similar in models further adjusting for eGFR and ACR.

Hydration and Biomarkers of Kidney Injury

There was no overall association between self-reported daily intake of water or electrolyte solution during the harvest and NGAL, IL-18, or NAG at the late-harvest time point, while controlling for the pre-harvest biomarker value (Table 4). However, when stratified by job, each electrolyte solution packet consumed by cane cutters during the workday was associated with a 23% decrease in mean NGAL (RM, 0.77; 95% CI, 0.61-0.98) and a 16% decrease in mean NAG (RM, 0.84; 95% CI, 0.70-1.01) (Table S5). Similarly, seed cutters had a 31% decrease in mean IL-18 (RM, 0.69; 95% CI, 0.46-1.04) and a 33% decrease in mean NAG (RM, 0.67; 95% CI, 0.44-1.02) with each additional electrolyte solution packet consumed (Table S5).

Relationships Between Biomarkers of Kidney Injury and eGFR

To determine whether marked increases in urinary biomarkers were associated with decreased eGFR, the change in each kidney injury biomarker was categorized into tertiles. During the six month harvest, workers with the largest increases in NGAL and NAG (upper tertile compared to lower two tertiles) had declines in eGFR of 4.6 (95% CI, −8.2 to −1.0) mL/min/1.73 m^2 and 3.1 (95% CI, −6.7 to 0.6) mL/min/1.73 m^2, respectively, while controlling for age, sex, and years worked. This relationship was not observed for IL-18. When examined as continuous variables, changes in biomarkers during the harvest season were not correlated with changes in eGFR, with the exception of NAG, which had a weak negative correlation with change in eGFR (Table 5). Although increases in all biomarkers were associated with increases in albuminuria, the increases in albuminuria were small; during the harvest, ACR remained >30 mg/g (microalbuminuria) in four workers and increased to >30 mg/g in five workers.

DISCUSSION

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

Our results indicate that, of the seven jobs studied, cane cutters are at highest risk of kidney injury during the harvest. These workers had consistently greater increases in both NGAL and IL-18 during the harvest season, suggesting more substantial kidney injury than workers in other jobs, although irrigators and seeders showed some evidence of injury as well. Cane cutters have the most physically strenuous sugarcane job; furthermore, they are paid based

on their daily work output, a compensation structure that may increase physical strain. Previous studies in Central America have determined that cane cutters are at risk for heat stress, documenting wet-bulb globe temperature indices that exceed internationally accepted heat exposure limits.[29,32-35] Seeders also have a demanding job in hot ambient conditions, and are paid based on a similar structure.[29] Our finding is in accordance with the hypothesis that heat stress might be playing a role, although other factors that differ by job could also have a part in the observed results (Table S1). Although agrichemicals have been suggested as an etiology of the CKD epidemic, compared to other field workers, agrichemical applicators did not have increases in kidney injury biomarkers. Despite this finding, the potential role of agrichemical exposure should be investigated further given the extensive use in the region.[36,37]

In combination with intense labor in high heat settings, repeated volume depletion may be an important contributor to kidney disease in the region.[5,10,12,17,38] In this study, self-reported intake of water or electrolyte solution was generally not associated with biomarkers of kidney injury. However, when stratified by job, we observed that electrolyte solution had a protective effect among cane cutters and seed cutters. Consistent with these findings, we previously noted in these cane cutters that late-harvest eGFR was higher by a mean of 6.1 mL/min/1.73 m^2 with each additional electrolyte solution packet consumed.[13] These findings suggest that for the most high-risk jobs such as cane cutting, intake of electrolyte solutions for rehydration during the workday might protect against kidney injury.

In this population, we previously reported mean decreases in eGFR during the harvest that varied by job.[13] Those results are consistent with findings from the present study and suggest that one or more risk factors for this kidney disease are occupational. Few of these workers had substantially decreased eGFR, however, highlighting the importance of examining kidney injury biomarkers. An evolving body of literature suggests that these markers may be useful as early indicators of AKI.[19,20,22,25,39] Less is known about their ability to predict subclinical AKI or future CKD; repeated incidents of subclinical kidney damage may increase CKD risk, but this relationship remains unclear.[40-42] The lack of overall correlation between changes in urinary biomarkers and changes in eGFR may reflect the possibility that changes in biomarkers precede changes in eGFR. However, workers with the greatest increases in NGAL and NAG during the harvest had greater declines in eGFR. Some biomarkers, particularly NGAL, may also be markers of disease severity and can predict CKD progression and mortality.[18,23,39,43] It is possible that among these workers, repeated subclinical kidney damage over multiple harvests leads to clinically apparent disease and progressive CKD.

Few workers had albuminuria; this is consistent with findings from other studies in the region,[6-10,12] but may be expected due to the relatively healthy nature of the study population. The presence of NGAL, IL-18, and NAG in the urine of these workers, however, provides evidence that the disease process may be primarily tubulointerstitial. While we cannot rule out systemic stress or inflammation as a cause of the elevated biomarkers,[20,24,26] these individuals are overtly healthy and exposed to environmental conditions that are hypothesized to cause kidney injury and damage.[44] Accordingly, it is most likely that the changes in these biomarkers are consistent with tubular injury. Kidney biopsy specimens

from patients with CKD in El Salvador indicated glomerular lesions in addition to tubulointerstitial damage,[45,46] which may suggest a primary tubulointerstitial disease with secondary glomerular manifestations.

Studies in Nicaragua suggest that men are 3-5 times more likely to be affected by Mesoamerican nephropathy compared with women.[8,12,16] We found that at both pre- and late-harvest, men had NGAL and IL-18 concentrations that were roughly one-third those of women, regardless of normalization for urine creatinine, but that the change in these biomarkers during the harvest was not different by sex. In other populations, there are inconsistent findings regarding inherent differences by sex, though one study reports higher NGAL levels in healthy female children and adults than in males of the same age.[47] A previous study of Nicaraguan adolescents also found that biomarker levels were higher in girls than boys.[48] These findings require further investigation.

One limitation of this research is the lack of established kidney injury biomarker levels that represent subclinical damage. Furthermore, we do not know the short-term intra-individual variability of these biomarkers. We were able to evaluate concentrations within and between workers during the six-month harvest, but the magnitude of the increase is difficult to interpret and the clinical relevance is unknown. When comparing our population to pre-operative cardiac surgery patients who did not develop AKI, mean and median concentrations of NGAL were similar, but concentrations of IL-18 were lower in our population.[49] There are limited comparable occupational cohorts that have measured these biomarkers in workers.[50] To our knowledge, only one previous study in this region has measured kidney injury biomarkers; NGAL levels were increased in 26% of CKD cases in El Salvador.[51] However, it is difficult to compare our population of healthy workers to a population of CKD cases.

There are several additional limitations to this study. First, we used job category as a surrogate exposure metric. Job category may represent one or more unidentified risk factors, possibly including occupational and/or non-occupational exposures. Future studies should directly measure exposure to putative causal agents. Second, biomarker levels could potentially be affected by storage of biological specimens. Though NGAL and IL-18 levels do not seem to be greatly affected by short-term handling variations,[52] levels may decline over time in frozen samples. In one study, NAG was shown to decline 28-fold over one year while stored at −80°C,[53] although this finding has not been confirmed. It is possible that this degradation issue influenced our null findings for NAG. Finally, the loss-to-follow-up in this study population is a potential issue and has been previously described in detail.[13] Loss-to-follow-up during the harvest could be categorized into two types: (1) 99 workers were no longer working when the late-harvest sampling was done, and (2) 499 workers were actively working but did not undergo sampling at late-harvest (Figure S1). If the reason for leaving work was related to kidney injury, it is possible that biomarker levels would have been higher than what we observed, with more pronounced differences by job task, as many of these workers were in higher risk jobs. We had financial and logistical restrictions that necessitated our selecting a random sample of those workers still active, and this would not be expected to influence our results.

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

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics and baseline kidney function of study population by job

Participants	Overall	Field Worker					Non – Field Worker	
		Cane cutter	Seeder	Seed cutter	Agrichemical applicator	Irrigator	Driver	Factory worker
No. (%)	284 (100%)	51 (18%)	36 (13%)	19 (7%)	29 (10%)	49 (17%)	41 (14%)	59 (21%)
Characteristics								
Male sex	251 (88%)	51 (100%)	15 (42%)	9 (47%)	29 (100%)	49 (100%)	41 (100%)	57 (97%)
Age (y)	33.6 ±10.4	30.5 ± 10.6	30.2 ± 8.7	30.3 ± 9.2	34.7 ± 8.2	30.3 ± 8.8	40.9 ± 11.2	36.5 ± 9.8
Duration working at company (y)	9.4 ± 9.0	3.6 ± 5.3	4.9 ± 4.8	3.7 ± 4.4	12.0 ± 8.0	9.7 ± 8.0	14.4 ± 8.9	14.2 ± 10.6
Water per day (L)	5.1 ± 2.2	6.3 ± 2.8	4.4 ± 2.2	5.8 ± 2.4	4.6 ± 1.8	6.3 ± 1.9	4.6 ± 1.6	4.0 ± 1.7
Electrolyte solution packets per day[a]	2.4 ± 1.9	3.6 ± 1.4	2.0 ± 1.5	2.5 ± 1.5	3.5 ± 1.9	2.3 ± 1.6	0.4 ± 1.2	2.7 ± 2.0
Kidney function at preharvest								
eGFR (mL/min/1.73 m^2)	114 ± 17	111 ±18	118 ± 19	116 ± 18	116 ± 14	120 ± 14	106 ± 17	113 ± 16
ACR (mg/g)	2.9 ± 3.5	3.8 ± 4.3	4.5 ± 4.4	2.9 ± 2.3	2.4 ± 4.5	2.2 ± 3.6	3.0 ± 2.7	2.5 ± 2.7
eGFR <60 mL/min/1.73 m^2	1 (0.4%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
ACR >30 mg/g	12 (4%)	3 (6%)	4 (11%)	0 (0%)	2 (7%)	2 (4%)	1 (2%)	0 (0%)

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

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

[a]Each packet is 100 mL.

Table 2

Creatinine-normalized biomarkers of kidney injury and function among sugarcane workers at pre- and late-harvest time points, stratified by sex

	No.	NGAL (μg/g)[a]			IL-18 (ng/g)[a]			NAG (U/g)[a]			eGFR (mL/min/1.73 m^2)[b]			ACR (mg/g)[a]		
		Pre	Late	*P*	Pre	Late	*P*	Pre	Late	*P*	Pre	Late	*P*	Pre	Late	*P*
Male																
Overall	251	7.5	10.4	<0.001	9.0	8.8	0.7	1.32	0.90	<0.001	113	112	0.3	2.8	2.4	0.07
Job																
Cane cutter	51	7.6	19.3	<0.001	9.1	12.6	0.1	1.17	1.54	0.08	111	108	0.3	3.8	2.0	0.01
Seeder	15	10.4	15.3	0.3	5.9	14.8	0.08	1.22	1.61	0.2	115	110	0.4	4.3	6.6	0.2
Seed cutter	9	10.1	6.2	0.01	9.2	5.4	0.4	1.54	0.63	0.05	119	112	0.08	3.0	1.0	0.02
Agrichemical applicator	29	7.0	6.9	0.9	9.7	8.5	0.6	1.09	0.93	0.6	116	114	0.1	2.4	2.7	0.8
Irrigator	49	7.2	14.7	<0.001	6.8	7.5	0.6	0.92	0.90	0.9	120	116	0.006	2.2	4.2	<0.001
Driver	41	6.9	7.5	0.8	14.0	9.9	0.2	1.27	0.69	0.07	106	110	0.003	3.0	2.0	0.08
Factory worker	57	7.2	6.8	0.8	8.8	6.4	0.07	2.23	0.60	<0.001	112	115	0.2	2.4	1.6	0.02
Female																
Overall	33	28.1	25.0	0.7	22.9	25.1	0.7	1.29	1.26	0.9	118	118	0.7	3.9	4.7	0.4
Job																
Cane cutter	-	-	-		-	-		-	-		-	-		-	-	
Seeder	21	32.2	24.7	0.5	26.4	29.8	0.7	1.19	1.26	0.7	120	122	0.1	4.6	5.6	0.6
Seed cutter	10	26.2	25.1	0.9	15.3	15.1	0.9	1.26	1.37	0.8	114	112	0.3	2.7	3.7	0.3
Agrichemical applicator	-	-	-		-	-		-	-		-	-		-	-	
Irrigator	-	-	-		-	-		-	-		-	-		-	-	
Driver	-	-	-		-	-		-	-		-	-		-	-	
Factory worker	2	9.3	28.4	0.6	38.1	51.4	0.6	3.32	0.80	0.05	116	113	0.2	4.2	2.4	0.7

Note: *P* is for comparison of pre- to late-harvest levels of each biomarker, using paired t-tests.

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

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

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

Table 3

Linear mixed effects models for change in urinary biomarkers of kidney injury among sugarcane workers

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

Note: Values are given as relative mean (95% confidence interval); relative mean (exponentiated β coefficient; ie, *e* to the power of β) represents the fold-change in biomarker during the harvest compared to the reference group, as outcome variables were ln-transformed prior to analyses. For example, field workers had a mean change in NGAL concentration during the harvest that was 1.49 times as high as the change among non-field workers.

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

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

[b] Model 2: all job categories were analyzed individually. Model parameters for each ln(outcome) include time, job, time×job, sex, age, and years worked. Results presented as "Change in biomarker during harvest" are the exponentiated β coefficients of the time×job variable. Full model results and p-values are presented in Table S4.

Table 4

Multivariable analysis of late-harvest biomarkers of kidney injury by hydration among sugarcane workers

Variable	NGAL [ln(μg/g)]	IL-18 [ln(ng/g)]	NAG [ln(U/g)]
Water consumption, per 1-L greater	0.99 (0.93-1.06)	1.02 (0.95-1.09)	1.02 (0.96-1.09)
Electrolyte solution consumption, per 1 packet more[a]	1.03 (0.94-1.12)	1.05 (0.96-1.14)	1.00 (0.92-1.07)

Note: Values are given as relative mean (95% confidence interval); relative mean (exponentiated β coefficient; ie, *e* to the power of β) represents the fold-change in biomarker per unit increase of consumption. Model parameters include water, electrolyte solution, job, sex, age, years worked, and pre-harvest biomarker value.

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

[a]Each packet is 100mL.

Table 5

Spearman correlations between biomarkers of kidney injury and function among sugarcane workers

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

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

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