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Heat stress, hydration, uric acid and Mesoamerican nephropathy: a cross-sectional study in workers of three occupations

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Running Title: Mesoamerican nephropathy and occupational heat stress

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Abstract

Objectives: To study Mesoamerican nephropathy (MeN) and its risk factors in three hot occupations.

Design: Cross-sectional.

Setting: Municipalities of Chinandega and León, Nicaraguan Pacific coast, January-February 2013.

Participants: 194 male workers aged 17-39: 86 sugarcane cutters, 56 construction workers and 52 small-scale farmers.

Outcome measures: i) Differences between the three occupational groups in prevalences/levels of socioeconomic, occupational, lifestyle and health risk factors for CKD; and in biomarkers of kidney function and hydration; ii) differences in prevalences/levels of CKD risk factors between workers with reduced estimated glomerular filtration rate (eGFR-EPI<80 ml/min/1.73m^2) and workers with normal kidney function (eGFR-EPI≥80 ml/min/1.73m^2).

Results: Sugarcane cutters were more exposed to heat and consumed more fluid on workdays; they also had a notably better metabolic profile. Reduced eGFR occurred in 16%, 9% and 2% of sugarcane cutters, construction workers and small-scale farmers, respectively (trend for cane>construction>farming p=0.003). Significant trends were also observed for high serum urea nitrogen (BUN>20 mg/dL), high serum creatinine (SCr>1.2 mg/dL) and, regarding dehydration markers, for low urinary pH (≤5.5) and high BUN/SCr ratio (>20) but not for high urinary specific gravity (USG≥1.030). Sugarcane cutters had also more often proteinuria, blood and leucocytes in urine. Workers with eGFR-EPI<80 ml/min/1.73m^2 reported higher intake of water and lower intake of sugary beverages. Serum uric acid levels related strongly and inversely to eGFR (adj. beta -10.4 ml/min/1.73m^2, 95%CI -12.2, -8.5, p<0.001). No associations were observed for other metabolic risk factors, pesticides, NSAIDs or alcohol. In analyses restricted to cane cutters, in addition, consumption of electrolyte hydration solution appeared preventive (adj. beta 8.1 ml/min/1.73m^2, 95% CI -1.2, 17.5; p=0.09).

Conclusion. Heat stress, dehydration and kidney dysfunction were most common among sugarcane cutters. Kidney dysfunction occurred in lesser extent also among construction workers, but hardly among small-scale farmers. High serum uric acid associated with kidney dysfunction.

Strengths and limitations of this study

- The study provides a detailed description of exposures to potential risk factors for MeN among workers in three occupations of special interest, subsistence farmers, construction workers and sugarcane cutters.
- The study established the prevalence of kidney dysfunction and dehydration among workers in these three distinct occupations at risk for MeN
- The cross-sectional design limits causal interpretations about associations between the potential risk factors and the markers of kidney function, but the study provides clues for possible pathways of kidney injury.
- Most exposures to risk factors are self-reported but much attention was payed to the quality of the questionnaires

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Competing interests:

The authors declare no competing interests.

INTRODUCTION

Mesoamerican nephropathy (MeN), an epidemic of chronic kidney disease (CKD), is a chronic tubulointerstitial disease unrelated to traditional CKD risk factors, affecting predominantly young, male workers in Pacific coastal communities of Central America and possibly southern Mexico.[1-4] Several tens of thousands of people have died of this disease.[3] Although MeN is often described as an epidemic of agricultural workers,[1, 5-8] in Central America sugarcane workers are clearly the most affected population.[1, 9, 10] Similar epidemics are occurring among farmers in Sri Lanka and India.[11, 12] It is possible, but to date unproven, that these outbreaks in different parts of the world are etiologically related.[13]

A consistent risk factor for MeN appears to be heavy manual labor in extreme heat.[1] Manual sugarcane cutters exert substantial amount of energy, often in environmental temperatures over 35^{o}C.[14-16] Besides heat stress, some sugarcane workers are also exposed to pesticides, either at sugarcane plantations or while laboring in other crops.[14, 17] Consumption of NSAIDs to manage muscle pain is common.[18] Exposure to heavy metals may occur through contaminated pesticide formulations and fertilizers, as has been shown in Sri Lanka,[19] contaminated drinking water,[20] or even during burning of the cane.[21] Overall, exposure of sugarcane workers to different potential CKD risk factors has not been described in detail.

A leading hypothesis is that recurrent dehydration, possibly in combination with exposure to other agents [e.g. nonsteroidal anti-inflammatory drugs (NSAIDs), heavy metals, agrochemicals, high fructose intake], may be a driving factor.[1, 4] Animal experiments have shown that dehydration and hyperosmolarity may induce tubular injury via activation of the polyol-fructokinase pathway in the kidney.[22] Recently, a mechanism of hyperuricemia and cyclical uricosuria associated with volume loss and dehydration has also been proposed.[23, 24]

Several studies suggest that MeN may also occur among miners and construction workers,[5, 25] cotton workers,[26] and subsistence farmers.[6] However, these cross-sectional data mostly consider current occupation and are therefore not conclusive. Cane cutting is seasonal and many sugarcane workers are also subsistence farmers or work in construction. Contrary to contracted workers, independent small-scale farmers have control over their work hours and are able to avoid the hottest temperatures. Prevalence studies have been recommended to assess exposure to CKD risk factors and kidney dysfunction in different occupations.[1]

The aim of this study was to compare prevalences of a range of potential CKD risk factors among sugarcane cutters, construction workers and small-scale farmers laboring in the same hot environment, along with biomarkers of hydration and kidney function. We hypothesize that sugarcane cutters experience more heat stress, more dehydration and more signs of kidney dysfunction and small scale farmers the least, with construction workers somewhere in between.

METHODS

Study population and recruitment

This is a cross sectional study. We recruited 194 male workers, aged 17-39 (response 93%) and all living in the municipalities of Chinandega and León in the Pacific region of Nicaragua, a major epicenter for the MeN epidemic. Of these, 86 were sugarcane cutters, 56 construction workers and 52 small-scale farmers. All cane cutters of several sugarcane villages were recruited with the help of community leaders; a trade union assisted in recruiting construction workers employed by private companies at three construction sites; and a rural farmer association to recruit associated farmers dedicated full time to the cultivation of subsistence crops.

The study was approved by the Ethical Review Board of UNAN-León, Nicaragua. All participants provided a written informed consent.

Data collection

Data were collected during January-February 2013, first for sugarcane cutters two months after the sugarcane harvest started, and immediately after for construction workers and farmers, under similar climatic conditions. In each of the sugarcane and farmer villages, a well-known public place was selected as the data collection station; construction workers were evaluated at their work site. Data collection started between 5:30 and 6:00 am on the morning after a workday, and blood and urine samples were collected after overnight fasting.

Medical measurements and biological samples. Blood pressure was measured with a calibrated digital sphygmomanometer with the participant seated after resting for 10 minutes. Weight was measured with a calibrated digital flat mobile scale, and height with a foldable stadiometer. Certified technicians collected blood samples in vacuum tubes for centrifugation and serum separation and in a tube with anticoagulant for blood cell count. Samples without coagulant were centrifuged on the spot at 3500 RPM for 10 minutes at room temperature. All samples were placed on ice and transported the same day to the laboratory at the Research Center on Health, Work and Environment (CISTA) at UNAN-León, where hematocrit and hemoglobin were determined with Mindray 2300 hematology analyzer and the serum samples were frozen at -50 ^{0}C. After finalizing all data collection, serum samples were transported to the National Diagnostic and Reference Center of the Ministry of Health (CNDR-MINSA) of Nicaragua, which takes part in an international interlaboratory quality control program. Serum glucose, lipid profile, serum uric acid (S-UA), serum creatinine (SCr) and serum urea nitrogen (BUN) were analyzed with Cobas Integra 400®, an automated equipment which uses a Jaffe compensated method for quantification of SCr and BUN. SCr was calibrated against IDMS-traceable creatinine. Blind spiked and duplicate blood samples from each 10th participant were in 95% within one standard deviation. A urinalysis dipstick was performed on a spot morning sample using a Bayer Clinitek 50 Urine Chemistry Analyzer with Multistix 10SG reagent strips (Siemens Diagnostics, United States) with semi-quantitative measurements of protein (≥30 to<300 mg/dL and ≥300 mg/dL, glucose (positive at ≥100 mg/dL), specific gravity (USG) (1.000 – 1.030), pH (5.0-8.5), blood (+ to +++), nitrite (positive), leukocyte esterase (+ to +++), bilirubin (+ to +++), ketone (≥5 mg/dl) and urobilinogen (≥2 Ehrlich Units).

Questionnaires. Questionnaires were applied by trained interviewers, with courses on bioethics and good clinical practices. A questionnaire on work and health obtained data on demographics and employment (age, education, drinking water source, income, type of contract, sub-employment, social security), lifestyle (smoking, alcohol, drugs, fluid intake on non-working days), health (medically diagnosed diseases, nephrotoxic medications), work history (industry, job titles, job duration, crops, pesticides), and occupational heat stress determinants (shift duration, breaks, shadow, work speed, heavy loads; for sugarcane workers, in addition, incentives to cut more cane, hours between cane burning and entering the field). This questionnaire was developed based on versions used in previous studies in the region.[5, 26, 27] A second questionnaire, developed at the National Institute of Public Health in Mexico, obtained data on the types and amounts of fluids and food items consumed during the day (always a workday) before the interview. The amount of fructose contained in the food and drinking items was estimated based on a fructose calculation list of the Mexican questionnaire,[28] and the USDA National Nutrient Database for Standard Reference for items not included in the Mexican questionnaire.[29]

Statistical analysis

Data were analyzed with SPSS Statistics 20. Descriptive statistics were computed for exposure characteristics of the three occupational groups.

Glomerular filtration rate estimated by the CKD-EPI equation ($eGFR_{CKD-EPI}$) was the main outcome measure, categorized into < and ≥ 80 mL/min/1.73 m2. This cutoff point was chosen instead of the traditional <60 because too few workers had eGFR<60. Prevalences of high serum urea nitrogen (BUN>20 mg/dL), high serum creatinine (SCr>1.2 mg/dL), high serum uric acid (S-UA>7.2 mg/dL) and of urinary markers (presence of protein >30mg/dL, blood, nitrites or leucocytes) were secondary measures of kidney dysfunction. Prevalences of high urinary specific gravity (USG≥1.030), low urinary pH (≤5.5) and high BUN/SCr ratio (>20) were used as indicators of dehydration.

Self-reported social and work history items, diseases and medications, and heat stress exposure variables were dichotomized. A category of heavy smoking was defined as ≥3 pack-years (upper quartile among ever smokers) and a category of heavy drinking composed of subjects in the upper tertiles of lifetime alcohol consumption (≥80,000 g) or average weekly consumption (≥125 g/wk). Total fluid intake was defined as drinking water plus sugary drinks (natural fruit refreshments, sodas, coffee, tea, and electrolyte solution) and reported as liters of total liquids consumed the previous (work)day and for comparison also for a typical non-work day, with subcategories into water only and sugary drinks. Total fructose intake was estimated from all food and fluids consumed including chewed cane, and stratified into fructose from food sources and added sugars. Fructose variables were categorized into quartiles. Cutoff for body mass index (BMI) were set at ≥25 kg/m^2. Hypertension was defined as systolic blood pressure ≥140 and/or diastolic blood pressure ≥90 mm Hg, or a self-reported medical history of hypertension. Diabetes was defined as serum glucose ≥125 mg/dL in the fasting serum sample or a self-reported medical history of diabetes. Use of nephrotoxic medications was recorded if taken at least three times per week for more than three months in the case of NSAIDs and other analgesics, or administered for at least a week in case of nephrotoxic antibiotics, during the last year. Blood and urine biochemical parameters were explored as continuous variables or defined as normal versus abnormal using standard clinical cutoff values.

Differences between occupations were assessed with ANOVA and Kruskal-Wallis tests for normally and not normally distributed continuous variables, respectively, and Pearson Chi-square test for categorical variables or Fisher's Exact Test when Chi-square was not applicable. With occupation as the main proxy for heat stress, we assessed trends for sugarcane cutters>construction workers>farmers for prevalences of markers of kidney dysfunction and dehydration over the ordered occupational groups (gamma statistic).

Differences in the distribution of risk factors between subjects with reduced and normal kidney function were explored for all occupations combined (n=194) and restricted to sugarcane cutters (n=86), with Whitney U-tests for continuous variables and Chi-square tests or Fisher's Exact Test for categorical variables. Exact p-values are reported and p-values ≤ 0.05 were considered statistically significant. Multivariate linear regression models were constructed, for all workers and restricted to sugarcane cutters, with factors that were different between subjects with reduced and normal kidney function at $p<0.10$. Residuals from the regressions were checked to assess the fit of the models.

RESULTS

Potential risk factors for CKD / MeN among the three occupations

Socioeconomic and health-related CKD risk factors

Socioeconomic CKD risk indicators were unfavorable for all workers, but somewhat less for construction workers (Table 1A). Farmers had the lowest income and, being mostly small landowners, they lacked most often contracts or social security with a third having no work for at least four months of the year. Sugarcane cutters were less educated with on average of 4 years of elementary schooling and had more temporary contracts and lack of social security than construction workers.

With regard to lifestyle and medical factors (Table 1B), sugarcane cutters had lower prevalences of heavy smoking and drinking. There were no major differences in use of nephrotoxic drugs between the groups. None of the workers had been diagnosed with diabetes and only five had hyperglycemia >125 mg/dL. Sugarcane cutters showed less obesity, better lipid profiles, lower heart rates and lower blood pressure, but more anemia (37% with hemoglobin <13 g/dL).

Table 1. Socioeconomic and health indicators relevant for CKD /MeN risk among workers in three occupations. Municipalities of Chinandega and León, Nicaragua, 2013

	Sugarcane (N = 86)	**Construction (N = 56)**	**Farming (N = 52)**	**P-value* differences between groups**
A. Demographics, employment and social indicators				
Age (yrs), mean ± SD	25.6 ± 5.5	27.3 ± 6.0	25.2 ± 5.1	0.11
Education (yrs), mean ± SD	3.9 ± 3.0	7.8 ± 3.6	8.0 ± 4.1	<0.001
Drinking water from well (%)	84.9	12.5	13.5	<0.001
Temporary contract (%)	93.0	74.0	21.1	<0.001
Without work ≥ 4 months/yr (%)	20.9	17.9	34.6	0.089
No current social security (%)	15.1	8.9	92.3	<0.001
Monthly household income per person in family, mean ± SD (25 córdobas = 1 US$)	1808 ± 1156	2267 ± 1124	1343 ± 1059	<0.001
B. Life style, medical history and health indicators				
Ever heavy smoker (%)	10.5	26.8	23.1	<0.001
Ever heavy drinker (%)	18.6	28.6	32.7	0.145
NSAIDs ≥ 3 months (%)	5.8	7.1	7.7	0.901
Nephrotoxic antibiotics (%)	1.2	1.8	0.0	0.648
History kidney stones (%)	1.2	5.4	1.9	0.287
History urinary tract infections (%)	23.3	33.9	42.3	0.058
Not feeling in good health (%)	10.5	37.5	17.5	<0.001
BMI >25 kg/m2 (%)	10.5	37.5	17.5	<0.001
Hypertension (BP>140/90) (%)	5.8	17.9	26.9	0.003
Heart rate (BPM), mean ± SD	62 ± 12	73 ± 14	72 ± 13	<0.001
Blood glucose (mg/dL) (mean ± SD)	89 ± 11	90 ± 14	90 ± 12	0.874
Triglycerides (mg/dL) (mean ± SD)	120 ± 67	168 ± 108	177 ± 124	<0.001
Cholesterol (mg/dL) (mean ± SD)	170 ± 36	188 ± 41	178 ± 44	0.032
HDL cholesterol (mg/dL) (mean ± SD)	48 ± 12	42 ± 10	38 ± 8	<0.001
LDL cholesterol (mg/dL) (mean ± SD)	93 ± 28	101 ± 33	91 ± 32	0.120
VLDL cholesterol (mg/dL) (mean ± SD)	24 ± 13	34 ± 22	35 ± 25	<0.001
Hematocrit (%), mean ± SD§	47.5 ± 6.2	48.4 ± 4.8	51.0 ± 3.6	0.002
Hemoglobin (g/dL), mean ± SD§	13.5 ± 1.7	14.8 ± 1.5	15.4 ± 1.2	<0.001
Hemoglobin <13 g/dL (%)§	37.0	9.1	2.1	<0.001

* P-value for differences between groups: ANOVA for normally distributed continuous variables, Kruskal-Wallis for not normally distributed continuous variables, Chi-square test for categorical variables.
§ 37 missing data, of which 32 for sugarcane workers, due to technical error.

Occupational heat exposure, fluid and fructose intake, and pesticides

Sugarcane cutters worked an average 7.7 hours, of which 6.5 hours were spent cutting cane. Farm workers worked least hours per day (mean 6 hrs) and construction workers worked the most (mean 9.4 hrs) but also had the longest total break time (Table 2A). A higher proportion of sugarcane workers perceived a very rapid work pace, and had to take rest breaks in the absence of shade; 83% received incentives for cutting more cane, and almost half started harvesting within 12 hours of burning the cane. Sugarcane cutters reported greater weight loss related to current job (over the last two months) and more frequently fainting on the job (6% as compared to 2% of farmers and no construction workers). Dysuria ('chistata'), a common symptom in MeN affected areas thought to be related to dehydration,[18,29] was not statistically different between the three groups.

With regard to fluid intake (Table 2B), sugarcane cutters reported on average 6.2 L of total fluid intake the previous (work) day, 70% (4.4 L) as water and almost 30% (1.8 L) as sugary drinks. This was higher than for construction workers and farmers. Intake of water and sugary beverages did not correlate (r_p= 0.01). In contrast, the three groups were not different for total fluid, water and sugary drinks intake on non-work days.

Fructose intake during the previous day was highest for sugarcane cutters compared to farmers and construction workers; and 41% of sugarcane cutters belonged to the category of highest quartile of consumption of total fructose (>107 g) (Table 2B). Fructose intake from fruits and food was low among sugarcane cutters and most fructose came from added sugars, specifically from sweetened beverages, electrolyte hydration solution (a third of the cutters) and cane chewing (about two thirds); most of that intake occurred during work hours. Fructose intake outside work hours was not different between the groups.

With regard to pesticide exposures (Table 2C), farmers used pesticides most frequently (71%), almost half of sugarcane cutters reported pesticide use, versus only 11% of construction workers. Glyphosate and 2,4-D use was more common among sugarcane cutters, whereas paraquat and the insecticides cypermethrin and chlorpyrifos were used more often by farmers. With the exception of cypermethrin, which had been used by almost half of the farmers, no specific pesticide exceeded 25% of users in any of the groups.

Table 2. Occupational heat stress, fluid and fructose intake and pesticide exposure indicators among workers in three occupations. Municipalities of Chinandega and León, Nicaragua, 2013

	Sugarcane (N = 86)	Construction (N = 56)	Farming (N = 52)	P-value*
A. Current occupational heat stress				
Work hours per day, mean ± SD	7.7 ± 1.2	9.4 ± 0.6	6.0 ± 2.5	<0.001
Total breaks per day (minutes), mean ± SD	58 ± 25	79 ± 19	42 ± 40	<0.001
Very rapid work pace (%)	74.4	53.6	40.4	<0.001
No shade during breaks (%)	20.9	1.8	11.5	0.004
Lifting weights >50 lbs (%)	18.6	66.1	65.4	<0.001
Awkward work postures (%)	58.1	76.8	69.2	0.063
Hours cutting cane, mean ± SD	6.5 ± 1.2	-	-	-
Incentives to cut more cane (%)	82.6	-	-	-
Hours post-burning at field entrance, mean ± SD	11.7 ± 6.2	-	-	-
Self-reported weight loss on the current job (last two months) (%)	77.9	39.3	36.5	<0.001
Fainted at work (%)	5.8	0	1.9	0.126
Dysuria ('chistata') (%)	43.0	48.2	44.2	0.827
B. Fluid and fructose intake				
Fluid intake previous day (workday)				
Total fluid (L) (mean ± SD)	6.2 ± 4.1	4.4 ± 2.1	4.0 ± 2.7	0.003
Water	4.4 ± 3.9	2.9 ± 2.1	2.8 ± 2.4	0.002
Sugary drinks	1.8 ± 1.8	1.5 ± 0.9	1.2 ± 0.8	0.208
Electrolyte solution (N=31)	1.2 ± 1.1	-	-	-
Lowest quartile total fluid (≤ 2.5 L) (%)	18.6	19.6	40.4	0.009
Highest quartile total fluid (≥7.0 L) (%)	40.7	8.9	13.5	<0.001
Fluid intake on typical non-work day				
Total fluid (L) (mean ± SD)	4.2 ± 2.3	3.8 ± 1.7	4.1 ± 2.2	0.503
Water	3.0 ± 2.0	2.2 ± 1.3	2.7 ± 2.0	0.053
Sugary drinks	1.2 ± 1.1	1.6 ± 1.1	1.4 ± 1.9	0.117
Fructose intake previous day (workday)				
Total fructose intake (g) (mean ± SD)	103.1 ± 72.1	80.1 ± 46.1	70.9 ± 36.8	0.008
From food sources	8.4 ± 10.7	15.9 ± 16.6	17.4 ± 16.7	<0.001
From added sugar	94.7 ± 70.5	64.2 ± 38.1	53.2 ± 30.7	<0.001
Fructose from added sugars (g) (mean ± SD)				
During work hours	58.6 ± 44.7	28.6 ± 21.4	26.1 ± 16.5	<0.001
Sugary drinks ('frescos', sodas, coffee)	22.5 ± 15.7	28.6 ± 21.4	26.1 ± 16.3	0.108
Sugarcane chewing (N=53)	35.0 ± 18.5	-	-	-
Electrolyte hydration solution (N=31)	40.3 ± 35.2	-	-	-
Before and after work hours	36.1 ± 39.3	35.6 ± 31.4	27.1 ± 25.9	0.350
Highest quartile total fructose intake (>107 g) (%)	40.7	19.6	15.7	0.002

	Sugarcane (N = 86)	Construction (N = 56)	Farming (N = 52)	P-value*
C. Work and pesticide use history				
Cumulative time on current job (months), mean ± SD	77 ± 60	68 ± 80	116 ± 67	0.001
Ever sugarcane work (%)	100.0	3.6	3.8	<0.001
Ever plantation (other than sugarcane) (%)	24.4	5.4	21.2	0.012
Ever work in small-scale agricultural (%)	61.6	25.0	100.0	<0.001
Ever construction work (%)	5.8	100.0	11.5	<0.001
Ever any pesticide use (%)	46.5	10.7	71.2	<0.001
Glyphosate (%)	19.8	0.0	3.8	<0.001
2,4-D (%)	23.3	0.0	9.6	<0.001
Paraquat (%)	9.3	3.6	25.0	0.002
Chlorpyrifos (%)	0.0	0.0	23.1	<0.001
Cypermethrin (%)	18.6	3.6	42.6	<0.001

* P-value for differences between groups: ANOVA for normally distributed continuous variables, Kruskal-Wallis for not normally distributed continuous variables, Chi-square test for categorical variables.

Status of kidney function and hydration by occupation

Kidney function biomarkers were more commonly abnormal among sugarcane cutters, with significant differences between the groups for prevalences of eGFR <80 ml/min/1.73m^2 (16%, 9% and 2%, in sugarcane, construction and small-scale farmers, respectively, p for trend=0.003), high SCr (p for trend=0.02) and high BUN (p for trend=0.003) (Table 3A). Likewise, proteinuria >30 mg/dL was approximately three times more prevalent in sugarcane workers compared to the other groups (15% vs 5-6%, p for trend=0.08), whereas leukocyturia was observed in 22% of sugarcane workers but in only 0-2% of the other heat-exposed groups (p<0.001). Microhematuria was also three times more prevalent in sugarcane workers but not statistically significant (6% vs 2%, p for trend=0.19). High S-UA was more common among sugarcane cutters (17%) and construction workers (16%) than among famers (6%).

Regarding markers of dehydration, prevalence of concentrated urine (USG ≥1.030) was lowest among sugarcane cutters and not statistically different between groups (Table 3B). Low urinary pH occurred in 29% of sugarcane cutters versus 12% of construction and farmers (p for trend=0.006) and sugarcane cutters more commonly had an elevated BUN/SCr ratio (26% vs 0 and 4% of construction workers and farmers, p<0.001). Trends over ordered categories were significant for urinary pH and BUN/SCr ratio. Although sugarcane cutters as group had a lower prevalence of concentrated urine, within the group low fluid intake was strongly associated with concentrated urine (OR 3.5, p=0.06) and acidic urine (OR 8.7, p<0.001) which was not the case among construction workers and farmers (Table 4).

Table 3. Biomarkers of kidney function and dehydration among workers in three occupations and trend over categories ordered by exposure to occupational heat stress (sugarcane > construction > farming). Municipalities of Chinandega and León, Nicaragua, 2013.

Variable	Sugarcane (N = 86)	Construction (N = 56)	Farming (N = 52)	P-value: differences between groups*	P-value: trend**
A. Indicators of kidney function					
BUN (mg/dL), mean ± SD (range)	13.9 ± 5.0 (6.0 – 28.4)	10.1 ± 5.1 (4.1 – 30.0)	9.2 ± 3.6 (4.0 – 22.0)	<0.001	
BUN >20 mg/dL (%)	15.1	5.4	1.9	0.017	0.003
SCr (mg/dL), mean ± SD (range)	0.84 ± 0.39 (0.44 – 2.39)	1.00 ± 1.16 (0.49 – 8.84)	0.78 ± 0.22 (0.51 – 1.83)	0.393	
SCr >1.2 mg/dL (%)	17.4	8.9	5.8	0.088	0.024
$eGFR_{CKD-EPI}$, mean ± SD (range)	121 ± 31 (34 – 160)	118 ± 30 (7 – 161)	125 ± 18 (49 – 158)	0.299	
$eGFR_{CKD-EPI}$ <80 ml/min/1.73m^2 (%)	16.3	8.9	1.9	0.025	0.003
S-UA (mg/dL), mean ± SD (range)	6.0 ± 1.7 (3.0 – 12.7)	5.8 ± 1.6 (3.6 – 11.0)	5.0 ± 1.1 (2.9 – 8.1)	0.001	
S-UA >7.2 mg/dL (%)	17.4	16.1	5.8	0.136	0.055
Proteinuria >30 mg/dL (%)	14.7	5.4	6.1	0.128	0.081
Leucocytes in urine (%)	22.1	0	1.9	<0.001	<0.001
Nitrites in urine (%)	0	0	0	-	-
Blood in urine (%)	5.8	1.8	1.9	0.339	0.186
B. Indicators of dehydration					
USG >1.025 (%)	15.3	28.6	20.4	0.161	0.255
Urinary pH ≤5.5 (%)	29.4	12.5	12.2	0.014	0.006
BUN/SCr ratio >20 (%)	25.6	0	3.8	<0.001	<0.001

* Chi-square test. **Gamma statistic for trend over ordered categories.

Table 4. Associations between low intake of fluids and markers of dehydration among sugarcane cutters (n=86) and non-sugarcane cutters (construction workers and small-scale farmers) (n=108): high urinary specific gravity (USG ≥1.030), acidic urine (urinary pH ≤5.5) and high blood urea nitrogen to serum creatinine ratio (BUN/SCr ratio >20). The odds ratios (OR) and 95% confidence intervals [CI, lower limit (LL) and upper limit (UL)] for water and sugary drinks are adjusted for each other.

Lowest quartiles of fluid intake		**USG ≥1.030**	**pH ≤5.5**	**BUN/SCr ratio >20**
		OR (95% CI: LL; UL) p-value		
Total fluids ≤2.5L	Sugarcane cutters	3.5 (1.0; 13)	8.7 (2.6; 29)	1.2 (0.3; 4.3)
		0.06	<0.001	0.67
	Construction workers and farmers	1.4 (0.5; 3.5)	2.3 (0.7; 7.5)	-[a]
		0.51	0.17	
Water ≤1.5L	Sugarcane cutters	3.0 (0.7; 12)	2.9 (0.9; 9.6)	2.3 (0.7; 7.3)
		0.14	0.08	0.17
	Construction workers and farmers	1.9 (0.7; 4.9)	1.7 (0.5; 5.6)	-a
		0.18	0.42	
Sugary drinks ≤0.75L	Sugarcane cutters	2.5 (0.7; 9.2)	2.5 (0.9; 7.1)	0.3 (0.2; 1.1)
		0.16	0.08	0.06
	Construction workers and farmers	1.8 (0.6; 5.2)	0.7 (0.2; 3.6)	-[a]
		0.28	0.69	

[a] Not computed because of too few workers with BUN/SCr ratio >20.

Risk factors for reduced kidney function

In bivariate analyses of differences in kidney, urinary and metabolic biomarkers, work practices, hydration practices and lifestyle characteristics between subjects with reduced kidney function and subjects with normal kidney function (Supplementary Table 1), reduced kidney function (eGFR <80 ml/min/1.73 m^2) was significantly associated with work as sugarcane cutter, high intake of water, low intake of sugary beverages, increasing age, low hemoglobin and history of heavy smoking. In analyses restricted to sugarcane cutters, results were similar and, in addition, workers with reduced kidney function had cut cane during considerably longer time than those with normal kidney function (cumulative time on the job: median 108 vs 60 months, p=0.06). Sugarcane cutters with reduced kidney function reported almost three times higher water intake and three times lower intake of sugary beverages as compared to cutters with normal kidney function, with only 1 of the 14 reporting intake of the electrolyte solution. In addition, the cane cutters with reduced kidney function had worse lipid profile than those with normal kidney function and more often hypertension, but none had diabetes or hyperglycemia and only one was overweight (Supplementary Table 1).

In backwards stepping multivariate linear regression analyses with inclusion of variables with p≤0.10 in the bivariate analyses (except hemoglobin due to missing data), age (beta -1.3, 95%CI -1.8, -0.8; p<0.001) and S-UA (beta -10.4, 95% CI -12.2, -8.5; p<0.001) associated significantly with reduced kidney function among all workers, identically in models with total fluid intake and with intake of water and sugary beverages separately (Table 5A). In the subset of sugarcane cutters, too many variables had a p-value ≤0.10 in bivariate analyses and therefore the regression was done in two steps (see supplementary Table 1). Hypertension and biomarkers of metabolic syndrome did not associate with reduced kidney function in a model also including age and serum uric acid (data not shown) and were not further considered. In a model with water intake, intake of sugary drinks (without electrolyte solution) and intake yes/no of electrolyte solution, age, S-UA, ever heavy smoking and ever heavy drinking (Table 5B), reduced kidney function associated significantly to age and uric acid and non-significantly to the intake of electrolyte solution (beta 8.1, 95% CI -1.2, 17.5, p=0.09). Age and cumulative months on the job were highly correlated (r_p 0.68, p<0.001), and substituting age with time cutting cane yielded similar results.

Table 5. Multivariate linear regression models of estimated glomerular filtration rate ($eGFR_{CKD-EPI}$) among all workers (sugarcane cutters, construction workers and farmers) and restricted to sugarcane cutters.

All subjects (N=194)		**Beta coefficient (95% CI: UL; LL)**	**Standardized beta coefficient**	**P-value**	**Adjusted R^2**
Step 1	Water intake (L)	-0.7 (-1.7; 0.3)	-0.08	0.15	0.47
	Sugary beverages intake (L)	1.2 (-0.8; 3.3)	0.06	0.24	
	Sugarcane cutter ever	3.6 (-2.5; 9.6)	0.07	0.25	
	Age (yrs)	-1.2 (-1.7; -0.6)	-0.24	<0.001	
	Serum uric acid (mg/dL)	-10.0 (-12.0; -8.1)	-0.57	<0.001	
	Heavy smoker ever	-4.5 (-11.6; 2.7)	-0.07	0.22	
	Heavy drinker ever	1.2 (-5.6; 8.1)	0.02	0.72	
Final step	Age (yrs)	-1.3 (-1.8; -0.8)	-0.27	<0.001	0.47
	Serum uric acid (mg/dL)	-10.4 (-12.2; -8.5)	-0.59	<0.001	
Sugarcane cutters (N=86)					
Step 1	Water intake (L)	-0.7 (-1.9; 0.5)	-0.09	0.25	0.58
	Sugary beverages intake (without electrolyte solution) (L)	1.2 (-3.7; 6.0)	0.04	0.63	
	Electrolyte solution (yes/no)	6.4 (-4.5; 17.3)	0.10	0.24	
	Age (yrs)	-1.7 (-2.5; -0.8)	-0.29	<0.001	
	Serum uric acid	-10.9 (-13.8: -8.1)	-0.59	<0.001	
	Heavy smoker ever	-10.1 (-22.5; 2.3)	-0.12	0.11	
	Heavy drinker ever	-7.8 (-19.5; 3.9)	-0.10	0.19	
Final step	Age (yrs)	-1.9 (-2.7; -1.1)	-0.34	<0.001	0.57
	Serum uric acid (mg/dL)	-11.3 (-14.0; -8.6)	-0.61	<0.001	
	Electrolyte solution (yes/no)	8.1 (-1.2; 17.5)	0.13	0.09	

DISCUSSION

This study found evidence for more frequent heat stress and kidney dysfunction among sugarcane cutters, as expected, and in lesser degree also reduced kidney function among construction workers but not among small-scale farmers. S-UA concentrations inversely associated with eGFR.

Evidence of reduced kidney function

We used a cutoff of eGFR of 80 ml/min/m^2 to evaluate differences in renal function because only 11 workers had eGFR below 60, due to young age (all under age 40) and also because sugarcane workers were screened by employers before the start of the harvest two months earlier and workers with SCr>1.2 mg/dL were not hired. Despite, approximately one-fourth of sugarcane cutters had evidence for either eGFR <80 ml/min/m^2, serum creatinine >1.2 mg/dL or proteinuria ≥30 mg, and these findings were, respectively, eight-, three- and two-fold more common than observed in subsistence agricultural workers and about two-fold greater than observed in construction workers (Table 3). However, although in lesser degree than cane cutters, construction workers also had an unusually high prevalence of decreased kidney function, which is in accordance with a previous unpublished study in the same area.[25] In contrast, the single small-scale farmer with reduced kidney function had worked previously in sugarcane. Thus, our results show that not all agricultural workers are at increased risk for CKD, as is commonly stated, but rather workers in certain types of agriculture and other hot jobs such as work in the construction industry. The absence of reduced kidney function among subsistence farmers is consistent with a study in a MeN epidemic area in El Salvador, where subsistence farmers without a history of plantation work had a significantly lower prevalence of abnormal SCr than men who had worked on sugar or cotton plantations (15% vs 33%).[20] Reduced kidney function was accompanied by a higher frequency of anemia among sugarcane cutters (37% versus <10% in other groups). The prevalence of anemia was higher than the prevalence of reduced kidney function and cannot be simply ascribed to the higher frequency of reduced renal function. Marked anemia, defined as Hb of <10 g/dL, was not observed in any of the groups.

The reduced kidney function did not associate with traditional risk factors for CKD. Notably, there was not one case of diabetes in the entire population. Importantly, sugarcane workers showed significantly worse renal function despite overall lower frequency of metabolic syndrome, hypertension and obesity compared to the other two groups (see Table 1). Increasing age, over the age of 50, is a known risk factor for CKD, but we found increasing age to be associated with decline of renal function, despite the young age of the study participants. This is possibly related to the increased risk that occurs with continued job exposure over time, in particular among the sugarcane cutters. Thus, our studies suggest that most, if not all cases of reduced kidney function, are related to Mesoamerican nephropathy and not classic CKD.

Evidence for heat stress

There was evidence for greater risk for heat stress among sugarcane cutters. Sugarcane cutters labored at a faster pace, had less exposure to shade, reported more weight loss during the ongoing harvest, and had more fainting episodes.

While sugarcane cutters had greater heat stress exposure, they also drank more fluids during the course of the day, amounting to an average of 6.2 L per day (although this varied considerably, with approximately 20 percent drinking <2.5 L/d and 40% >7 L/day). However, the type of exertion and sweating that occurs with cane harvesting[14-16] could still result in dramatic loss of fluids such that dehydration can occur despite high fluid consumption. Cade et al. found that college football players could lose as much as 8 quarts (about 7.6 L) of water in a 2 hour period, associated with loss of salt, a decrease in blood glucose, and a fall in blood pressure.[30]

Although self-reported, our heat exposure and hydration data were collected through carefully designed questionnaires. Our results add to knowledge on working conditions and perceptions of workers at risk for MeN.

Potential role for uric acid for kidney disease

Heat stress is known to raise serum uric acid levels, in part from subclinical rhabdomyolysis,[31] but also from reduced renal blood flow.[32] In turn, hyperuricemia is a well-known risk factor for CKD[33] and mediates both glomerular and tubulointerstitial disease in animals.[34-36] Interestingly, serum uric acid levels tended to be highest in both sugarcane workers and construction workers, with 16-17% of these individuals having hyperuricemia (defined as >7.2 mg/dl) compared to 6% in subsistence farmers. Furthermore, we found that the presence of hyperuricemia was independently associated with declining renal function (see Table 5). However, since reduced renal function can also result in increased uric acid levels due to impaired excretion, the causal role of uric acid in the reduced kidney function cannot be determined.

Recently we hypothesized that renal injury could be occurring in sugarcane workers due to cyclical uricosuria with crystal formation.[23, 24] According to this hypothesis, serum uric acid might rise as a consequence of subclinical rhabdomyolysis, followed by its crystallization in the urine. One factor that increases the risk for urate crystal formation is acidic urine that could result from the release of lactic acid associated with strenuous exercise and by the effects of dehydration to reclaim sodium with hydrogen ion excretion. Urine pH was significantly lower in the sugarcane workers compared to other groups (see Table 3) and was strongly associated with low fluid intake the previous (work) day in the subset of sugarcane workers (see Table 4). This might reflect the effects of greater volume depletion (with aldosterone stimulation), lactic acid generation during the prior day, or other mechanisms.

Hydration and fructose

We had expected that low water intake, or high sugary fluid intake, would be associated with reduced renal function, based on studies in animals.[22, 37] However, workers with eGFR <80 ml/min/m^2 drank more water and consumed less sugar-based drinks during the workday compared to subjects with normal kidney function. This was particularly so among sugarcane cutters (respectively 6.3 L vs 2.2 L water and 0.5 L vs 1.6 L sugary drinks), although the difference of total fluid intake between cutters with and without reduced kidney function was not significant (6.3 L vs 4.5 L, p=0.16) (see Supplementary Table 1). These findings are counterintuitive, but may be partially explained by very high water requirements during the heavy labor of sugarcane cutting.[15, 16] Although low fluid intake clearly associated with concentrated urine among sugarcane cutters (OR USG≥1.030=3.5, p=0.06) (see Table 4), cutters in the quartile with the highest fluid intake did not have a decreased risk (OR=1.3, p=0.70) while high fluid intake among non-sugarcane workers appeared to be preventive (OR 0.10, p=0.06). Salvadorian cane cutters who consumed amounts of fluid comparable to our Nicaraguan cutters, were found to have insufficient fluid intake under their work conditions.[14]

Sugary beverages that contain fructose are known to increase the risk for CKD,[38] and can induce renal injury in laboratory animals.[37] However, fructose is also a component of sports drinks and fluid resuscitation packets containing glucose and electrolytes that might be beneficial to the volume and water depleted, such as by providing glucose that may prevent or treat any associated hypoglycemia, or by helping to maintain blood pressure due to the fructose component.[39, 40] In our study, the intake of electrolyte solutions tended to be associated (p=0.09) with improved kidney function in multivariate analyses (see Table 4). One study in Nicaragua found that the eGFR of cane cutters increased by 7 ml/min/1.73 m2 for each 100 cc electrolyte hydration packet over the course of one harvest season.[20] These issues need to be assessed with prospective studies that examine overall fluid balance by measuring fluid intake as well as losses during work, such as pre- and post-shift weight and serum and urine osmolarity.

Other risk factors for kidney disease

There was no association with NSAIDs or alcohol intake. A history of heavy smoking was more frequent among subjects with reduced kidney function (p=0.02) but lost significance in multivariate analyses. A history of pesticide exposure was more common among farmers, although exposure to herbicides was more common among sugarcane cutters, especially glyphosate and 2,4-D, both of special interest. However, analyses failed to identify pesticide exposures as an independent risk factor for reduced kidney function (see Supplementary Table 1).

CONCLUSIONS

In summary, sugarcane workers have higher heat stress and worse renal function despite a better metabolic profile, compared to construction workers and, in particular, subsistence farmers from the same MeN epidemic region of Nicaragua. Our study supports the need for improved work practices and even more hydration with adequate access to water for sugarcane cutters, as well as for workers in other hot occupations such as construction. The associations between intake of water and sugary drinks and kidney function as well as the role of hyperuricemia need to be assessed in carefully designed follow-up studies.

AUTHORS' CONTRIBUTIONS:

Concept and design: Aurora Aragón, Catharina Wesseling, Marvin González, Richard J Johnson, Jason Glaser, Ricardo Correa-Rotter

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Manuscript preparation: Catharina Wesseling, Richard J. Johnson

Critical revision and approval of manuscript: All authors

DATA SHARING STATEMENT:

We are willing to share parts of the database, if there is research that could benefit from it.

STROBE CHECKLIST: see separate file

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Supplementary Table 1. Comparison of kidney, urinary and metabolic biomarkers, work practices, hydration practices and lifestyle characteristics of subjects with reduced kidney function (<80ml/min/1.73 m^2) and subjects with normal kidney function (≥80ml/min/1.73 m^2), all study participants (N=194) and sugarcane workers (N=86)

	All workers (N=194)			**Sugarcane cutters (N=86)**		
	eGFR <80ml/min/1.73 m^2 N=20	eGFR ≥80ml/min/1.73 m^2 N=174	P-value*	eGFR <80ml/min/1.73 m^2 N=14	eGFR ≥80ml/min/1.73 m^2 N=72	P-value*
Kidney function						
eGFR (ml/min/1.73 m^2), median (10%; 90%)	55 (27; 76)	129 (112; 147)	<0.001	63 (39, 78)	134 (102, 153)	<0.001
S creatinine (mg/dL), median (10%; 90%)	1.67 (0.24; 3.05)	0.72 (0.56; 0.94)	<0.001	1.47 (1.24; 2.21)	0.70 (0.51; 0.95)	<0.001
S urea nitrogen (BUN) (mg/dL), median (10%; 90%)	22.6 (13.5; 28.3)	10.0 (6.1; 15.0)	<0.001	22.6 (14.4; 28.1)	12.1 (7.9; 18.0)	<0.001
S uric acid (mg/dL), median (10%; 90%)	8.2 (6.7; 11.0)	5.2 (3.9; 6.8)	<0.001	8.1 (6.9, 11.8)	5.4 (3.9, 6.9)	<0.001
S uric acid >7.2 mg/dL, % (# cases)	85.0 (17)	5.7 (10)	<0.001	85.7 (12)	4.2 (3)	<0.001
Protein >30 mg, % (# cases) (N=180)	20.0 (4)	7.5 (13)	0.09	23.1 (3)	2.9 (8)	0.39
Leukocytes positive at dipstick, % (# cases)	30.0 (6)	8.0 (14)	0.009	42.9 (6)	18.1 (13)	0.07
Blood, traces and higher at dipstick, % (# cases)	20.0 (4)	1.7 (3)	0.002	28.6 (4)	1.4 (1)	0.002
Hydration						
USG ≥1030, % (# cases) (N=190)	10.0 (2)	21.8 (37)	0.38	7.1 (1)	16.9 (12)	0.69
USG ≤1005, % (# cases) (N=190)	20.0 (4)	16.1 (28)	0.75	28.6 (4)	12.7 (9)	0.22
pH ≤5.5, % (# cases) (N=190)	30.0 (6)	18.4 (32)	0.24	42.9 (6)	26.8 (19)	0.34
BUN/serum creatinine ratio>20	0 (0)	13.8 (24)	0.14	0 (0)	30.6 (22)	0.02
Work practices						
Sugarcane cutter ever, % (# cases)	75.0 (15)	43.1 (75)	0.007	n.a.	n.a.	n.a.
Cumulative time in job (months), median (10%; 90%)	90 (2; 235)	62.5 (4; 180)	0.39	108 (30; 216)	60 (30; 216)	0.06
Work day (hours), median (10%; 90%)	7.75 (6.0; 10.0)	8.0 (4.0; 10.0)	0.85	7.0 (5.5; 10.0)	8.0 (6.0; 9.0)	0.03
Hours cutting cane, median (10%; 90%)	n.a.	n.a.	n.a.	6.0 (4.6; 7.5)	6.8 (5.0; 8.0)	0.02
Total break time (min), median (10%; 90%)	60 (20; 113)	60 (20; 104)	0.55	60.0 (20, 112)	60 (25, 90)	0.30
Breaks ≤2/d, % (# cases)	65.0 (13)	70.7 (123)	0.60	57.1 (8)	63.9 (46)	0.63

	All workers (N=194)			Sugarcane cutters (N=86)		
	eGFR <80ml/min/1.73 m^2 N=20	eGFR ≥80ml/min/1.73 m^2 N=174	P-value*	eGFR <80ml/min/1.73 m^2 N=14	eGFR ≥80ml/min/1.73 m^2 N=72	P-value*
No shade during breaks, % (# cases)	20.0 (4)	12.1 (21)	0.30	28.6 (4)	19.4 (14)	0.48
High speed perception, % (# cases)	75.0 (15)	57.5 (100)	0.13	85.7 (12)	72.2 (52)	0.50
Production (tons/d), median (10%; 90%)	n.a.	n.a.	n.a.	6.5 (5.0; 10.0)	7.0 (5.0; 10.0)	0.66
Incentives to cut more, % (N)	n.a.	n.a.	n.a.	71.4 (10)	84.7 (61)	0.26
History of pesticide use, % (# cases)	30.0 (6)	44.3 (77)	0.22	42.9 (6)	47.2 (34)	1.00
Chlorpyrifos, % (# cases)	0 (0)	6.9 (12)	0.62	0 (0)	0 (0)	-
Cypermethrin, % (# cases)	10.0 (2)	23.0 (40)	0.26	14.3 (2)	19.4 (14)	1.00
Paraquat, % (# cases)	0 (0)	13.2 (23)	0.14	0 (0)	11.1 (8)	0.34
2,4-D, % (# cases)	20.0 (4)	12.1 (21)	0.30	28.6 (4)	22.2 (16)	0.73
Glyphosate, % (# cases)	15.0 (3)	9.2 (16)	0.42	21.3 (3)	19.4 (14)	1.00
Hydration practices						
Total fluid intake (L), median (10%; 90%)	5.0 (1.1; 13.8)	3.8 (1.9; 10.0)	0.08	6.7 (1.4; 14.6)	4.5 (2.2; 12.4)	0.16
Low total fluid intake ≤2.5 L/d, % (# cases)	15.0 (3)	25.9 (45)	0.41	14.2 (2)	19.4 (14)	1.00
High total fluid intake ≥7.0 L/d, % (# cases)	40.0 (7)	22.4 (39)	0.10	50.0 (7)	38.9 (28)	0.44
Water (L), median (10%; 90%)	4.5 (0.3; 13.3)	2.2 (0.5; 8.0)	0.03	6.3 (0.6; 14.3)	2.2 (0.7; 10.0)	0.03
Low water intake < 1.5 L/d, % (# cases)	20.0 (4)	25.3 (44)	0.79	14.2 (2)	19.4 (14)	1.00
High water intake > 4.5 L/d, % (# cases)	50.0 (10)	22.4 (39)	0.007	64.3 (9)	30.6 (22)	0.02
Sugary beverage, median (10%; 90%)	0.6 (0.4; 2.4)	1.25 (0.5; 3.0)	0.001	0.5 (0.3; 1.9)	1.6 (0.4; 4.5)	0.002
Low sugary drink intake <0.75 L/d, % (# cases)	50.0 (10)	22.4 (39)	0.007	64.3 (9)	26.4 (19)	0.01
High sugary drink intake ≥2.0 L/d, % (# cases)	10.0 (2)	28.7 (50)	0.07	7.1 (1)	40.3 (29)	0.03
Intake electrolyte solution, % (N)	n.a.	n.a.	n.a.	7.1 (1)	41.7 (30)	0.01
Age, metabolic and lifestyle risk factors						
Age (yrs), median (10%; 90%)	29.5 (21; 37)	25 (19; 33)	0.002	29 (20; 36.5)	24 (18; 33)	0.01
Hypertension, % (# cases)	15.0 (3)	19.5 (34)	0.77	21.4 (3)	4.2 (3)	0.05
Body mass index (BMI, kg/m^2), median (10%; 90%)	24.0 (19.9; 28.9)	23.3 (19.9; 29.7)	0.49	23.0 (19.2; 29.7)	22.2 (19.6; 26.4)	0.23

	All workers (N=194)			Sugarcane cutters (N=86)		
	eGFR <80ml/min/1.73 m^2 N=20	eGFR ≥80ml/min/1.73 m^2 N=174	P-value*	eGFR <80ml/min/1.73 m^2 N=14	eGFR ≥80ml/min/1.73 m^2 N=72	P-value*
BMI >25 kg/m^2, % (# cases)	40.0 (8)	35.1 (61)	0.81	21.4 (3)	16.7 (12)	0.70
BMI >30 kg/m^2, % (# cases)	5.0 (1)	15 (8.6)	1.00	7.1 (1)	1.4 (1)	0.30
Heart rate >80 pulses/min, % (# cases)	25.0 (5)	15.5 (27)	0.34	28.6 (4)	5.6 (4)	0.02
Glycemia (mg/dL), median (10%; 90%)	90.4 (78.0; 103.1)	87.9 (75.3; 104.2)	0.64	90.8 (79.3; 103.5)	88.4 (75.2; 103.6)	0.43
Glycemia ≥ 100 mg/dL, % (# cases)	15.0 (3)	16.1 (28)	1.00	14.3 (2)	15.3 (11)	1.00
Hypertension (HT) and hyperglycemia (≥100mg/dL), % (# cases)	0 (0)	2.9 (5)	1.00	0 (0)	0 (0)	-
Triglycerides (mg/dL), median (10%; 90%)	144.7 (76.4; 293.2)	122.7 (59.2; 268.1)	0.15	139.1 (74.4; 304.9)	101.7 (48.3; 188.1)	0.01
LDL cholesterol (mg/dL), median (10%; 90%)	89.9 (53.5; 138.7)	93.7 (58.7; 135.1)	0.78	96.4 (63.4; 134.0)	91.0 (57.1; 131.8)	0.44
HDL cholesterol (mg/dL), median (10%; 90%)	39.9 (20.7; 56.1)	43.0 (32.2; 60.1)	0.23	42.2 (23.1; 58.4)	47.7 (33.9; 67.8)	0.06
VLDL cholesterol (mg/dL), median (10%; 90%)	28.9 (15.3; 58.6)	24.8 (11.9; 53.6)	0.15	27.8 (14.9; 61.0)	20.3 (9.7; 37.6)	0.01
Hemoglobin (mg/dL), median (10%; 90%) (N=157)	11.3 (10.6; 15.2)	15.0 (12.8; 16.4)	<0.001	11.1 (10.5; 11.7)	14.0 (12.0; 16.1)	0.03
Hb < 13 mg/dL, % (# cases) (N=157)	55.0 (11)	8.6 (15)		100 (9/9)	24.4 (11/45)	<0.001
Smoking ≥3 pack years, % (# cases)	45.0 (9)	20.0 (35)	0.02	41.7 (5)	11.3 (8)	0.02
Heavy drinker ever, % (# cases)	10.0 (2)	27.0 (47)	0.10	7.1 (1)	20.8 (15)	0.21
Regular use of nephrotoxic medications, % (# cases)	10.0 (2)	6.3 (11)	0.63	7.1 (1)	5.6 (4)	1.00

*Mann Whitney U test for medians; Chi-square test (Fisher exact test when 1 or more cells with count<5) for categorical data
n.a.: not applicable

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*
Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any pre-specified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-8
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-8*
Bias	9	Describe any efforts to address potential sources of bias	3, 19-22
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7-8
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	Not applicable

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	Not applicable
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-13
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	14-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	16-17
		(*b*) Report category boundaries when continuous variables were categorized	7-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	19
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	3, 19-22
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22
Generalisability	21	Discuss the generalisability (external validity) of the study results	22
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Heat stress, hydration and uric acid: a cross-sectional study in workers of three occupations in a hotspot of Mesoamerican nephropathy in Nicaragua

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Heat stress, hydration and uric acid: a cross-sectional study in workers of three occupations in a hotspot of Mesoamerican nephropathy in Nicaragua

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Running Title: Mesoamerican nephropathy and occupational heat stress

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Key terms: Mesoamerican nephropathy, chronic kidney disease, occupational heat stress, hydration, serum uric acid

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Abstract

Objectives: To study Mesoamerican nephropathy (MeN) and its risk factors in three hot occupations.

Design: Cross-sectional.

Setting: Chinandega and León municipalities, a MeN hotspot in the Nicaraguan Pacific coast, January-February 2013.

Participants: 194 male workers aged 17-39: 86 sugarcane cutters, 56 construction workers and 52 small-scale farmers.

Outcome measures: i) Differences between the three occupational groups in prevalences/levels of socioeconomic, occupational, lifestyle and health risk factors for chronic kidney disease (CKD); and in biomarkers of kidney function and hydration; ii) differences in prevalences/levels of CKD risk factors between workers with reduced estimated glomerular filtration rate ($eGFR_{CKD-EPI}$<80 ml/min/1.73m^2) and workers with normal kidney function ($eGFR_{CKD-EPI}\geq$80 ml/min/1.73m^2).

Results: Sugarcane cutters were more exposed to heat and consumed more fluid on workdays; and had less obesity, lower blood sugar, lower blood pressure and better lipid profile. Reduced eGFR occurred in 16%, 9% and 2% of sugarcane cutters, construction workers and farmers, respectively (trend cane>construction>farming p=0.003). Significant trends were also observed for high serum urea nitrogen (BUN>20 mg/dL), high serum creatinine (SCr>1.2 mg/dL), low urinary pH (≤5.5) and high BUN/SCr ratio (>20) but not for high urinary specific gravity (USG≥1.030). Sugarcane cutters had also more often proteinuria, and blood and leucocytes in urine. Workers with eGFR<80 ml/min/1.73m^2 reported higher intake of water and lower intake of sugary beverages. Serum uric acid levels related strongly and inversely to eGFR levels (adj. beta -10.4 ml/min/1.73m^2, 95%CI -12.2, -8.5, p<0.001). No associations were observed for other metabolic risk factors, pesticides, nonsteroidal anti-inflammatory drugs or alcohol. Among cane cutters, consumption of electrolyte hydration solution appeared preventive (adj. beta 8.1 ml/min/1.73m^2, p=0.09).

Conclusion. Heat stress, dehydration and kidney dysfunction were most common among sugarcane cutters. Kidney dysfunction occurred in lesser extent also among construction workers, but hardly among small-scale farmers. High serum uric acid associated with reduced kidney function.

Strengths and limitations of this study

- The study provides a detailed description of exposures to potential risk factors for Mesoamerican nephropathy (MeN) among workers in three occupations of special interest, subsistence farmers, construction workers and sugarcane cutters.
- The study established the prevalence of kidney dysfunction and dehydration among workers in these three distinct occupations at risk for MeN.
- The cross-sectional design limits causal interpretations about associations between the potential risk factors and the markers of kidney function, but the study provides clues for etiology and possible pathways of kidney injury.
- Most exposures to risk factors are self-reported but much attention was payed to the quality of the questionnaires

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Competing interests:

The authors declare no competing interests.

INTRODUCTION

Mesoamerican nephropathy (MeN), an epidemic of chronic kidney disease (CKD), is a chronic tubulointerstitial disease unrelated to traditional CKD risk factors, affecting predominantly young, male workers in Pacific coastal communities of Central America and possibly southern Mexico.[1-4] Several tens of thousands of people have died of this disease.[3] Although MeN is often described as an epidemic of agricultural workers,[1, 5-8] in Central America sugarcane workers are clearly the most affected population.[1, 9, 10]

A consistent risk factor for MeN appears to be heavy manual labor in extreme heat.[1] Manual sugarcane cutters exert substantial amount of energy, often in environmental temperatures over 35^{o}C and high humidity.[11-13] Besides heat stress, some sugarcane workers are also exposed to pesticides, either at sugarcane plantations or while laboring in other crops.[11,14] Consumption of nonsteroidal anti-inflammatory drugs (NSAIDs) to manage muscle pain is common.[15] Exposure to heavy metals may occur through contaminated pesticide formulations and fertilizers, as has been shown in Sri Lanka,[16] contaminated drinking water,[17] or even during burning of the cane.[18] Overall, exposure of sugarcane workers to different potential CKD risk factors has not been described in detail.

A leading hypothesis is that recurrent dehydration, possibly in combination with exposure to other agents (e.g. NSAIDs, heavy metals, agrochemicals, high fructose intake), may be a driving factor.[1, 4] Animal experiments have shown that dehydration and hyperosmolarity may induce tubular injury via activation of the polyol-fructokinase pathway in the kidney.[19] Recently, a mechanism of hyperuricemia and cyclical uricosuria associated with volume loss and dehydration has also been proposed.[20, 21]

Studies suggest that MeN may also occur among miners and construction workers,[5, 22] cotton workers,[23] and subsistence farmers.[6] However, these cross-sectional data mostly consider current occupation and are therefore not conclusive. Cane cutting is seasonal and many sugarcane workers are also subsistence farmers or work in construction. Contrary to contracted workers, independent small-scale farmers have control over their work hours and are able to avoid the hottest temperatures. Prevalence studies have been recommended to assess exposure to CKD risk factors and kidney dysfunction in different occupations.[1]

The aim of this study was to compare the prevalence of a range of potential CKD risk factors among sugarcane cutters, construction workers and small-scale farmers laboring in the same hot environment, along with biomarkers of hydration and kidney function. We hypothesize that sugarcane cutters experience more heat stress, more dehydration and more signs of kidney dysfunction than small scale farmers, with construction workers somewhere in between.

METHODS

Study population and recruitment

This is a cross sectional study. We recruited 194 male workers, aged 17-39, all living in the municipalities of Chinandega and León in the Pacific region of Nicaragua, a major epicenter for the MeN epidemic. Of these, 86 were sugarcane cutters, 56 construction workers and 52 small-scale farmers. Cane cutters of several sugarcane villages were recruited with the help of community leaders; a trade union assisted in recruiting construction workers employed by private companies at three construction sites; and a rural farmer association to recruit associated farmers dedicated full time to the cultivation of subsistence crops. The response rate was 86% among cane cutters and there were no refusals among construction workers and farmers.

The study was approved by the Ethical Review Board of UNAN-León, Nicaragua. All participants provided a written informed consent.

Data collection

Data were collected for sugarcane cutters during January 2013, two months after the sugarcane harvest started, and during February 2013 for construction workers and farmers, under similar climatic conditions. In each of the sugarcane and farmer villages, a well-known public place was selected as the data collection station; construction workers were evaluated at their work site. Data collection started between 5:30 and 6:00 am on the morning after a workday, and blood and urine samples were collected after overnight fasting.

Medical measurements and biological samples. Blood pressure was measured with a calibrated digital sphygmomanometer with the participant seated after resting for 10 minutes. Weight was measured with a calibrated digital flat mobile scale, and height with a foldable stadiometer. Certified technicians collected blood samples in vacuum tubes for centrifugation and serum separation and in a tube with anticoagulant for blood cell count. Samples without coagulant were centrifuged on the spot at 3500 RPM for 10 minutes at room temperature. All samples were placed on ice and transported the same day to the laboratory at the Research Center on Health, Work and Environment (CISTA) at UNAN-León, where hematocrit and hemoglobin were determined with a Mindray 2300 hematology analyzer and the serum samples were frozen at -80 ^{0}C. After finalizing all data collection, serum samples were transported to the National Diagnostic and Reference Center of the Ministry of Health (CNDR-MINSA) of Nicaragua, which takes part in an international interlaboratory quality control program. Samples were analyzed with Cobas Integra 400®, an automated equipment which uses a photometric test to determine levels of serum glucose, lipid profile, serum uric acid (S-UA), and blood urea nitrogen (BUN) and a Jaffe compensated method for quantification of serum creatinine (SCr). SCr was calibrated against IDMS-traceable creatinine. Blind spiked and duplicate blood samples from each 10th participant were in 95% within one standard deviation. A urinalysis dipstick was performed on a spot morning sample using a Bayer Clinitek 50 Urine Chemistry Analyzer with Multistix 10SG reagent strips (Siemens Diagnostics, United States) with semi-quantitative measurements of protein (≥30 to<300 mg/dL and ≥300 mg/dL, glucose (positive at ≥100 mg/dL), specific gravity (USG) (1.000 – 1.030), pH (5.0-8.5), blood (+ to +++), nitrite (positive), leukocyte esterase (+ to +++), bilirubin (+ to +++), ketone (≥5 mg/dl) and urobilinogen (≥2 Ehrlich Units).

Questionnaires. Questionnaires were applied by trained interviewers, with courses on bioethics and good clinical practices. A questionnaire on work and health obtained data on demographics and employment (age, education, drinking water source, income, type of contract, sub-employment, social security), lifestyle (smoking, alcohol, drugs, fluid intake on non-working days), health (medically diagnosed diseases, nephrotoxic medications), work history (industry, job titles, job duration, crops, pesticides), and occupational heat stress determinants (shift duration, breaks, shadow, work speed, heavy loads; for sugarcane workers, in addition, incentives to cut more cane, hours between cane burning and entering the field). This questionnaire was developed based on versions used in previous studies in the region.[5, 23, 24] A second questionnaire, developed at the National Institute of Public Health in Mexico, obtained data on the types and amounts of fluids and food items consumed during the day (always a workday) before the interview. The amount of fructose contained in the food and drinking items was estimated based on a fructose calculation list of the Mexican questionnaire,[25] and the USDA National Nutrient Database for Standard Reference for items not included in the Mexican questionnaire.[26]

Statistical analysis

Data were analyzed with SPSS Statistics 20. Glomerular filtration rate estimated by the CKD-EPI equation ($eGFR_{CKD\text{-}EPI}$) was the main outcome measure, categorized into < and ≥80 mL/min/1.73m^2. This cutoff point was chosen instead of the traditional <60 because too few workers had eGFR<60. Prevalences of high BUN (>20 mg/dL), high SCr (>1.2 mg/dL), high S-UA (>7.2 mg/dL), and protein >30mg/dL, blood, nitrites or leucocytes in urine were secondary measures of kidney dysfunction. Prevalences of high urinary specific gravity (USG≥1.030), low urinary pH (≤5.5) and high BUN/SCr ratio (>20) were used as indicators of dehydration.

Self-reported social and work history items, diseases and medications, and heat stress exposure variables were dichotomized. A category of high tobacco consumption was created with subjects in the upper quartile of ever smokers (≥3 pack-years) and a category of high alcohol consumption composed of subjects in the upper tertiles of lifetime alcohol consumption (≥80,000 g) or average weekly consumption (≥125 g). Total fluid intake was defined as drinking water plus sugary drinks (natural fruit refreshments, sodas, coffee, tea and electrolyte solution) and reported as liters of total liquids consumed the previous (work) day and for comparison also for a typical non-work day, with subcategories into water only and sugary drinks. Total fructose intake was estimated from all food and fluids consumed including chewed cane, and stratified into fructose from food sources and added sugars. Fructose variables were categorized into quartiles. Cutoff for body mass index (BMI) were set at ≥25 kg/m^2. Hypertension was defined as systolic blood pressure ≥140 and/or diastolic blood pressure ≥90 mm Hg, or a self-reported medical history of hypertension. Diabetes was defined as serum glucose ≥125 mg/dL in the fasting serum sample or a self-reported medical history of diabetes. Use of nephrotoxic medications was recorded if taken at least three times per week for more than three months in the case of NSAIDs and other analgesics, or administered for at least a week in case of nephrotoxic antibiotics, during the last year. Blood and urine biochemical parameters were explored as continuous variables or defined as normal versus abnormal using standard clinical cutoff values.

Differences between occupations were assessed with ANOVA and Kruskal-Wallis tests for normally and not normally distributed continuous variables, respectively, and Pearson Chi-square test for categorical variables or Fisher's Exact Test when Chi-square was not applicable. Post hoc tests were performed with Tukey's HSD test for continuous results, and post-hoc Chi-square as described by Franke et al. (2012).[27] With occupation as the main proxy for heat stress, we assessed trends for sugarcane cutters>construction workers>farmers for prevalences of markers of kidney dysfunction and dehydration over the ordered occupational groups with the gamma statistic.

Differences in the distribution of risk factors between subjects with reduced and normal kidney function were explored for all occupations combined (n=194) and restricted to sugarcane cutters (n=86), with Whitney U-tests for continuous variables and Chi-square tests or Fisher's Exact Test for categorical variables. Exact p-values are reported and p-values ≤0.05 were considered statistically significant. Multivariate linear regression models were constructed, for all workers and restricted to sugarcane cutters, with factors that were different between subjects with reduced and normal kidney function at p<0.10. Residuals from the regressions were checked to assess the fit of the models.

RESULTS

Potential risk factors for CKD / MeN among the three occupations

Socioeconomic and health-related CKD risk factors

Socioeconomic CKD risk indicators were unfavorable for all workers, but somewhat less for construction workers (Table 1A). Farmers had the lowest income and sugarcane cutters were significantly less educated with on average of 4 years of elementary schooling. With regard to lifestyle and medical factors (Table 1B), sugarcane cutters had lower prevalences of high tobacco and alcohol consumption. There were no major differences in use of nephrotoxic drugs between the groups. None of the workers had been previously diagnosed with diabetes and only five had hyperglycemia >125 mg/dL, two cutters, two construction workers and 1 farmer. Sugarcane cutters showed less obesity, better lipid profiles, lower heart rates and lower blood pressure, but more anemia (36% with hemoglobin <13 g/dL). There were no differences in total leukocyte count between occupations.

Table 1. Socioeconomic and health indicators relevant for CKD /MeN risk among workers in three occupations. Municipalities of Chinandega and León, Nicaragua, 2013

	Sugarcane (N = 86)	**Construction (N = 56)**	**Farming (N = 52)**	**P-value* differences between groups**
A. Demographics, employment and social indicators				
Age (yrs), mean ± SD	25.6 ± 5.5	27.3 ± 6.0	25.2 ± 5.1	0.11
Education (yrs), mean ± SD	3.9 ± 3.0 [a]	7.8 ± 3.6	8.0 ± 4.1	<0.001
Drinking water from well (%)	84.9 [a]	12.5	13.5	<0.001
Temporary contract, %	93.0 [a]	75.0 [a]	21.1 [a]	<0.001
Without work ≥ 4 months/yr, %	20.9	17.9	34.6 [a]	0.089
No current social security, %	15.1	8.9	92.3 [a]	<0.001
Monthly household income per person in family, mean ± SD (25 córdobas = 1 US$), mean ± SD	1808 ± 1156 [a]	2267 ± 1124 [a]	1343 ± 1059 [a]	<0.001
B. Life style, medical history and health indicators				
High tobacco consumption, %	10.5 [a]	26.8	23.1	0.031
High alcohol consumption, %	18.6	28.6	32.7	0.145
Nonsteroidal anti-inflammatory drugs ≥ 3 months, %	5.8	7.1	7.7	0.901
Nephrotoxic antibiotics, %	1.2	1.8	0.0	0.648
History kidney stones, %	1.2	5.4	1.9	0.287
History urinary tract infections, %	23.3 [b]	33.9	42.3 [b]	0.058
Not feeling in good health, %	10.5 [c]	37.5 [c]	17.5	<0.001
Body mass index ≥25 kg/m^2, %	17.4 [c]	58.9 [c]	40.4	<0.001
Blood pressure>140/90, %	5.8 [a]	17.9	26.9	0.003
Heart rate (beats per minute)), mean ± SD	62 ± 12 [a]	73 ± 14	72 ± 13	<0.001
Blood glucose (mg/dL), mean ± SD	89 ± 11	90 ± 14	90 ± 12	0.874
Triglycerides (mg/dL), mean ± SD	120 ± 67 [a]	168 ± 108	177 ± 124	<0.001
Cholesterol (mg/dL), mean ± SD	170 ± 36 [c]	188 ± 41 [c]	178 ± 44	0.032
High density lipoprotein (HDL) cholesterol (mg/dL), mean ± SD	48 ± 12 [a]	42 ± 10	38 ± 8	<0.001
Low density lipoprotein (LDL) cholesterol (mg/dL), mean ± SD	93 ± 28	101 ± 33	91 ± 32	0.120
Very low density lipoprotein (VLDL) cholesterol (mg/dL), mean ± SD	24 ± 13 [a]	34 ± 22	35 ± 25	<0.001
Hematocrit (%), mean ± SD [d]	46.8 ± 5.9	48.5 ± 4.8	50.8 ± 4.0 [a]	<0.001
Hemoglobin (g/dL), mean ± SD [d]	13.4 ± 1.6 [a]	14.8 ± 1.5	15.4 ± 1.3	<0.001

Hemoglobin <13 g/dL, % [d]	35.8 [a]	8.9	3.8	<0.001
White blood cells/µL, mean ± SD [d,e]	7184 ± 2048	7307 ± 1656	7580 ± 1882	0.503
% neutrophils, mean ± SD [d,e]	38.6 ± 10.6	38.6 ± 8.8	36.5 ± 9.0	0.421
% lymphocytes, mean ± SD [d,e]	21.2 ± 6.6 [a]	18.5 ± 4.9 [a]	14.5 ± 4.7 [a]	<0.001
% other cells, mean ± SD [d,e]	40.2 ± 10.0	43.0 ± 8.2	49.1 ± 10.6 [a]	<0.001
Erythrocytes $*10^6$/µL, mean ± SD [d]	4.87 ± 0.59 [a]	5.27 ± 0.47 [a]	5.53 ± 0.50 [a]	<0.001
Platelets $*10^3$/µL, mean ± SD [d]	299.4 ± 76.7	315.6 ± 67.7	292.8 ± 62.8	0.218

* P-value for differences between groups: ANOVA for normally distributed continuous variables, Kruskal-Wallis for not normally distributed continuous variables, Chi-square test for categorical variables.

[a] Significantly different from the other two categories in post hoc tests.

[b] Significant difference only between sugarcane cutters and farmers

[c] Significant difference only between sugarcane cutters and construction workers.

[d] 5 missing data for sugarcane workers, due to technical error

[e] Exclusion of one farmer with outlier for white blood cell count (WBC count = 17.500).

Occupational heat exposure, fluid and fructose intake, and pesticides

On average, construction workers had an effective work time of eight hours and farmers the shortest with five hours, whereas sugarcane workers actively cut cane during 6.5 hours per day (Table 2A). A higher proportion of sugarcane workers perceived a very rapid work pace, and had to take rest breaks in the absence of shade; 83% received incentives for cutting more cane, and almost half started harvesting within 12 hours of burning the cane. Sugarcane cutters reported more often weight loss related to current job (over the last two months) and fainting on the job (6% as compared to 2% of farmers and no construction workers). Dysuria (‘chistata’), a common symptom in MeN affected areas thought to be related to dehydration,[15, 24] was not different between the three groups.

With regard to fluid intake (Table 2B), sugarcane cutters reported on average 6.2 L of total fluid intake the previous (work) day, 70% (4.4 L) as water and almost 30% (1.8 L) as sugary drinks. This was higher than for construction workers and farmers. Intake of water and sugary beverages were not correlated (r_p= 0.01). In contrast, the three groups were not different for total fluid, water and sugary drinks intake on non-work days.

Fructose intake during the previous day was highest for sugarcane cutters compared to farmers and construction workers; and 41% of sugarcane cutters belonged to the category of highest quartile of consumption of total fructose (>107 g) (Table 2B). Fructose intake from food was low among sugarcane cutters and most came from added sugars during work hours, specifically from sweetened beverages, electrolyte hydration solution (a third of the cutters) and cane chewing (about two thirds). Fructose intake outside work hours was not different between the groups.

With regard to pesticide exposures (Table 2C), farmers used pesticides most frequently (71%), almost half of sugarcane cutters reported pesticide use, versus only 11% of construction workers. Glyphosate and 2,4-D use was more common among sugarcane cutters, whereas paraquat and the insecticides cypermethrin and chlorpyrifos were used more often by farmers. With the exception of cypermethrin, which had been used by almost half of the farmers, no specific pesticide exceeded 25% of users in any of the groups.

Table 2. Occupational heat stress, fluid and fructose intake and pesticide exposure indicators among workers in three occupations. Municipalities of Chinandega and León, Nicaragua, 2013

	Sugarcane (N = 86)	**Construction (N = 56)**	**Farming (N = 52)**	**P-value***
A. Current occupational heat stress				
Effective work hours per day (work hours minus breaks), mean ± SD	6.5 ± 1.2 [a]	8.1 ± 0.7 [a]	5.3 ± 2.0 [a]	<0.001
Very rapid work pace, %	74.4 [a]	53.6	40.4	<0.001
No shade during breaks, %	20.9 [b]	1.8 [b]	11.5	0.004
Lifting weights >50 lbs., %	18.6 [a]	66.1	65.4	<0.001
Awkward work postures, %	58.1	76.8	69.2	0.063
Incentives to cut more cane, %	82.6	-	-	-
Hours post-burning at field entrance, mean ± SD	11.7 ± 6.2	-	-	-
Self-reported weight loss on the current job (last two months), %	77.9 [a]	39.3	36.5	<0.001
Fainted at work, %	5.8	0	1.9	0.126
Dysuria (‘chistata’), %	43.0	48.2	44.2	0.827
B. Fluid and fructose intake				
Fluid intake previous day (workday)				
Total fluid (L), mean ± SD	6.2 ± 4.1[a]	4.4 ± 2.1	4.0 ± 2.7	0.003
Water	4.4 ± 3.9 [a]	2.9 ± 2.1	2.8 ± 2.4	0.002
Sugary drinks without electrolyte hydration solution	1.8 ± 1.8	1.5 ± 0.9	1.2 ± 0.8	0.208
Electrolyte solution (N=31)	1.2 ± 1.1	-	-	-
Lowest quartile total fluid (≤ 2.5 L), %	18.6	19.6	40.4 [a]	0.009
Highest quartile total fluid (≥7.0 L), %	40.7 [a]	8.9	13.5	<0.001
Fluid intake on typical non-work day				
Total fluid (L), mean ± SD	4.2 ± 2.3	3.8 ± 1.7	4.1 ± 2.2	0.503
Water	3.0 ± 2.0	2.2 ± 1.3	2.7 ± 2.0	0.053

Sugary drinks	1.2 ± 1.1	1.6 ± 1.1	1.4 ± 1.9	0.117
Fructose intake previous day (workday)				
Total fructose intake (g), mean ± SD	103.1 ± 72.1 [a]	80.1 ± 46.1	70.9 ± 36.8	0.008
From food sources	8.4 ± 10.7 [a]	15.9 ± 16.6	17.4 ± 16.7	<0.001
From added sugar	94.7 ± 70.5 [a]	64.2 ± 38.1	53.2 ± 30.7	<0.001
During work hours	58.6 ± 44.7 [a]	28.6 ± 21.4	26.1 ± 16.5	<0.001
Sugary drinks (‘frescos’, sodas, coffee)	22.5 ± 15.7	28.6 ± 21.4	26.1 ± 16.3	0.108
Sugarcane chewing (N=53)	35.0 ± 18.5	-	-	-
Electrolyte solution (N=31)	40.3 ± 35.2	-	-	-
Outside (before and after) work hours	36.1 ± 39.3	35.6 ± 31.4	27.1 ± 25.9	0.350
Highest quartile total fructose intake (>107 g), %	40.7 [a]	19.6	15.7	0.002
C. Work and pesticide use history				
Cumulative time on current job (months), mean ± SD	77 ± 60	68 ± 80	116 ± 67 [a]	0.001
Ever sugarcane work, %	100.0 [a]	3.6	3.8	<0.001
Ever plantation (other than sugarcane), %	24.4	5.4 [a]	21.2	0.012
Ever work in small-scale agricultural (%)	61.6 [a]	25.0 [a]	100.0 [a]	<0.001
Ever construction work, %	5.8	100.0 [a]	11.5	<0.001
Ever any pesticide use, %	46.5 [a]	10.7 [a]	71.2 [a]	<0.001
Glyphosate, %	19.8 [a]	0.0	3.8	<0.001
2,4-D, %	23.3 [a]	0.0 [a]	9.6 [a]	<0.001
Paraquat, %	9.3	3.6	25.0 [a]	0.002
Chlorpyrifos, %	0.0	0.0	23.1 [a]	<0.001
Cypermethrin, %	18.6 [a]	3.6 [a]	42.6 [a]	<0.001

* P-value for differences between groups: ANOVA for normally distributed continuous variables, Kruskal-Wallis for not normally distributed continuous variables, Chi-square test for categorical variables.

[a] Significantly different from the other two categories in post hoc tests.

[b] Significant difference only between sugarcane cutters and construction workers.

Status of kidney function and hydration by occupation

Kidney function biomarkers were more commonly abnormal among sugarcane cutters, with significant differences between the groups for prevalences of eGFR<80 ml/min/1.73m^2 (16%, 9% and 2%, in sugarcane, construction and small-scale farmers, respectively, p for trend=0.003), high SCr (p for trend=0.02) and high BUN (p for trend=0.003) (Table 3A). Likewise, proteinuria >30 mg/dL was approximately three times more prevalent in sugarcane workers compared to the other groups (15% vs 5-6%, p for trend=0.08), whereas leukocyturia was observed in 22% of sugarcane workers but in only 0-2% of the other heat-exposed groups (p<0.001). Microhematuria was also three times more prevalent in sugarcane workers but not statistically significant (6% vs 2%, p for trend=0.19). High S-UA was more common among sugarcane cutters (17%) and construction workers (16%) than among famers (6%).

Table 3. Biomarkers of kidney function and dehydration among workers in three occupations and trend over categories ordered by exposure to occupational heat stress (sugarcane > construction > farming). Municipalities of Chinandega and León, Nicaragua, 2013.

Variable	Sugarcane (N = 86)	Construction (N = 56)	Farming (N = 52)	P-value: differences between groups*	P-value: trend**
A. Indicators of kidney function					
Blood urea nitrogen (BUN) (mg/dL), mean ± SD (range)	13.9 ± 5.0 [a] (6.0 – 28.4)	10.1 ± 5.1 (4.1 – 30.0)	9.2 ± 3.6 (4.0 – 22.0)	<0.001	
BUN >20 mg/dL (%)	15.1 [a]	5.4	1.9	0.017	0.003
Serum creatinine (SCr) (mg/dL), mean ± SD (range)	0.84 ± 0.39 (0.44 – 2.39)	1.00 ± 1.16 (0.49 – 8.84)	0.78 ± 0.22 (0.51 – 1.83)	0.393	
SCr >1.2 mg/dL, %	17.4 [c]	8.9	5.8 [c]	0.088	0.024
Estimated glomerular filtration rate ($eGFR_{CKD-EPI}$), mean ± SD (range)	121 ± 31 (34 – 160)	118 ± 30 (7 – 161)	125 ± 18 (49 – 158)	0.299	
$eGFR_{CKD-EPI}$ <80 ml/min/1.73m^2, %	16.3 [c]	8.9	1.9 [c]	0.025	0.003
Serum uric acid (S-UA) (mg/dL), mean ± SD (range)	6.0 ± 1.7 (3.0 – 12.7)	5.8 ± 1.6 (3.6 – 11.0)	5.0 ± 1.1 [a] (2.9 – 8.1)	0.001	
S-UA >7.2 mg/dL, %	17.4	16.1	5.8	0.136	0.055
Proteinuria >30 mg/dL, %	14.7	5.4	6.1	0.128	0.081
Leucocytes in urine, %	22.1 [a]	0	1.9	<0.001	<0.001
Nitrites in urine, %	0	0	0	-	-
Blood in urine, %	5.8	1.8	1.9	0.339	0.186
B. Indicators of dehydration					
Urinary specific gravity (USG) ≥1.030, %	15.3	28.6	20.4	0.161	0.255
Urinary pH ≤5.5, %	29.4 [a]	12.5	12.2	0.014	0.006
BUN/SCr ratio >20, %	25.6 [a]	0	3.8	<0.001	<0.001

* P-value for differences between groups: ANOVA for normally distributed continuous variables, Kruskal-Wallis for not normally distributed continuous variables, Chi-square test for categorical variables.
**Gamma statistic for trend over ordered categories.
[a] Significantly different from the other two categories in post hoc tests.
[b] Significant difference only between sugarcane cutters and construction workers.

Regarding markers of dehydration, prevalence of concentrated urine (USG ≥1.030) was not statistically different between groups (Table 3B). Low urinary pH occurred in 29% of sugarcane cutters versus 12% of construction workers and farmers (p=0.01) and sugarcane cutters more commonly had an elevated BUN/SCr ratio (26% vs 0 and 4% of construction workers and farmers, p<0.001). Trends over ordered categories were significant for urinary pH and BUN/SCr ratio. Although sugarcane cutters as a group had a lower prevalence of concentrated urine, within the group low fluid intake was strongly associated with concentrated urine (OR 3.5, p=0.06) and acidic urine (OR 8.7, p<0.001) which was not the case among construction workers and farmers (Table 4).

Table 4. Associations between low intake of fluids and markers of dehydration among sugarcane cutters (n=86) and non-cutters (construction workers and small-scale farmers) (n=108)

Lowest quartiles of fluid intake		**USG ≥1.030** [a]	**pH ≤5.5** [a]	**BUN/SCr ratio >20** [a]
		OR (95% CI: LL; UL) p-value [b]		
Total fluids ≤2.5L	Sugarcane cutters (n=16)	3.5 (1.0; 13) 0.06	8.7 (2.6; 29) <0.001	1.2 (0.3; 4.3) 0.67
	Construction workers and farmers (n=32)	1.4 (0.5; 3.5) 0.51	2.3 (0.7; 7.5) 0.17	-[c]
Water ≤1.5L	Sugarcane cutters (n=16)	3.0 (0.7; 12) 0.14	2.9 (0.9; 9.6) 0.08	2.3 (0.7; 7.3) 0.17
	Construction workers and farmers (n=32)	1.9 (0.7; 4.9) 0.18	1.7 (0.5; 5.6) 0.42	-[c]
Sugary drinks ≤0.75L	Sugarcane cutters (n=28)	2.5 (0.7; 9.2) 0.16	2.5 (0.9; 7.1) 0.08	0.3 (0.2; 1.1) 0.06
	Construction workers and farmers (n=21)	1.8 (0.6; 5.2) 0.28	0.7 (0.2; 3.6) 0.69	-[c]

[a] Markers of dehydration: high urinary specific gravity (USG≥1.030); acidic urine (urinary pH ≤5.5); high serum urea nitrogen to serum creatinine ratio (BUN/SCr ratio >20).
[b] The odds ratios (OR) and 95% confidence intervals [CI, lower limit (LL) and upper limit (UL)] for water and sugary drinks are adjusted for each other.
[c] Not computed because only two non-cutters had BUN/SCr ratio >20.

Risk factors for reduced kidney function

In bivariate analyses of differences in kidney, urinary and metabolic biomarkers, work practices, hydration practices and lifestyle characteristics between subjects with reduced kidney function (eGFR <80 ml/min/1.73 m^2) and subjects with normal kidney function (eGFR ≥80 ml/min/1.73 m^2) (Supplementary Table 1), reduced kidney function was significantly associated with work as sugarcane cutter, high intake of water, low intake of sugary beverages, increasing age, low hemoglobin and high tobacco consumption. In analyses restricted to sugarcane cutters, results were similar and, in addition, workers with reduced kidney function had cut cane during considerably longer time than those with normal kidney function (cumulative time on the job: median 108 vs 60 months, p=0.06). Sugarcane cutters with reduced kidney function reported almost three times higher water intake and three times lower intake of sugary beverages as compared to cutters with normal kidney function, with only 1 of the 14 reporting intake of the electrolyte solution. In addition, the cane cutters with reduced kidney function had a worse lipid profile than those with normal kidney function and more often hypertension, but none had diabetes or hyperglycemia and only one was overweight (Supplementary Table 1).

In backwards stepping multivariate linear regression analyses with inclusion of variables with p≤0.10 in the bivariate analyses (except hemoglobin due to missing data), age (beta -1.3, 95%CI -1.8, -0.8; p<0.001) and S-UA (beta -10.4, 95% CI -12.2, -8.5; p<0.001) associated significantly with reduced kidney function among all workers, identically in models with total fluid intake and with intake of water and sugary beverages separately (Table 5A). In the subset of sugarcane cutters, too many variables had a p-value ≤0.10 in bivariate analyses (see supplementary Table 1) and therefore the regression was done in two steps. Hypertension, lipid profile tests and blood sugar did not associate with reduced kidney function in a model also including age and S-UA (data not shown) and were not further considered. In a model with water intake, intake of sugary drinks (without electrolyte solution) and intake yes/no of electrolyte solution, age, S-UA, high tobacco consumption and high alcohol consumption (Table 5B), reduced kidney function associated significantly with age and S-UA and non-significantly with the intake of electrolyte solution (beta 8.1, 95% CI -1.2, 17.5, p=0.09). Age and cumulative months on the job correlated (r_p 0.68, p<0.001), and substituting age with time cutting cane yielded similar results.

Table 5. Multivariate linear regression models of estimated glomerular filtration rate ($eGFR_{CKD-EPI}$) among all workers (sugarcane cutters, construction workers and farmers) and restricted to sugarcane cutters.

A. All subjects (N=194)		**Beta coefficient (95% CI: UL; LL)**	**Standardized beta coefficient**	**P-value**	**Adjusted R^2**
Step 1	Water intake (L)	-0.7 (-1.7; 0.3)	-0.08	0.15	0.47
	Sugary beverages intake (L)	1.2 (-0.8; 3.3)	0.06	0.24	
	Sugarcane cutter ever	3.6 (-2.5; 9.6)	0.07	0.25	
	Age (yrs)	-1.2 (-1.7; -0.6)	-0.24	<0.001	
	Serum uric acid (mg/dL)	-10.0 (-12.0; -8.1)	-0.57	<0.001	
	High tobacco consumption	-4.5 (-11.6; 2.7)	-0.07	0.22	
	High alcohol consumption	1.2 (-5.6; 8.1)	0.02	0.72	
Final step	Age (yrs)	-1.3 (-1.8; -0.8)	-0.27	<0.001	0.47
	Serum uric acid (mg/dL)	-10.4 (-12.2; -8.5)	-0.59	<0.001	
B. Sugarcane cutters (N=86)					
Step 1	Water intake (L)	-0.7 (-1.9; 0.5)	-0.09	0.25	0.58
	Sugary beverages intake (without electrolyte solution) (L)	1.2 (-3.7; 6.0)	0.04	0.63	
	Electrolyte solution (yes/no)	6.4 (-4.5; 17.3)	0.10	0.24	
	Age (yrs)	-1.7 (-2.5; -0.8)	-0.29	<0.001	
	Serum uric acid	-10.9 (-13.8: -8.1)	-0.59	<0.001	
	High tobacco consumption	-10.1 (-22.5; 2.3)	-0.12	0.11	
	High alcohol consumption	-7.8 (-19.5; 3.9)	-0.10	0.19	
Final step	Age (yrs)	-1.9 (-2.7; -1.1)	-0.34	<0.001	0.57
	Serum uric acid (mg/dL)	-11.3 (-14.0; -8.6)	-0.61	<0.001	
	Electrolyte solution (yes/no)	8.1 (-1.2; 17.5)	0.13	0.09	

DISCUSSION

This study found evidence for more frequent heat stress, dehydration and kidney dysfunction among sugarcane cutters, as expected, and in lesser degree also reduced kidney function among construction workers but not among small-scale farmers. Also, as expected, serum uric acid levels increased with decreasing eGFR.

Evidence of reduced kidney function

We used a cutoff of eGFR of 80 ml/min/1.73m^2 to evaluate differences in renal function because only 11 workers had eGFR below 60, due to young age (all under age 40) and also because sugarcane workers were screened by employers before the start of the harvest two months earlier and workers with SCr>1.2 mg/dL were not hired and, thus, not part of our study population. Despite, approximately one-fourth of sugarcane cutters had evidence for either eGFR <80 ml/min/1.73m^2, SCr >1.2 mg/dL or proteinuria ≥30 mg, and these findings were, respectively, eight-, three- and two-fold more common than observed in subsistence farmers and about two-fold more common than in construction workers (Table 3). However, although in lesser degree than cane cutters, construction workers also had an unusually high prevalence of decreased kidney function, which is in accordance with a previous unpublished study in the same area.[25] In contrast, the single small-scale farmer with reduced kidney function had worked previously in sugarcane. Thus, our results show that not all agricultural workers are at increased risk for CKD, as is commonly stated, but rather workers in certain types of agriculture and other hot jobs such as work in the construction industry. The absence of reduced kidney function among subsistence farmers is consistent with a study in a MeN epidemic area in El Salvador, where subsistence farmers without a history of plantation work had a significantly lower prevalence of abnormal SCr than men who had worked on sugar or cotton plantations (15% vs 33%).[20] Reduced kidney function was accompanied by a higher frequency of anemia among sugarcane cutters (36% versus 4-9% in the other groups). The prevalence of anemia was higher than the prevalence of reduced kidney function and cannot be simply ascribed to the higher frequency of reduced renal function. Marked anemia, defined as Hb of <10 g/dL, was not observed in any of the groups.

The reduced kidney function did not associate with traditional risk factors for CKD. Notably, there was not one case of confirmed diabetes in the entire population. Importantly, sugarcane workers showed significantly worse renal function despite overall lower frequency of abnormal lipid profile, hypertension and obesity compared to the other two groups (see Table 1). Increasing age - over 50 - is a known risk factor for CKD, but in our study increasing age was associated with decline of renal function despite the young age of the study participants. This is possibly related to an increased risk with continued job exposure over time, in particular among the sugarcane cutters. Thus, our study suggests that most cases of reduced kidney function are related to Mesoamerican nephropathy and not classic CKD.

Evidence for heat stress

There was evidence for greater risk for heat stress among sugarcane cutters. Sugarcane cutters labored at a faster pace, had less exposure to shade, reported more weight loss during the ongoing harvest, and had more fainting episodes. While sugarcane cutters had greater heat stress exposure, they also drank more fluids during the course of the day, amounting to an average of 6.2 L per day (although this varied considerably, with approximately 20 percent drinking <2.5 L/d and 40% >7 L/day). However, the type of exertion and sweating that occurs with cane harvesting[11-13] could still result in dramatic loss of fluids such that dehydration can occur despite high fluid consumption. Cade et al. found that college football players could lose as much as 8 quarts (about 7.6 L) of water in a 2 hour period, associated with loss of salt, a decrease in blood glucose, and a fall in blood pressure.[28]

Potential mechanisms involved in inducing kidney damage

Daily heat stress and dehydration may cause repeated renal hypoperfusion episodes, and intermittent subclinical rhabdomyolysis associated with excessive exertion may also induce repeated acute kidney injury (AKI) through the release of inflammatory mediators including oxidants, cytokines and uric acid, which over time leads to CKD [18]. Experimental evidence has shown that repeated exposure to heat stress caused a reduction of renal function accompanied by histological evidence of tubulointerstitial damage.[19] Heat stress is known to raise serum uric acid levels, in part from subclinical rhabdomyolysis,[29] but also from reduced renal blood flow.[30] In turn, hyperuricemia is a well-known risk factor for CKD[31] and mediates both glomerular and tubulointerstitial disease in animals.[32-34] Interestingly, serum uric acid levels tended to be highest in both sugarcane workers and construction workers, with 16-17% of these individuals having hyperuricemia compared to 6% in subsistence farmers. Furthermore, we found that the presence of hyperuricemia was independently and strongly associated with declining renal function, i.e. for each increase of 1 mg/dl of S-UA there was an average decline of 10 ml/min in kidney filtration (see Table 5). However, since reduced renal function can also result in increased uric acid levels due to impaired excretion, the causal role of uric acid in the reduced kidney function cannot be determined.

Recently we hypothesized that renal injury could be occurring in sugarcane workers due to cyclical uricosuria with crystal formation.[20, 21] According to this hypothesis, serum uric acid might rise as a consequence of subclinical rhabdomyolysis, followed by its crystallization in the urine. One factor that increases the risk for urate crystal formation is acidic urine that could result from the release of lactic acid associated with strenuous exercise and by the effects of dehydration to reclaim sodium with hydrogen ion excretion. Urine pH was significantly lower in the sugarcane workers compared to other groups (see Table 3) and was strongly associated with low fluid intake the previous (work) day in the subset of sugarcane workers (see Table 4). This might reflect the effects of greater volume depletion (with aldosterone stimulation), lactic acid generation during the prior day, or other mechanisms.

Hydration and fructose

We had expected that low water intake, or high sugary fluid intake, would be associated with reduced renal function, based on studies in animals.[19, 35] However, workers with eGFR <80 ml/min/1.73m^2 drank more water and consumed less sugar-based drinks during the workday compared to subjects with normal kidney function (4.5 L vs 2.2 L water, p=0.08; 0.6 L vs 1.25 L sugary beverages, p=0.001) (see Supplementary Table 1). This was particularly so among the sugarcane cutters with reduced kidney function, who drank about 4 L more water and 1 L less sugary beverages. Excessive thirst from decreased concentration capacity of impaired kidneys may partially explain these counterintuitive findings, as well as the very high water requirements during the heavy labor of sugarcane cutting.[12, 13] Although low fluid intake clearly associated with concentrated urine among sugarcane cutters (OR USG≥1.030=3.5, p=0.06) (see Table 4), cutters in the quartile with the highest fluid intake did not have a decreased risk of concentrated urine (OR=1.3, p=0.70) while high fluid intake among non-sugarcane workers appeared to be preventive (OR 0.10, p=0.06). Salvadorian cane cutters who consumed amounts of fluid comparable to our Nicaraguan cutters, were found to have insufficient fluid intake under their work conditions.[11]

Sugary beverages that contain fructose are known to increase the risk for albuminuria,[36] and can induce renal injury in laboratory animals.[35] However, fructose is also a component of sports drinks and fluid resuscitation packets containing glucose and electrolytes that might be beneficial to the volume and water depleted, such as by providing glucose that may prevent or treat any associated hypoglycemia, or by helping to maintain blood pressure due to the fructose component.[37, 38] In our study, the intake of electrolyte solutions tended to be associated (p=0.09) with improved kidney function in multivariate analyses (see Table 5). One study in Nicaragua found that, for each 100 cc electrolyte hydration packet consumed during the workday, the eGFR of cane cutters increased by 7 ml/min/1.73m2 over the course of one harvest season.[17] These issues need to be assessed with prospective studies that examine overall fluid balance by measuring fluid intake as well as losses during work, such as pre- and post-shift weight and serum and urine osmolarity.

Other risk factors for kidney disease

There was no association with NSAIDs or alcohol intake. A history of high tobacco consumption was more frequent among subjects with reduced kidney function ($p=0.02$) but lost significance in multivariate analyses. A history of pesticide exposure was more common among farmers, although exposure to herbicides was more common among sugarcane cutters, especially glyphosate and 2,4-D, both of special interest. However, analyses failed to identify pesticide exposures as an independent risk factor for reduced kidney function (see Supplementary Table 1).

Study limitations

The main limitation of our study is its cross-sectional design. However, in the same region, at the time of this study, we also followed a small group of heat-exposed sugarcane cutters and a group of control workers unexposed to heat over the harvest season. The cutters showed an important decline in kidney function,[39] which provides support for the cross-sectional findings, although no cohort data exist for construction workers or farmers. Another limitation is that our heat exposure and hydration data were self-reported, but these data were collected through carefully designed questionnaires. Our sample size was based on a pre-study power calculation of 80% to detect CKD among 100 sugarcane cutters and100 non-cutters at alpha 0.05. Post hoc, we achieved a power of 0.68 for increased risk of reduced eGFR among cutters versus non-cutters, but the post hoc power of the comparison between cutters and farmers was 80%. Therefore, our results seem sufficiently reliable, also considering the significant trends for indicators of heat stress, dehydration and kidney dysfunction in support our main hypothesis of cane cutting >construction>farming. Finally, we did not have resources for examining biomarkers of early damage such as NGAL or NAG, which are important to include in future studies.

CONCLUSIONS

In summary, compared to construction workers and, in particular, subsistence farmers from the same MeN epidemic region of Nicaragua, sugarcane cutters have higher heat stress, more dehydration and worse renal function, despite that other health indicators of the cutters were significantly better. Our study supports the need for improved work practices and even more hydration with adequate access to water for sugarcane cutters, as well as for workers in other hot occupations such as construction. The associations between intake of water and sugary drinks and kidney function as well as the role of hyperuricemia need to be assessed in carefully designed follow-up studies.

AUTHORS' CONTRIBUTIONS:

Concept and design: Aurora Aragón, Catharina Wesseling, Marvin González, Richard J Johnson, Jason Glaser, Ricardo Correa-Rotter

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Data analysis: Catharina Wesseling

Data interpretation: All authors

Manuscript preparation: Catharina Wesseling, Richard J. Johnson

Critical revision and approval of manuscript: All authors

STROBE CHECKLIST: see separate file

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Supplementary Table 1. Comparison of kidney, urinary and metabolic biomarkers, work practices, hydration practices and lifestyle characteristics of subjects with reduced kidney function (<80ml/min/1.73 m^2) and subjects with normal kidney function (≥80ml/min/1.73 m^2), all study participants (N=194) and sugarcane workers (N=86)

	All workers (N=194)			**Sugarcane cutters (N=86)**		
	eGFR <80ml/min/1.73 m^2 N=20	eGFR ≥80ml/min/1.73 m^2 N=174	P-value*	eGFR <80ml/min/1.73 m^2 N=14	eGFR ≥80ml/min/1.73 m^2 N=72	P-value*
Kidney function						
eGFR (ml/min/1.73 m^2), median (10%; 90%)	55 (27; 76)	129 (112; 147)	<0.001	63 (39, 78)	134 (102, 153)	<0.001
S creatinine (mg/dL), median (10%; 90%)	1.67 (0.24; 3.05)	0.72 (0.56; 0.94)	<0.001	1.47 (1.24; 2.21)	0.70 (0.51; 0.95)	<0.001
S urea nitrogen (BUN) (mg/dL), median (10%; 90%)	22.6 (13.5; 28.3)	10.0 (6.1; 15.0)	<0.001	22.6 (14.4; 28.1)	12.1 (7.9; 18.0)	<0.001
S uric acid (mg/dL), median (10%; 90%)	8.2 (6.7; 11.0)	5.2 (3.9; 6.8)	<0.001	8.1 (6.9, 11.8)	5.4 (3.9, 6.9)	<0.001
S uric acid >7.2 mg/dL, % (# cases)	85.0 (17)	5.7 (10)	<0.001	85.7 (12)	4.2 (3)	<0.001
Protein >30 mg, % (# cases) (N=180)	20.0 (4)	7.5 (13)	0.09	23.1 (3)	2.9 (8)	0.39
Leukocytes positive at dipstick, % (# cases)	30.0 (6)	8.0 (14)	0.009	42.9 (6)	18.1 (13)	0.07
Blood, traces and higher at dipstick, % (# cases)	20.0 (4)	1.7 (3)	0.002	28.6 (4)	1.4 (1)	0.002
Hydration						
USG ≥1030, % (# cases) (N=190)	10.0 (2)	21.8 (37)	0.38	7.1 (1)	16.9 (12)	0.69
USG ≤1005, % (# cases) (N=190)	20.0 (4)	16.1 (28)	0.75	28.6 (4)	12.7 (9)	0.22
pH ≤5.5, % (# cases) (N=190)	30.0 (6)	18.4 (32)	0.24	42.9 (6)	26.8 (19)	0.34
BUN/serum creatinine ratio>20	0 (0)	13.8 (24)	0.14	0 (0)	30.6 (22)	0.02
Work practices						
Sugarcane cutter ever, % (# cases)	75.0 (15)	43.1 (75)	0.007	n.a. [a]	n.a.	n.a.
Cumulative time in job (months), median (10%; 90%)	90 (2; 235)	62.5 (4; 180)	0.39	108 (30; 216)	60 (30; 216)	0.06
Work day (hours), median (10%; 90%)	7.75 (6.0; 10.0)	8.0 (4.0; 10.0)	0.85	7.0 (5.5; 10.0)	8.0 (6.0; 9.0)	0.03
Hours cutting cane, median (10%; 90%)	n.a. [a]	n.a.	n.a.	6.0 (4.6; 7.5)	6.8 (5.0; 8.0)	0.02
Total break time (min), median (10%; 90%)	60 (20; 113)	60 (20; 104)	0.55	60.0 (20, 112)	60 (25, 90)	0.30
Breaks ≤2/d, % (# cases)	65.0 (13)	70.7 (123)	0.60	57.1 (8)	63.9 (46)	0.63

	All workers (N=194)			Sugarcane cutters (N=86)		
	eGFR <80ml/min/1.73 m^2 N=20	eGFR ≥80ml/min/1.73 m^2 N=174	P-value*	eGFR <80ml/min/1.73 m^2 N=14	eGFR ≥80ml/min/1.73 m^2 N=72	P-value*
No shade during breaks, % (# cases)	20.0 (4)	12.1 (21)	0.30	28.6 (4)	19.4 (14)	0.48
High speed perception, % (# cases)	75.0 (15)	57.5 (100)	0.13	85.7 (12)	72.2 (52)	0.50
Production (tons/d), median (10%; 90%)	n.a.	n.a.	n.a.	6.5 (5.0; 10.0)	7.0 (5.0; 10.0)	0.66
Incentives to cut more, % (N)	n.a.	n.a.	n.a.	71.4 (10)	84.7 (61)	0.26
History of pesticide use, % (# cases)	30.0 (6)	44.3 (77)	0.22	42.9 (6)	47.2 (34)	1.00
Chlorpyrifos, % (# cases)	0 (0)	6.9 (12)	0.62	0 (0)	0 (0)	-
Cypermethrin, % (# cases)	10.0 (2)	23.0 (40)	0.26	14.3 (2)	19.4 (14)	1.00
Paraquat, % (# cases)	0 (0)	13.2 (23)	0.14	0 (0)	11.1 (8)	0.34
2,4-D, % (# cases)	20.0 (4)	12.1 (21)	0.30	28.6 (4)	22.2 (16)	0.73
Glyphosate, % (# cases)	15.0 (3)	9.2 (16)	0.42	21.3 (3)	19.4 (14)	1.00
Hydration practices						
Total fluid intake (L), median (10%; 90%)	5.0 (1.1; 13.8)	3.8 (1.9; 10.0)	0.08	6.7 (1.4; 14.6)	4.5 (2.2; 12.4)	0.16
Low total fluid intake ≤2.5 L/d, % (# cases)	15.0 (3)	25.9 (45)	0.41	14.2 (2)	19.4 (14)	1.00
High total fluid intake ≥7.0 L/d, % (# cases)	40.0 (7)	22.4 (39)	0.10	50.0 (7)	38.9 (28)	0.44
Water (L), median (10%; 90%)	4.5 (0.3; 13.3)	2.2 (0.5; 8.0)	0.03	6.3 (0.6; 14.3)	2.2 (0.7; 10.0)	0.03
Low water intake < 1.5 L/d, % (# cases)	20.0 (4)	25.3 (44)	0.79	14.2 (2)	19.4 (14)	1.00
High water intake > 4.5 L/d, % (# cases)	50.0 (10)	22.4 (39)	0.007	64.3 (9)	30.6 (22)	0.02
Sugary beverage, median (10%; 90%)	0.6 (0.4; 2.4)	1.25 (0.5; 3.0)	0.001	0.5 (0.3; 1.9)	1.6 (0.4; 4.5)	0.002
Low sugary drink intake <0.75 L/d, % (# cases)	50.0 (10)	22.4 (39)	0.007	64.3 (9)	26.4 (19)	0.01
High sugary drink intake ≥2.0 L/d, % (# cases)	10.0 (2)	28.7 (50)	0.07	7.1 (1)	40.3 (29)	0.03
Intake electrolyte solution, % (N)	n.a.	n.a.	n.a.	7.1 (1)	41.7 (30)	0.01
Age, metabolic and lifestyle risk factors						
Age (yrs), median (10%; 90%)	29.5 (21; 37)	25 (19; 33)	0.002	29 (20; 36.5)	24 (18; 33)	0.01
Hypertension (history or exam), % (# cases)	15.0 (3)	19.5 (34)	0.77	21.4 (3)	4.2 (3)	0.05
Body mass index (BMI, kg/m^2), median (10%; 90%)	24.0 (19.9; 28.9)	23.3 (19.9; 29.7)	0.49	23.0 (19.2; 29.7)	22.2 (19.6; 26.4)	0.23

	All workers (N=194)			Sugarcane cutters (N=86)		
	eGFR <80ml/min/1.73 m^2 N=20	eGFR ≥80ml/min/1.73 m^2 N=174	P-value*	eGFR <80ml/min/1.73 m^2 N=14	eGFR ≥80ml/min/1.73 m^2 N=72	P-value*
BMI >25 kg/m^2, % (# cases)	40.0 (8)	35.1 (61)	0.81	21.4 (3)	16.7 (12)	0.70
BMI >30 kg/m^2, % (# cases)	5.0 (1)	15 (8.6)	1.00	7.1 (1)	1.4 (1)	0.30
Heart rate >80 pulses/min, % (# cases)	25.0 (5)	15.5 (27)	0.34	28.6 (4)	5.6 (4)	0.02
Glycemia (mg/dL), median (10%; 90%)	90.4 (78.0; 103.1)	87.9 (75.3; 104.2)	0.64	90.8 (79.3; 103.5)	88.4 (75.2; 103.6)	0.43
Glycemia ≥ 100 mg/dL, % (# cases)	15.0 (3)	16.1 (28)	1.00	14.3 (2)	15.3 (11)	1.00
Hypertension (HT) and hyperglycemia (≥100mg/dL), % (# cases)	0 (0)	2.9 (5)	1.00	0 (0)	0 (0)	-
Triglycerides (mg/dL), median (10%; 90%)	144.7 (76.4; 293.2)	122.7 (59.2; 268.1)	0.15	139.1 (74.4; 304.9)	101.7 (48.3; 188.1)	0.01
LDL cholesterol (mg/dL), median (10%; 90%)	89.9 (53.5; 138.7)	93.7 (58.7; 135.1)	0.78	96.4 (63.4; 134.0)	91.0 (57.1; 131.8)	0.44
HDL cholesterol (mg/dL), median (10%; 90%)	39.9 (20.7; 56.1)	43.0 (32.2; 60.1)	0.23	42.2 (23.1; 58.4)	47.7 (33.9; 67.8)	0.06
VLDL cholesterol (mg/dL), median (10%; 90%)	28.9 (15.3; 58.6)	24.8 (11.9; 53.6)	0.15	27.8 (14.9; 61.0)	20.3 (9.7; 37.6)	0.01
Hemoglobin (mg/dL), median (10%; 90%) [b]	11.2 (10.5; 14.9)	14.8 (12.5; 16.43	0.001	11.1 (10.2; 13.1)	13.8 (11.9; 15.8)	<0.001
Hb < 13 mg/dL, % (# cases) [b]	75.0 (15)	12.4 (21)	<0.001	85.7 (12/14)	25.4 (17/67)	<0.001
White blood cell count (#/µL), median (10%; 90%) [b]	7600 (6200; 11.790)	7300 (5000; 9500)	0.68	7500 (5750; 12.250)	7200 (4400; 9480)	0.95
High tobacco consumption, % (# cases)	45.0 (9)	20.0 (35)	0.02	41.7 (5)	11.3 (8)	0.02
High alcohol consumption, % (# cases)	10.0 (2)	27.0 (47)	0.10	7.1 (1)	20.8 (15)	0.21
Regular use of nephrotoxic medications, % (# cases)	10.0 (2)	6.3 (11)	0.63	7.1 (1)	5.6 (4)	1.00

*Mann Whitney U test for medians; Chi-square test (Fisher exact test when 1 or more cells with count<5) for categorical data

[a] n.a.: not applicable

[b] 5 missing values for sugarcane cutters due to technical error

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*
Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any pre-specified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-8
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-8*
Bias	9	Describe any efforts to address potential sources of bias	3, 19-22
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7-8
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	Not applicable

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	Not applicable
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-13
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	14-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	16-17
		(*b*) Report category boundaries when continuous variables were categorized	7-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	19
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	3, 19-22
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22
Generalisability	21	Discuss the generalisability (external validity) of the study results	22
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Heat stress, hydration and uric acid: a cross-sectional study in workers of three occupations in a hotspot of Mesoamerican nephropathy in Nicaragua

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Heat stress, hydration and uric acid: a cross-sectional study in workers of three occupations in a hotspot of Mesoamerican nephropathy in Nicaragua

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Running Title: Mesoamerican nephropathy and occupational heat stress

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Key terms: Mesoamerican nephropathy, chronic kidney disease, occupational heat stress, hydration, serum uric acid

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Abstract

Objectives: To study Mesoamerican nephropathy (MeN) and its risk factors in three hot occupations.

Design: Cross-sectional.

Setting: Chinandega and León municipalities, a MeN hotspot in the Nicaraguan Pacific coast, January-February 2013.

Participants: 194 male workers aged 17-39: 86 sugarcane cutters, 56 construction workers, 52 small-scale farmers.

Outcome measures: i) Differences between the three occupational groups in prevalences/levels of socioeconomic, occupational, lifestyle and health risk factors for chronic kidney disease (CKD); and in biomarkers of kidney function and hydration; ii) differences in prevalences/levels of CKD risk factors between workers with reduced estimated glomerular filtration rate ($eGFR_{CKD-EPI}$<80 ml/min/1.73m^2) and workers with normal kidney function ($eGFR_{CKD-EPI}$≥80 ml/min/1.73m^2).

Results: Sugarcane cutters were more exposed to heat and consumed more fluid on workdays; and had less obesity, lower blood sugar, lower blood pressure and better lipid profile. Reduced eGFR occurred in 16%, 9% and 2% of sugarcane cutters, construction workers and farmers, respectively (trend cane>construction>farming p=0.003). Significant trends (cane>construction>farming) were also observed for high serum urea nitrogen (BUN>20 mg/dL), high serum creatinine (SCr>1.2 mg/dL), low urinary pH (≤5.5) and high BUN/SCr ratio (>20) but not for high urinary specific gravity (USG≥1.030). Sugarcane cutters had also more often proteinuria, and blood and leucocytes in urine. Workers with eGFR<80 ml/min/1.73m^2 reported higher intake of water and lower intake of sugary beverages. Serum uric acid levels related strongly and inversely to eGFR levels (adj. beta -10.4 ml/min/1.73m^2, 95%CI -12.2, -8.5, p<0.001). No associations were observed for other metabolic risk factors, pesticides, nonsteroidal anti-inflammatory drugs or alcohol. Among cane cutters, consumption of electrolyte hydration solution appeared preventive (adj. beta 8.1 ml/min/1.73m^2, p=0.09).

Conclusion. Heat stress, dehydration and kidney dysfunction were most common among sugarcane cutters. Kidney dysfunction occurred in lesser extent also among construction workers, but hardly among small-scale farmers. High serum uric acid associated with reduced kidney function.

Strengths and limitations of this study

- The study provides a detailed description of exposures to potential risk factors for Mesoamerican nephropathy (MeN) among workers in three occupations of special interest, subsistence farmers, construction workers and sugarcane cutters.
- The study established the prevalence of kidney dysfunction and dehydration among workers in these three distinct occupations at risk for MeN.
- The cross-sectional design limits causal interpretations about associations between the potential risk factors and the markers of kidney function, but the study provides clues for etiology and possible pathways of kidney injury.
- Most exposures to risk factors are self-reported but much attention was payed to the quality of the questionnaires

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Competing interests:

The authors declare no competing interests.

INTRODUCTION

Mesoamerican nephropathy (MeN), an epidemic of chronic kidney disease (CKD), is a chronic tubulointerstitial disease unrelated to traditional CKD risk factors, affecting predominantly young, male workers in Pacific coastal communities of Central America and possibly southern Mexico.[1-4] Several tens of thousands of people have died of this disease.[3] Although MeN is often described as an epidemic of agricultural workers,[1, 5-8] in Central America sugarcane workers are clearly the most affected population.[1, 9, 10]

A consistent risk factor for MeN appears to be heavy manual labor in extreme heat.[1] Manual sugarcane cutters exert substantial amount of energy, often in environmental temperatures over 35°C and high humidity.[11-13] Besides heat stress, some sugarcane workers are also exposed to pesticides, either at sugarcane plantations or while laboring in other crops.[11,14] Consumption of nonsteroidal anti-inflammatory drugs (NSAIDs) to manage muscle pain is common.[15] Exposure to heavy metals may occur through contaminated pesticide formulations and fertilizers, as has been shown in Sri Lanka,[16] contaminated drinking water,[17] or even during burning of the cane.[18] Overall, exposure of sugarcane workers to different potential CKD risk factors has not been described in detail.

A leading hypothesis is that recurrent dehydration, possibly in combination with exposure to other agents (e.g. NSAIDs, heavy metals, agrochemicals, high fructose intake), may be a driving factor.[1, 4] Animal experiments have shown that dehydration and hyperosmolarity may induce tubular injury via activation of the polyol-fructokinase pathway in the kidney.[19] Recently, a mechanism of hyperuricemia and cyclical uricosuria associated with volume loss and dehydration has also been proposed.[20, 21]

Studies suggest that MeN may also occur among miners and construction workers,[5, 22] cotton workers,[23] and subsistence farmers.[6] However, these cross-sectional data mostly consider current occupation and are therefore not conclusive. Cane cutting is seasonal and many sugarcane workers are also subsistence farmers or work in construction. Contrary to contracted workers, independent small-scale farmers have control over their work hours and are able to avoid the hottest temperatures. Prevalence studies have been recommended to assess exposure to CKD risk factors and kidney dysfunction in different occupations.[1]

The aim of this study was to compare the prevalence of a range of potential CKD risk factors among sugarcane cutters, construction workers and small-scale farmers laboring in the same hot environment, along with biomarkers of hydration and kidney function. We hypothesize that sugarcane cutters experience more heat stress, more dehydration and more signs of kidney dysfunction than small scale farmers, with construction workers somewhere in between.

METHODS

Study population and recruitment

This is a cross sectional study. We recruited 194 male workers, aged 17-39, all living in the municipalities of Chinandega and León in the Pacific region of Nicaragua, a major epicenter for the MeN epidemic. Of these, 86 were sugarcane cutters, 56 construction workers and 52 small-scale farmers. Cane cutters of several sugarcane villages were recruited with the help of community leaders; a trade union assisted in recruiting construction workers employed by private companies at three construction sites; and a rural farmer association to recruit associated farmers dedicated full time to the cultivation of subsistence crops. The response rate was 86% among cane cutters and there were no refusals among construction workers and farmers.

The study was approved by the Ethical Review Board of UNAN-León, Nicaragua. All participants provided a written informed consent.

Data collection

Data were collected for sugarcane cutters during January 2013, two months after the sugarcane harvest started, and during February 2013 for construction workers and farmers, under similar climatic conditions. In each of the sugarcane and farmer villages, a well-known public place was selected as the data collection station; construction workers were evaluated at their work site. Data collection started between 5:30 and 6:00 am on the morning after a workday, and blood and urine samples were collected after overnight fasting.

Medical measurements and biological samples. Blood pressure was measured with a calibrated digital sphygmomanometer with the participant seated after resting for 10 minutes. Weight was measured with a calibrated digital flat mobile scale, and height with a foldable stadiometer. Certified technicians collected blood samples in vacuum tubes for centrifugation and serum separation and in a tube with anticoagulant for blood cell count. Samples without coagulant were centrifuged on the spot at 3500 RPM for 10 minutes at room temperature. All samples were placed on ice and transported the same day to the laboratory at the Research Center on Health, Work and Environment (CISTA) at UNAN-León, where hematocrit and hemoglobin were determined with a Mindray 2300 hematology analyzer and the serum samples were frozen at -80 ^{0}C. After finalizing all data collection, serum samples were transported to the National Diagnostic and Reference Center of the Ministry of Health (CNDR-MINSA) of Nicaragua, which takes part in an international interlaboratory quality control program. Samples were analyzed with Cobas Integra 400®, an automated equipment which uses a photometric test to determine levels of serum glucose, lipid profile, serum uric acid (S-UA), and blood urea nitrogen (BUN) and a Jaffe compensated method for quantification of serum creatinine (SCr). SCr was calibrated against IDMS-traceable creatinine. Blind spiked and duplicate blood samples from each 10th participant were in 95% within one standard deviation. A urinalysis dipstick was performed on a spot morning sample using a Bayer Clinitek 50 Urine Chemistry Analyzer with Multistix 10SG reagent strips (Siemens Diagnostics, United States) with semi-quantitative measurements of protein (≥30 to<300 mg/dL and ≥300 mg/dL, glucose (positive at ≥100 mg/dL), specific gravity (USG) (1.000 – 1.030), pH (5.0-8.5), blood (+ to +++), nitrite (positive), leukocyte esterase (+ to +++), bilirubin (+ to +++), ketone (≥5 mg/dl) and urobilinogen (≥2 Ehrlich Units).

Questionnaires. Questionnaires were applied by trained interviewers, with courses on bioethics and good clinical practices. A questionnaire on work and health obtained data on demographics and employment (age, education, drinking water source, income, type of contract, sub-employment, social security), lifestyle (smoking, alcohol, drugs, fluid intake on non-working days), health (medically diagnosed diseases, nephrotoxic medications), work history (industry, job titles, job duration, crops, pesticides), and occupational heat stress determinants (shift duration, breaks, shadow, work speed, heavy loads; for sugarcane workers, in addition, incentives to cut more cane, hours between cane burning and entering the field). This questionnaire was developed based on versions used in previous studies in the region.[5, 23, 24] A second questionnaire, developed at the National Institute of Public Health in Mexico, obtained data on the types and amounts of fluids and food items consumed during the day (always a workday) before the interview. The amount of fructose contained in the food and drinking items was estimated based on a fructose calculation list of the Mexican questionnaire,[25] and the USDA National Nutrient Database for Standard Reference for items not included in the Mexican questionnaire.[26]

Statistical analysis

Data were analyzed with SPSS Statistics 20. Glomerular filtration rate estimated by the CKD-EPI equation ($eGFR_{CKD-EPI}$) was the main outcome measure, categorized into < and ≥80 mL/min/1.73m^2. This cutoff point was chosen instead of the traditional <60 because too few workers had eGFR<60. Prevalences of high BUN (>20 mg/dL), high SCr (>1.2 mg/dL), high S-UA (>7.2 mg/dL), and protein >30mg/dL, blood, nitrites or leucocytes in urine were secondary measures of kidney dysfunction. Prevalences of high urinary specific gravity (USG≥1.030), low urinary pH (≤5.5) and high BUN/SCr ratio (>20) were used as indicators of dehydration.

Self-reported social and work history items, diseases and medications, and heat stress exposure variables were dichotomized. A category of high tobacco consumption was created with subjects in the upper quartile of ever smokers (≥3 pack-years) and a category of high alcohol consumption composed of subjects in the upper tertiles of lifetime alcohol consumption (≥80,000 g) or average weekly consumption (≥125 g). Total fluid intake was defined as drinking water plus sugary drinks (natural fruit refreshments, sodas, coffee, tea and electrolyte solution) and reported as liters of total liquids consumed the previous (work) day and for comparison also for a typical non-work day, with subcategories into water only and sugary drinks. Total fructose intake was estimated from all food and fluids consumed including chewed cane, and stratified into fructose from food sources and added sugars. Fructose variables were categorized into quartiles. Cutoff for body mass index (BMI) were set at ≥25 kg/m^2. Hypertension was defined as systolic blood pressure ≥140 and/or diastolic blood pressure ≥90 mm Hg, or a self-reported medical history of hypertension. Diabetes was defined as serum glucose ≥125 mg/dL in the fasting serum sample or a self-reported medical history of diabetes. Use of nephrotoxic medications was recorded if taken at least three times per week for more than three months in the case of NSAIDs and other analgesics, or administered for at least a week in case of nephrotoxic antibiotics, during the last year. Blood and urine biochemical parameters were explored as continuous variables or defined as normal versus abnormal using standard clinical cutoff values.

Differences between occupations were assessed with ANOVA and Kruskal-Wallis tests for normally and not normally distributed continuous variables, respectively, and Pearson Chi-square test for categorical variables or Fisher's Exact Test when Chi-square was not applicable. Post hoc tests were performed with Tukey's HSD test for continuous results, and post-hoc Chi-square as described by Franke et al. (2012).[27] With occupation as the main proxy for heat stress, we assessed trends for sugarcane cutters>construction workers>farmers for prevalences of markers of kidney dysfunction and dehydration over the ordered occupational groups with the gamma statistic.

Differences in the distribution of risk factors between subjects with reduced and normal kidney function were explored for all occupations combined (n=194) and restricted to sugarcane cutters (n=86), with Whitney U-tests for continuous variables and Chi-square tests or Fisher's Exact Test for categorical variables. Exact p-values are reported and p-values ≤0.05 were considered statistically significant. Multivariate linear regression models were constructed, for all workers and restricted to sugarcane cutters, with factors that were different between subjects with reduced and normal kidney function at p<0.10. Residuals from the regressions were checked to assess the fit of the models.

RESULTS

Potential risk factors for CKD / MeN among the three occupations

Socioeconomic and health-related CKD risk factors

Socioeconomic CKD risk indicators were unfavorable for all workers, but somewhat less for construction workers (Table 1A). Farmers had the lowest income and sugarcane cutters were significantly less educated with on average of 4 years of elementary schooling. With regard to lifestyle and medical factors (Table 1B), sugarcane cutters had lower prevalences of high tobacco and alcohol consumption. There were no major differences in use of nephrotoxic drugs between the groups. None of the workers had been previously diagnosed with diabetes and only five had hyperglycemia >125 mg/dL, two cutters, two construction workers and 1 farmer. Sugarcane cutters showed less obesity, better lipid profiles, lower heart rates and lower blood pressure, but more anemia (36% with hemoglobin <13 g/dL). There were no differences in total leukocyte count between occupations.

Table 1. Socioeconomic and health indicators relevant for CKD /MeN risk among workers in three occupations. Municipalities of Chinandega and León, Nicaragua, 2013

	Sugarcane (N = 86)	**Construction (N = 56)**	**Farming (N = 52)**	**P-value* differences between groups**
A. Demographics, employment and social indicators				
Age (yrs), mean ± SD	25.6 ± 5.5	27.3 ± 6.0	25.2 ± 5.1	0.11
Education (yrs), mean ± SD	3.9 ± 3.0 [a]	7.8 ± 3.6	8.0 ± 4.1	<0.001
Drinking water from well (%)	84.9 [a]	12.5	13.5	<0.001
Temporary contract, %	93.0 [a]	75.0 [a]	21.1 [a]	<0.001
Without work ≥ 4 months/yr, %	20.9	17.9	34.6 [a]	0.089
No current social security, %	15.1	8.9	92.3 [a]	<0.001
Monthly household income per person in family, mean ± SD (25 córdobas = 1 US$), mean ± SD	1808 ± 1156 [a]	2267 ± 1124 [a]	1343 ± 1059 [a]	<0.001
B. Life style, medical history and health indicators				
High tobacco consumption, %	10.5 [a]	26.8	23.1	0.031
High alcohol consumption, %	18.6	28.6	32.7	0.145
Nonsteroidal anti-inflammatory drugs ≥ 3 months, %	5.8	7.1	7.7	0.901
Nephrotoxic antibiotics, %	1.2	1.8	0.0	0.648
History kidney stones, %	1.2	5.4	1.9	0.287
History urinary tract infections, %	23.3 [b]	33.9	42.3 [b]	0.058
Not feeling in good health, %	10.5 [c]	37.5 [c]	17.5	<0.001
Body mass index ≥25 kg/m^2, %	17.4 [c]	58.9 [c]	40.4	<0.001
Blood pressure>140/90, %	5.8 [a]	17.9	26.9	0.003
Heart rate (beats per minute)), mean ± SD	62 ± 12 [a]	73 ± 14	72 ± 13	<0.001
Blood glucose (mg/dL), mean ± SD	89 ± 11	90 ± 14	90 ± 12	0.874
Triglycerides (mg/dL), mean ± SD	120 ± 67 [a]	168 ± 108	177 ± 124	<0.001
Cholesterol (mg/dL), mean ± SD	170 ± 36 [c]	188 ± 41 [c]	178 ± 44	0.032
High density lipoprotein (HDL) cholesterol (mg/dL), mean ± SD	48 ± 12 [a]	42 ± 10	38 ± 8	<0.001
Low density lipoprotein (LDL) cholesterol (mg/dL), mean ± SD	93 ± 28	101 ± 33	91 ± 32	0.120
Very low density lipoprotein (VLDL) cholesterol (mg/dL), mean ± SD	24 ± 13 [a]	34 ± 22	35 ± 25	<0.001
Hematocrit (%), mean ± SD [d]	46.8 ± 5.9	48.5 ± 4.8	50.8 ± 4.0 [a]	<0.001
Hemoglobin (g/dL), mean ± SD [d]	13.4 ± 1.6 [a]	14.8 ± 1.5	15.4 ± 1.3	<0.001

Hemoglobin <13 g/dL, % [d]	35.8 [a]	8.9	3.8	<0.001
White blood cells/µL, mean ± SD [d,e]	7184 ± 2048	7307 ± 1656	7580 ± 1882	0.503
% neutrophils, mean ± SD [d,e]	38.6 ± 10.6	38.6 ± 8.8	36.5 ± 9.0	0.421
% lymphocytes, mean ± SD [d,e]	21.2 ± 6.6 [a]	18.5 ± 4.9 [a]	14.5 ± 4.7 [a]	<0.001
% other cells, mean ± SD [d,e]	40.2 ± 10.0	43.0 ± 8.2	49.1 ± 10.6 [a]	<0.001
Erythrocytes $*10^6$/µL, mean ± SD [d]	4.87 ± 0.59 [a]	5.27 ± 0.47 [a]	5.53 ± 0.50 [a]	<0.001
Platelets $*10^3$/µL, mean ± SD [d]	299.4 ± 76.7	315.6 ± 67.7	292.8 ± 62.8	0.218

* P-value for differences between groups: ANOVA for normally distributed continuous variables, Kruskal-Wallis for not normally distributed continuous variables, Chi-square test for categorical variables.

[a] Significantly different from the other two categories in post hoc tests.

[b] Significant difference only between sugarcane cutters and farmers

[c] Significant difference only between sugarcane cutters and construction workers.

[d] 5 missing data for sugarcane workers, due to technical error

[e] Exclusion of one farmer with outlier for white blood cell count (WBC count = 17.500).

Occupational heat exposure, fluid and fructose intake, and pesticides

On average, construction workers had an effective work time of eight hours and farmers the shortest with five hours, whereas sugarcane workers actively cut cane during 6.5 hours per day (Table 2A). A higher proportion of sugarcane workers perceived a very rapid work pace, and had to take rest breaks in the absence of shade; 83% received incentives for cutting more cane, and almost half started harvesting within 12 hours of burning the cane. Sugarcane cutters reported more often weight loss related to current job (over the last two months) and fainting on the job (6% as compared to 2% of farmers and no construction workers). Dysuria ('chistata'), a common symptom in MeN affected areas thought to be related to dehydration,[15, 24] was not different between the three groups.

With regard to fluid intake (Table 2B), sugarcane cutters reported on average 6.2 L of total fluid intake the previous (work) day, 70% (4.4 L) as water and almost 30% (1.8 L) as sugary drinks. This was higher than for construction workers and farmers. Intake of water and sugary beverages were not correlated (r_p= 0.01). In contrast, the three groups were not different for total fluid, water and sugary drinks intake on non-work days.

Fructose intake during the previous day was highest for sugarcane cutters compared to farmers and construction workers; and 41% of sugarcane cutters belonged to the category of highest quartile of consumption of total fructose (>107 g) (Table 2B). Fructose intake from food was low among sugarcane cutters and most came from added sugars during work hours, specifically from sweetened beverages, electrolyte hydration solution (a third of the cutters) and cane chewing (about two thirds). Fructose intake outside work hours was not different between the groups.

With regard to pesticide exposures (Table 2C), farmers used pesticides most frequently (71%), almost half of sugarcane cutters reported pesticide use, versus only 11% of construction workers. Glyphosate and 2,4-D use was more common among sugarcane cutters, whereas paraquat and the insecticides cypermethrin and chlorpyrifos were used more often by farmers. With the exception of cypermethrin, which had been used by almost half of the farmers, no specific pesticide exceeded 25% of users in any of the groups.

Table 2. Occupational heat stress, fluid and fructose intake and pesticide exposure indicators among workers in three occupations. Municipalities of Chinandega and León, Nicaragua, 2013

	Sugarcane (N = 86)	**Construction (N = 56)**	**Farming (N = 52)**	**P-value***
A. Current occupational heat stress				
Effective work hours per day (work hours minus breaks), mean ± SD	6.5 ± 1.2 [a]	8.1 ± 0.7 [a]	5.3 ± 2.0 [a]	<0.001
Very rapid work pace, %	74.4 [a]	53.6	40.4	<0.001
No shade during breaks, %	20.9 [b]	1.8 [b]	11.5	0.004
Lifting weights >50 lbs., %	18.6 [a]	66.1	65.4	<0.001
Awkward work postures, %	58.1	76.8	69.2	0.063
Incentives to cut more cane, %	82.6	-	-	-
Hours post-burning at field entrance, mean ± SD	11.7 ± 6.2	-	-	-
Self-reported weight loss on the current job (last two months), %	77.9 [a]	39.3	36.5	<0.001
Fainted at work, %	5.8	0	1.9	0.126
Dysuria (‘chistata’), %	43.0	48.2	44.2	0.827
B. Fluid and fructose intake				
Fluid intake previous day (workday)				
Total fluid (L), mean ± SD	6.2 ± 4.1 [a]	4.4 ± 2.1	4.0 ± 2.7	0.003
Water	4.4 ± 3.9 [a]	2.9 ± 2.1	2.8 ± 2.4	0.002
Sugary drinks without electrolyte hydration solution	1.8 ± 1.8	1.5 ± 0.9	1.2 ± 0.8	0.208
Electrolyte solution (N=31)	1.2 ± 1.1	-	-	-
Lowest quartile total fluid (≤ 2.5 L), %	18.6	19.6	40.4 [a]	0.009
Highest quartile total fluid (≥7.0 L), %	40.7 [a]	8.9	13.5	<0.001
Fluid intake on typical non-work day				
Total fluid (L), mean ± SD	4.2 ± 2.3	3.8 ± 1.7	4.1 ± 2.2	0.503
Water	3.0 ± 2.0	2.2 ± 1.3	2.7 ± 2.0	0.053

Sugary drinks	1.2 ± 1.1	1.6 ± 1.1	1.4 ± 1.9	0.117
Fructose intake previous day (workday)				
Total fructose intake (g), mean ± SD	103.1 ± 72.1 [a]	80.1 ± 46.1	70.9 ± 36.8	0.008
From food sources	8.4 ± 10.7 [a]	15.9 ± 16.6	17.4 ± 16.7	<0.001
From added sugar	94.7 ± 70.5 [a]	64.2 ± 38.1	53.2 ± 30.7	<0.001
During work hours	58.6 ± 44.7 [a]	28.6 ± 21.4	26.1 ± 16.5	<0.001
Sugary drinks ('frescos', sodas, coffee)	22.5 ± 15.7	28.6 ± 21.4	26.1 ± 16.3	0.108
Sugarcane chewing (N=53)	35.0 ± 18.5	-	-	-
Electrolyte solution (N=31)	40.3 ± 35.2	-	-	-
Outside (before and after) work hours	36.1 ± 39.3	35.6 ± 31.4	27.1 ± 25.9	0.350
Highest quartile total fructose intake (>107 g), %	40.7 [a]	19.6	15.7	0.002
C. Work and pesticide use history				
Cumulative time on current job (months), mean ± SD	77 ± 60	68 ± 80	116 ± 67 [a]	0.001
Ever sugarcane work, %	100.0 [a]	3.6	3.8	<0.001
Ever plantation (other than sugarcane), %	24.4	5.4 [a]	21.2	0.012
Ever work in small-scale agricultural (%)	61.6 [a]	25.0 [a]	100.0 [a]	<0.001
Ever construction work, %	5.8	100.0 [a]	11.5	<0.001
Ever any pesticide use, %	46.5 [a]	10.7 [a]	71.2 [a]	<0.001
Glyphosate, %	19.8 [a]	0.0	3.8	<0.001
2,4-D, %	23.3 [a]	0.0 [a]	9.6 [a]	<0.001
Paraquat, %	9.3	3.6	25.0 [a]	0.002
Chlorpyrifos, %	0.0	0.0	23.1 [a]	<0.001
Cypermethrin, %	18.6 [a]	3.6 [a]	42.6 [a]	<0.001

* P-value for differences between groups: ANOVA for normally distributed continuous variables, Kruskal-Wallis for not normally distributed continuous variables, Chi-square test for categorical variables.

[a] Significantly different from the other two categories in post hoc tests.

[b] Significant difference only between sugarcane cutters and construction workers.

Status of kidney function and hydration by occupation

Kidney function biomarkers were more commonly abnormal among sugarcane cutters, with significant differences between the groups for prevalences of eGFR<80 ml/min/1.73m^2 (16%, 9% and 2%, in sugarcane, construction and small-scale farmers, respectively, p for trend=0.003), high SCr (p for trend=0.02) and high BUN (p for trend=0.003) (Table 3A). Likewise, proteinuria >30 mg/dL was approximately three times more prevalent in sugarcane workers compared to the other groups (15% vs 5-6%, p for trend=0.08), whereas leukocyturia was observed in 22% of sugarcane workers but in only 0-2% of the other heat-exposed groups (p<0.001). Microhematuria was also three times more prevalent in sugarcane workers but not statistically significant (6% vs 2%, p for trend=0.19). High S-UA was more common among sugarcane cutters (17%) and construction workers (16%) than among famers (6%).

Table 3. Biomarkers of kidney function and dehydration among workers in three occupations and trend over categories ordered by exposure to occupational heat stress (sugarcane > construction > farming). Municipalities of Chinandega and León, Nicaragua, 2013.

Variable	Sugarcane (N = 86)	Construction (N = 56)	Farming (N = 52)	P-value: differences between groups*	P-value: trend**
A. Indicators of kidney function					
Blood urea nitrogen (BUN) (mg/dL), mean ± SD (range)	13.9 ± 5.0 [a] (6.0 – 28.4)	10.1 ± 5.1 (4.1 – 30.0)	9.2 ± 3.6 (4.0 – 22.0)	<0.001	
BUN >20 mg/dL (%)	15.1 [a]	5.4	1.9	0.017	0.003
Serum creatinine (SCr) (mg/dL), mean ± SD (range)	0.84 ± 0.39 (0.44 – 2.39)	1.00 ± 1.16 (0.49 – 8.84)	0.78 ± 0.22 (0.51 – 1.83)	0.393	
SCr >1.2 mg/dL, %	17.4 [c]	8.9	5.8 [c]	0.088	0.024
Estimated glomerular filtration rate ($eGFR_{CKD-EPI}$), mean ± SD (range)	121 ± 31 (34 – 160)	118 ± 30 (7 – 161)	125 ± 18 (49 – 158)	0.299	
$eGFR_{CKD-EPI}$ <80 ml/min/1.73m^2, %	16.3 [c]	8.9	1.9 [c]	0.025	0.003
Serum uric acid (S-UA) (mg/dL), mean ± SD (range)	6.0 ± 1.7 (3.0 – 12.7)	5.8 ± 1.6 (3.6 – 11.0)	5.0 ± 1.1 [a] (2.9 – 8.1)	0.001	
S-UA >7.2 mg/dL, %	17.4	16.1	5.8	0.136	0.055
Proteinuria >30 mg/dL, %	14.7	5.4	6.1	0.128	0.081
Leucocytes in urine, %	22.1 [a]	0	1.9	<0.001	<0.001
Nitrites in urine, %	0	0	0	-	-
Blood in urine, %	5.8	1.8	1.9	0.339	0.186
B. Indicators of dehydration					
Urinary specific gravity (USG) ≥1.030, %	15.3	28.6	20.4	0.161	0.255
Urinary pH ≤5.5, %	29.4 [a]	12.5	12.2	0.014	0.006
BUN/SCr ratio >20, %	25.6 [a]	0	3.8	<0.001	<0.001

* P-value for differences between groups: ANOVA for normally distributed continuous variables, Kruskal-Wallis for not normally distributed continuous variables, Chi-square test for categorical variables.
**Gamma statistic for trend over ordered categories.
[a] Significantly different from the other two categories in post hoc tests.
[b] Significant difference only between sugarcane cutters and construction workers.

Regarding markers of dehydration, prevalence of concentrated urine (USG ≥1.030) was not statistically different between groups (Table 3B). Low urinary pH occurred in 29% of sugarcane cutters versus 12% of construction workers and farmers (p=0.01) and sugarcane cutters more commonly had an elevated BUN/SCr ratio (26% vs 0 and 4% of construction workers and farmers, p<0.001). Trends over ordered categories were significant for urinary pH and BUN/SCr ratio. Although sugarcane cutters as a group had a lower prevalence of concentrated urine, within the group low fluid intake was strongly associated with concentrated urine (OR 3.5, p=0.06) and acidic urine (OR 8.7, p<0.001) which was not the case among construction workers and farmers (Table 4).

Table 4. Associations between low intake of fluids and markers of dehydration among sugarcane cutters (n=86) and non-cutters (construction workers and small-scale farmers) (n=108)

Lowest quartiles of fluid intake		**USG ≥1.030** [a]	**pH ≤5.5** [a]	**BUN/SCr ratio >20** [a]
		OR (95% CI: LL; UL) p-value [b]		
Total fluids ≤2.5L	Sugarcane cutters (n=16)	3.5 (1.0; 13) 0.06	8.7 (2.6; 29) <0.001	1.2 (0.3; 4.3) 0.67
	Construction workers and farmers (n=32)	1.4 (0.5; 3.5) 0.51	2.3 (0.7; 7.5) 0.17	- [c]
Water ≤1.5L	Sugarcane cutters (n=16)	3.0 (0.7; 12) 0.14	2.9 (0.9; 9.6) 0.08	2.3 (0.7; 7.3) 0.17
	Construction workers and farmers (n=32)	1.9 (0.7; 4.9) 0.18	1.7 (0.5; 5.6) 0.42	- [c]
Sugary drinks ≤0.75L	Sugarcane cutters (n=28)	2.5 (0.7; 9.2) 0.16	2.5 (0.9; 7.1) 0.08	0.3 (0.2; 1.1) 0.06
	Construction workers and farmers (n=21)	1.8 (0.6; 5.2) 0.28	0.7 (0.2; 3.6) 0.69	- [c]

[a] Markers of dehydration: high urinary specific gravity (USG≥1.030); acidic urine (urinary pH ≤5.5); high serum urea nitrogen to serum creatinine ratio (BUN/SCr ratio >20).
[b] The odds ratios (OR) and 95% confidence intervals [CI, lower limit (LL) and upper limit (UL)] for water and sugary drinks are adjusted for each other.
[c] Not computed because only two non-cutters had BUN/SCr ratio >20.

Risk factors for reduced kidney function

In bivariate analyses of differences in kidney, urinary and metabolic biomarkers, work practices, hydration practices and lifestyle characteristics between subjects with reduced kidney function (eGFR <80 ml/min/1.73 m^2) and subjects with normal kidney function (eGFR ≥80 ml/min/1.73 m^2) (Supplementary Table 1), reduced kidney function was significantly associated with work as sugarcane cutter, high intake of water, low intake of sugary beverages, increasing age, low hemoglobin and high tobacco consumption. In analyses restricted to sugarcane cutters, results were similar and, in addition, workers with reduced kidney function had cut cane during considerably longer time than those with normal kidney function (cumulative time on the job: median 108 vs 60 months, p=0.06). Sugarcane cutters with reduced kidney function reported almost three times higher water intake and three times lower intake of sugary beverages as compared to cutters with normal kidney function, with only 1 of the 14 reporting intake of the electrolyte solution. In addition, the cane cutters with reduced kidney function had a worse lipid profile than those with normal kidney function and more often hypertension, but none had diabetes or hyperglycemia and only one was overweight (Supplementary Table 1).

In backwards stepping multivariate linear regression analyses with inclusion of variables with p≤0.10 in the bivariate analyses (except hemoglobin due to missing data), age (beta -1.3, 95%CI -1.8, -0.8; p<0.001) and S-UA (beta -10.4, 95% CI -12.2, -8.5; p<0.001) associated significantly with reduced kidney function among all workers, identically in models with total fluid intake and with intake of water and sugary beverages separately (Table 5A). In the subset of sugarcane cutters, too many variables had a p-value ≤0.10 in bivariate analyses (see supplementary Table 1) and therefore the regression was done in two steps. Hypertension, lipid profile tests and blood sugar did not associate with reduced kidney function in a model also including age and S-UA (data not shown) and were not further considered. In a model with water intake, intake of sugary drinks (without electrolyte solution) and intake yes/no of electrolyte solution, age, S-UA, high tobacco consumption and high alcohol consumption (Table 5B), reduced kidney function associated significantly with age and S-UA and non-significantly with the intake of electrolyte solution (beta 8.1, 95% CI -1.2, 17.5, p=0.09). Age and cumulative months on the job correlated (r_p 0.68, p<0.001), and substituting age with time cutting cane yielded similar results.

Table 5. Multivariate linear regression models of estimated glomerular filtration rate ($eGFR_{CKD-EPI}$) among all workers (sugarcane cutters, construction workers and farmers) and restricted to sugarcane cutters.

A. All subjects (N=194)		**Beta coefficient (95% CI: UL; LL)**	**Standardized beta coefficient**	**P-value**	**Adjusted R^2**
Step 1	Water intake (L)	-0.7 (-1.7; 0.3)	-0.08	0.15	0.47
	Sugary beverages intake (L)	1.2 (-0.8; 3.3)	0.06	0.24	
	Sugarcane cutter ever	3.6 (-2.5; 9.6)	0.07	0.25	
	Age (yrs)	-1.2 (-1.7; -0.6)	-0.24	<0.001	
	Serum uric acid (mg/dL)	-10.0 (-12.0; -8.1)	-0.57	<0.001	
	High tobacco consumption	-4.5 (-11.6; 2.7)	-0.07	0.22	
	High alcohol consumption	1.2 (-5.6; 8.1)	0.02	0.72	
Final step	Age (yrs)	-1.3 (-1.8; -0.8)	-0.27	<0.001	0.47
	Serum uric acid (mg/dL)	-10.4 (-12.2; -8.5)	-0.59	<0.001	
B. Sugarcane cutters (N=86)					
Step 1	Water intake (L)	-0.7 (-1.9; 0.5)	-0.09	0.25	0.58
	Sugary beverages intake (without electrolyte solution) (L)	1.2 (-3.7; 6.0)	0.04	0.63	
	Electrolyte solution (yes/no)	6.4 (-4.5; 17.3)	0.10	0.24	
	Age (yrs)	-1.7 (-2.5; -0.8)	-0.29	<0.001	
	Serum uric acid	-10.9 (-13.8: -8.1)	-0.59	<0.001	
	High tobacco consumption	-10.1 (-22.5; 2.3)	-0.12	0.11	
	High alcohol consumption	-7.8 (-19.5; 3.9)	-0.10	0.19	
Final step	Age (yrs)	-1.9 (-2.7; -1.1)	-0.34	<0.001	0.57
	Serum uric acid (mg/dL)	-11.3 (-14.0; -8.6)	-0.61	<0.001	
	Electrolyte solution (yes/no)	8.1 (-1.2; 17.5)	0.13	0.09	

DISCUSSION

This study found evidence for more frequent heat stress, dehydration and kidney dysfunction among sugarcane cutters, as expected, and in lesser degree also reduced kidney function among construction workers but not among small-scale farmers. Also, as expected, serum uric acid levels increased with decreasing eGFR.

Evidence of reduced kidney function

We used a cutoff of eGFR of 80 ml/min/1.73m^2 to evaluate differences in renal function because only 11 workers had eGFR below 60, due to young age (all under age 40) and also because sugarcane workers were screened by employers before the start of the harvest two months earlier and workers with SCr>1.2 mg/dL were not hired and, thus, not part of our study population. Despite, approximately one-fourth of sugarcane cutters had evidence for either eGFR <80 ml/min/1.73m^2, SCr >1.2 mg/dL or proteinuria ≥30 mg, and these findings were, respectively, eight-, three- and two-fold more common than observed in subsistence farmers and about two-fold more common than in construction workers (Table 3). However, although in lesser degree than cane cutters, construction workers also had an unusually high prevalence of decreased kidney function, which is in accordance with a previous unpublished study in the same area.[25] In contrast, the single small-scale farmer with reduced kidney function had worked previously in sugarcane. Thus, our results show that not all agricultural workers are at increased risk for CKD, as is commonly stated, but rather workers in certain types of agriculture and other hot jobs such as work in the construction industry. The absence of reduced kidney function among subsistence farmers is consistent with a study in a MeN epidemic area in El Salvador, where subsistence farmers without a history of plantation work had a significantly lower prevalence of abnormal SCr than men who had worked on sugar or cotton plantations (15% vs 33%).[20] Reduced kidney function was accompanied by a higher frequency of anemia among sugarcane cutters (36% versus 4-9% in the other groups). The prevalence of anemia was higher than the prevalence of reduced kidney function and cannot be simply ascribed to the higher frequency of reduced renal function. Marked anemia, defined as Hb of <10 g/dL, was not observed in any of the groups.

The reduced kidney function did not associate with traditional risk factors for CKD. Notably, there was not one case of confirmed diabetes in the entire population. Importantly, sugarcane workers showed significantly worse renal function despite overall lower frequency of abnormal lipid profile, hypertension and obesity compared to the other two groups (see Table 1). Increasing age - over 50 - is a known risk factor for CKD, but in our study increasing age was associated with decline of renal function despite the young age of the study participants. This is possibly related to an increased risk with continued job exposure over time, in particular among the sugarcane cutters. Thus, our study suggests that most cases of reduced kidney function are related to Mesoamerican nephropathy and not classic CKD.

Evidence for heat stress

There was evidence for greater risk for heat stress among sugarcane cutters. Sugarcane cutters labored at a faster pace, had less exposure to shade, reported more weight loss during the ongoing harvest, and had more fainting episodes. While sugarcane cutters had greater heat stress exposure, they also drank more fluids during the course of the day, amounting to an average of 6.2 L per day (although this varied considerably, with approximately 20 percent drinking <2.5 L/d and 40% >7 L/day). However, the type of exertion and sweating that occurs with cane harvesting[11-13] could still result in dramatic loss of fluids such that dehydration can occur despite high fluid consumption. Cade et al. found that college football players could lose as much as 8 quarts (about 7.6 L) of water in a 2 hour period, associated with loss of salt, a decrease in blood glucose, and a fall in blood pressure.[28]

Potential mechanisms involved in inducing kidney damage

Daily heat stress and dehydration may cause repeated renal hypoperfusion episodes, and intermittent subclinical rhabdomyolysis associated with excessive exertion may also induce repeated acute kidney injury (AKI) through the release of inflammatory mediators including oxidants, cytokines and uric acid, which over time leads to CKD [18]. Experimental evidence has shown that repeated exposure to heat stress caused a reduction of renal function accompanied by histological evidence of tubulointerstitial damage.[19] Heat stress is known to raise serum uric acid levels, in part from subclinical rhabdomyolysis,[29] but also from reduced renal blood flow.[30] In turn, hyperuricemia is a well-known risk factor for CKD[31] and mediates both glomerular and tubulointerstitial disease in animals.[32-34] Interestingly, serum uric acid levels tended to be highest in both sugarcane workers and construction workers, with 16-17% of these individuals having hyperuricemia compared to 6% in subsistence farmers. Furthermore, we found that the presence of hyperuricemia was independently and strongly associated with declining renal function, i.e. for each increase of 1 mg/dl of S-UA there was an average decline of 10 ml/min in kidney filtration (see Table 5). However, since reduced renal function can also result in increased uric acid levels due to impaired excretion, the causal role of uric acid in the reduced kidney function cannot be determined.

Recently we hypothesized that renal injury could be occurring in sugarcane workers due to cyclical uricosuria with crystal formation.[20, 21] According to this hypothesis, serum uric acid might rise as a consequence of subclinical rhabdomyolysis, followed by its crystallization in the urine. One factor that increases the risk for urate crystal formation is acidic urine that could result from the release of lactic acid associated with strenuous exercise and by the effects of dehydration to reclaim sodium with hydrogen ion excretion. Urine pH was significantly lower in the sugarcane workers compared to other groups (see Table 3) and was strongly associated with low fluid intake the previous (work) day in the subset of sugarcane workers (see Table 4). This might reflect the effects of greater volume depletion (with aldosterone stimulation), lactic acid generation during the prior day, or other mechanisms.

Hydration and fructose

We had expected that low water intake, or high sugary fluid intake, would be associated with reduced renal function, based on studies in animals.[19, 35] However, workers with eGFR <80 ml/min/1.73m^2 drank more water and consumed less sugar-based drinks during the workday compared to subjects with normal kidney function (4.5 L vs 2.2 L water, p=0.08; 0.6 L vs 1.25 L sugary beverages, p=0.001) (see Supplementary Table 1). This was particularly so among the sugarcane cutters with reduced kidney function, who drank about 4 L more water and 1 L less sugary beverages. Excessive thirst from decreased concentration capacity of impaired kidneys may partially explain these counterintuitive findings, as well as the very high water requirements during the heavy labor of sugarcane cutting.[12, 13] Although low fluid intake clearly associated with concentrated urine among sugarcane cutters (OR USG≥1.030=3.5, p=0.06) (see Table 4), cutters in the quartile with the highest fluid intake did not have a decreased risk of concentrated urine (OR=1.3, p=0.70) while high fluid intake among non-sugarcane workers appeared to be preventive (OR 0.10, p=0.06). Salvadorian cane cutters who consumed amounts of fluid comparable to our Nicaraguan cutters, were found to have insufficient fluid intake under their work conditions.[11]

Sugary beverages that contain fructose are known to increase the risk for albuminuria,[36] and can induce renal injury in laboratory animals.[35] However, fructose is also a component of sports drinks and fluid resuscitation packets containing glucose and electrolytes that might be beneficial to the volume and water depleted, such as by providing glucose that may prevent or treat any associated hypoglycemia, or by helping to maintain blood pressure due to the fructose component.[37, 38] In our study, the intake of electrolyte solutions tended to be associated (p=0.09) with improved kidney function in multivariate analyses (see Table 5). One study in Nicaragua found that, for each 100 cc electrolyte hydration packet consumed during the workday, the eGFR of cane cutters increased by 7 ml/min/1.73m2 over the course of one harvest season.[17] These issues need to be assessed with prospective studies that examine overall fluid balance by measuring fluid intake as well as losses during work, such as pre- and post-shift weight and serum and urine osmolarity.

Other risk factors for kidney disease

There was no association with NSAIDs or alcohol intake. A history of high tobacco consumption was more frequent among subjects with reduced kidney function (p=0.02) but lost significance in multivariate analyses. A history of pesticide exposure was more common among farmers, although exposure to herbicides was more common among sugarcane cutters, especially glyphosate and 2,4-D, both of special interest. However, analyses failed to identify pesticide exposures as an independent risk factor for reduced kidney function (see Supplementary Table 1).

Study limitations

The main limitation of our study is its cross-sectional design. The kidney function parameters are based on single determinations in blood and urine without a chronicity criterion (presence during at least 3 months) for a proper clinical diagnosis of CKD.[39]. Recently, attention has been drawn to the fact that single biomarker determinations and consequent categorizations into CKD-stages based on a cut-off value, without consideration of age- and sex-specific criteria for GFR, are inadequate as the basis for population-based CKD prevalences, because these practices can lead to overdiagnosis among the elderly and underdiagnosis in younger age groups with large unexplained differences between nations.[40, 41] However, the main purpose of our study is not a clinical diagnosis but to distinguish differences in kidney function parameters between three occupational groups of the same sex and same young age distribution, and comparisons remain therefore valid on the group level. In addition, in the same region, at the time of this study, we also followed a small group of heat-exposed sugarcane cutters and a group of control workers unexposed to heat over the harvest season. The cutters showed an important decline in kidney function,[42] which provides support for the cross-sectional findings, although no cohort data exist for construction workers or farmers.

Another limitation is that our heat exposure and hydration data were self-reported, but these data were collected through carefully designed questionnaires. Workers were asked to fast and did not consume any food before providing blood and spot urine samples between 5.30 and 6.00 am (see methods), but they did ingest water or other fluids during the evening, night and early morning. Nonetheless, we observed lower U-pH and more frequent high BUN/SCr ratio among cane cutters, and to a lesser extent among construction workers as compared to subsistence farmers, which is an indication of incomplete recovery of adequate hydration status after the previous work day among the more heat stress exposed workers.

Our sample size was based on a pre-study power calculation of 80% to detect CKD among 100 sugarcane cutters and100 non-cutters at alpha 0.05. Post hoc, we achieved a power of 0.68 for increased risk of reduced eGFR among cutters versus non-cutters, but the post hoc power of the comparison between cutters and farmers was 80%. Therefore, our results seem sufficiently reliable, also considering the significant trends for indicators of heat stress, dehydration and kidney dysfunction in support our main hypothesis of cane cutting >construction>farming. Finally, we did not have resources for examining biomarkers of early damage such as NGAL or NAG, which are important to include in future studies.

CONCLUSIONS

In summary, compared to construction workers and, in particular, subsistence farmers from the same MeN epidemic region of Nicaragua, sugarcane cutters have higher heat stress, more dehydration and worse renal function, despite that other health indicators of the cutters were significantly better. Our study supports the need for improved work practices and even more hydration with adequate access to water for sugarcane cutters, as well as for workers in other hot occupations such as construction. The associations between intake of water and sugary drinks and kidney function as well as the role of hyperuricemia need to be assessed in carefully designed follow-up studies.

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Supplementary Table 1. Comparison of kidney, urinary and metabolic biomarkers, work practices, hydration practices and lifestyle characteristics of subjects with reduced kidney function (<80ml/min/1.73 m^2) and subjects with normal kidney function (≥80ml/min/1.73 m^2), all study participants (N=194) and sugarcane workers (N=86)

	All workers (N=194)			**Sugarcane cutters (N=86)**		
	eGFR <80ml/min/1.73 m^2 N=20	eGFR ≥80ml/min/1.73 m^2 N=174	P-value*	eGFR <80ml/min/1.73 m^2 N=14	eGFR ≥80ml/min/1.73 m^2 N=72	P-value*
Kidney function						
eGFR (ml/min/1.73 m^2), median (10%; 90%)	55 (27; 76)	129 (112; 147)	<0.001	63 (39, 78)	134 (102, 153)	<0.001
S creatinine (mg/dL), median (10%; 90%)	1.67 (0.24; 3.05)	0.72 (0.56; 0.94)	<0.001	1.47 (1.24; 2.21)	0.70 (0.51; 0.95)	<0.001
S urea nitrogen (BUN) (mg/dL), median (10%; 90%)	22.6 (13.5; 28.3)	10.0 (6.1; 15.0)	<0.001	22.6 (14.4; 28.1)	12.1 (7.9; 18.0)	<0.001
S uric acid (mg/dL), median (10%; 90%)	8.2 (6.7; 11.0)	5.2 (3.9; 6.8)	<0.001	8.1 (6.9, 11.8)	5.4 (3.9, 6.9)	<0.001
S uric acid >7.2 mg/dL, % (# cases)	85.0 (17)	5.7 (10)	<0.001	85.7 (12)	4.2 (3)	<0.001
Protein >30 mg, % (# cases) (N=180)	20.0 (4)	7.5 (13)	0.09	23.1 (3)	2.9 (8)	0.39
Leukocytes positive at dipstick, % (# cases)	30.0 (6)	8.0 (14)	0.009	42.9 (6)	18.1 (13)	0.07
Blood, traces and higher at dipstick, % (# cases)	20.0 (4)	1.7 (3)	0.002	28.6 (4)	1.4 (1)	0.002
Hydration						
USG ≥1030, % (# cases) (N=190)	10.0 (2)	21.8 (37)	0.38	7.1 (1)	16.9 (12)	0.69
USG ≤1005, % (# cases) (N=190)	20.0 (4)	16.1 (28)	0.75	28.6 (4)	12.7 (9)	0.22
pH ≤5.5, % (# cases) (N=190)	30.0 (6)	18.4 (32)	0.24	42.9 (6)	26.8 (19)	0.34
BUN/serum creatinine ratio>20	0 (0)	13.8 (24)	0.14	0 (0)	30.6 (22)	0.02
Work practices						
Sugarcane cutter ever, % (# cases)	75.0 (15)	43.1 (75)	0.007	n.a. [a]	n.a.	n.a.
Cumulative time in job (months), median (10%; 90%)	90 (2; 235)	62.5 (4; 180)	0.39	108 (30; 216)	60 (30; 216)	0.06
Work day (hours), median (10%; 90%)	7.75 (6.0; 10.0)	8.0 (4.0; 10.0)	0.85	7.0 (5.5; 10.0)	8.0 (6.0; 9.0)	0.03
Hours cutting cane, median (10%; 90%)	n.a. [a]	n.a.	n.a.	6.0 (4.6; 7.5)	6.8 (5.0; 8.0)	0.02
Total break time (min), median (10%; 90%)	60 (20; 113)	60 (20; 104)	0.55	60.0 (20, 112)	60 (25, 90)	0.30
Breaks ≤2/d, % (# cases)	65.0 (13)	70.7 (123)	0.60	57.1 (8)	63.9 (46)	0.63

	All workers (N=194)			Sugarcane cutters (N=86)		
	eGFR <80ml/min/1.73 m^2 N=20	eGFR ≥80ml/min/1.73 m^2 N=174	P-value*	eGFR <80ml/min/1.73 m^2 N=14	eGFR ≥80ml/min/1.73 m^2 N=72	P-value*
No shade during breaks, % (# cases)	20.0 (4)	12.1 (21)	0.30	28.6 (4)	19.4 (14)	0.48
High speed perception, % (# cases)	75.0 (15)	57.5 (100)	0.13	85.7 (12)	72.2 (52)	0.50
Production (tons/d), median (10%; 90%)	n.a.	n.a.	n.a.	6.5 (5.0; 10.0)	7.0 (5.0; 10.0)	0.66
Incentives to cut more, % (N)	n.a.	n.a.	n.a.	71.4 (10)	84.7 (61)	0.26
History of pesticide use, % (# cases)	30.0 (6)	44.3 (77)	0.22	42.9 (6)	47.2 (34)	1.00
Chlorpyrifos, % (# cases)	0 (0)	6.9 (12)	0.62	0 (0)	0 (0)	-
Cypermethrin, % (# cases)	10.0 (2)	23.0 (40)	0.26	14.3 (2)	19.4 (14)	1.00
Paraquat, % (# cases)	0 (0)	13.2 (23)	0.14	0 (0)	11.1 (8)	0.34
2,4-D, % (# cases)	20.0 (4)	12.1 (21)	0.30	28.6 (4)	22.2 (16)	0.73
Glyphosate, % (# cases)	15.0 (3)	9.2 (16)	0.42	21.3 (3)	19.4 (14)	1.00
Hydration practices						
Total fluid intake (L), median (10%; 90%)	5.0 (1.1; 13.8)	3.8 (1.9; 10.0)	0.08	6.7 (1.4; 14.6)	4.5 (2.2; 12.4)	0.16
Low total fluid intake ≤2.5 L/d, % (# cases)	15.0 (3)	25.9 (45)	0.41	14.2 (2)	19.4 (14)	1.00
High total fluid intake ≥7.0 L/d, % (# cases)	40.0 (7)	22.4 (39)	0.10	50.0 (7)	38.9 (28)	0.44
Water (L), median (10%; 90%)	4.5 (0.3; 13.3)	2.2 (0.5; 8.0)	0.03	6.3 (0.6; 14.3)	2.2 (0.7; 10.0)	0.03
Low water intake < 1.5 L/d, % (# cases)	20.0 (4)	25.3 (44)	0.79	14.2 (2)	19.4 (14)	1.00
High water intake > 4.5 L/d, % (# cases)	50.0 (10)	22.4 (39)	0.007	64.3 (9)	30.6 (22)	0.02
Sugary beverage, median (10%; 90%)	0.6 (0.4; 2.4)	1.25 (0.5; 3.0)	0.001	0.5 (0.3; 1.9)	1.6 (0.4; 4.5)	0.002
Low sugary drink intake <0.75 L/d, % (# cases)	50.0 (10)	22.4 (39)	0.007	64.3 (9)	26.4 (19)	0.01
High sugary drink intake ≥2.0 L/d, % (# cases)	10.0 (2)	28.7 (50)	0.07	7.1 (1)	40.3 (29)	0.03
Intake electrolyte solution, % (N)	n.a.	n.a.	n.a.	7.1 (1)	41.7 (30)	0.01
Age, metabolic and lifestyle risk factors						
Age (yrs), median (10%; 90%)	29.5 (21; 37)	25 (19; 33)	0.002	29 (20; 36.5)	24 (18; 33)	0.01
Hypertension (history or exam), % (# cases)	15.0 (3)	19.5 (34)	0.77	21.4 (3)	4.2 (3)	0.05
Body mass index (BMI, kg/m^2), median (10%; 90%)	24.0 (19.9; 28.9)	23.3 (19.9; 29.7)	0.49	23.0 (19.2; 29.7)	22.2 (19.6; 26.4)	0.23

	All workers (N=194)			Sugarcane cutters (N=86)		
	eGFR <80ml/min/1.73 m^2 N=20	eGFR ≥80ml/min/1.73 m^2 N=174	P-value*	eGFR <80ml/min/1.73 m^2 N=14	eGFR ≥80ml/min/1.73 m^2 N=72	P-value*
BMI >25 kg/m^2, % (# cases)	40.0 (8)	35.1 (61)	0.81	21.4 (3)	16.7 (12)	0.70
BMI >30 kg/m^2, % (# cases)	5.0 (1)	15 (8.6)	1.00	7.1 (1)	1.4 (1)	0.30
Heart rate >80 pulses/min, % (# cases)	25.0 (5)	15.5 (27)	0.34	28.6 (4)	5.6 (4)	0.02
Glycemia (mg/dL), median (10%; 90%)	90.4 (78.0; 103.1)	87.9 (75.3; 104.2)	0.64	90.8 (79.3; 103.5)	88.4 (75.2; 103.6)	0.43
Glycemia ≥ 100 mg/dL, % (# cases)	15.0 (3)	16.1 (28)	1.00	14.3 (2)	15.3 (11)	1.00
Hypertension (HT) and hyperglycemia (≥100mg/dL), % (# cases)	0 (0)	2.9 (5)	1.00	0 (0)	0 (0)	-
Triglycerides (mg/dL), median (10%; 90%)	144.7 (76.4; 293.2)	122.7 (59.2; 268.1)	0.15	139.1 (74.4; 304.9)	101.7 (48.3; 188.1)	0.01
LDL cholesterol (mg/dL), median (10%; 90%)	89.9 (53.5; 138.7)	93.7 (58.7; 135.1)	0.78	96.4 (63.4; 134.0)	91.0 (57.1; 131.8)	0.44
HDL cholesterol (mg/dL), median (10%; 90%)	39.9 (20.7; 56.1)	43.0 (32.2; 60.1)	0.23	42.2 (23.1; 58.4)	47.7 (33.9; 67.8)	0.06
VLDL cholesterol (mg/dL), median (10%; 90%)	28.9 (15.3; 58.6)	24.8 (11.9; 53.6)	0.15	27.8 (14.9; 61.0)	20.3 (9.7; 37.6)	0.01
Hemoglobin (mg/dL), median (10%; 90%) [b]	11.2 (10.5; 14.9)	14.8 (12.5; 16.43	0.001	11.1 (10.2; 13.1)	13.8 (11.9; 15.8)	<0.001
Hb < 13 mg/dL, % (# cases) [b]	75.0 (15)	12.4 (21)	<0.001	85.7 (12/14)	25.4 (17/67)	<0.001
White blood cell count (#/µL), median (10%; 90%) [b]	7600 (6200; 11.790)	7300 (5000; 9500)	0.68	7500 (5750; 12.250)	7200 (4400; 9480)	0.95
High tobacco consumption, % (# cases)	45.0 (9)	20.0 (35)	0.02	41.7 (5)	11.3 (8)	0.02
High alcohol consumption, % (# cases)	10.0 (2)	27.0 (47)	0.10	7.1 (1)	20.8 (15)	0.21
Regular use of nephrotoxic medications, % (# cases)	10.0 (2)	6.3 (11)	0.63	7.1 (1)	5.6 (4)	1.00

*Mann Whitney U test for medians; Chi-square test (Fisher exact test when 1 or more cells with count<5) for categorical data

[a] n.a.: not applicable

[b] 5 missing values for sugarcane cutters due to technical error

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Correction

Wesseling C, Aragón A, González M, *et al.* Heat stress, hydration and uric acid: a cross-sectional study in workers of three occupations in a hotspot of Mesoamerican nephropathy in Nicaragua. *BMJ Open* 2016;6:e011034.

There is a mistake in the layout of Table 2, beginning in Part B. The main categories should be: (A) Current occupational heat stress, (B) Fluid and fructose intake and (C) Work and pesticide use history. The correct table layout is shown below:

	Sugarcane (N=86)	Construction (N=56)	Farming (N=52)	P-value*
A. Current occupational heat stress				
Effective work hours per day (work hours minus breaks), mean±SD	6.5±1.2†	8.1±0.7†	5.3±2.0†	<0.001
Very rapid work pace, %	74.4†	53.6	40.4	<0.001
No shade during breaks, %	20.9‡	1.8‡	11.5	0.004
Lifting weights >50 lbs., %	18.6†	66.1	65.4	<0.001
Awkward work postures, %	58.1	76.8	69.2	0.063
Incentives to cut more cane, %	82.6	–	–	–
Hours post-burning at field entrance, mean±SD	11.7±6.2	–	–	–
Self-reported weight loss on the current job (last two months), %	77.9†	39.3	36.5	<0.001
Fainted at work, %	5.8	0	1.9	0.126
Dysuria (‘chistata’), %	43.0	48.2	44.2	0.827
B. Fluid and fructose intake				
Fluid intake previous day (workday)				
Total fluid (L), mean±SD	6.2±4.1†	4.4±2.1	4.0±2.7	0.003
Water	4.4±3.9†	2.9±2.1	2.8±2.4	0.002
Sugary drinks without electrolyte hydration solution	1.8±1.8	1.5±0.9	1.2±0.8	0.208
Electrolyte solution (N=31)	1.2±1.1	–	–	–
Lowest quartile total fluid (≤2.5 L), %	18.6	19.6	40.4†	0.009
Highest quartile total fluid (≥7.0 L), %	40.7†	8.9	13.5	<0.001
Fluid intake on typical non-work day				
Total fluid (L), mean±SD	4.2±2.3	3.8±1.7	4.1±2.2	0.503
Water	3.0±2.0	2.2±1.3	2.7±2.0	0.053
Sugary drinks	1.2±1.1	1.6±1.1	1.4±1.9	0.117
Fructose intake previous day (workday)				
Total fructose intake (g), mean±SD	103.1±72.1†	80.1±46.1	70.9±36.8	0.008
From food sources	8.4±10.7†	15.9±16.6	17.4±16.7	<0.001
From added sugar	94.7±70.5†	64.2±38.1	53.2±30.7	<0.001
During work hours	58.6±44.7†	28.6±21.4	26.1±16.5	<0.001
Sugary drinks (‘frescos’, sodas, coffee)	22.5±15.7	28.6±21.4	26.1±16.3	0.108
Sugarcane chewing (N=53)	35.0±18.5	–	–	–
Electrolyte solution (N=31)	40.3±35.2	–	–	–
Outside (before and after) work hours	36.1±39.3	35.6±31.4	27.1±25.9	0.350
Highest quartile total fructose intake (>107 g), %	40.7†	19.6	15.7	0.002
C. Work and pesticide use history				
Cumulative time on current job (months), mean±SD	77±60	68±80	116±67†	0.001
Ever sugarcane work, %	100.0†	3.6	3.8	<0.001
	24.4	5.4†	21.2	0.012

Continued

Continued

	Sugarcane (N=86)	Construction (N=56)	Farming (N=52)	P-value*
Ever plantation (other than sugarcane), %				
Ever work in small-scale agricultural (%)	61.6†	25.0†	100.0†	<0.001
Ever construction work, %	5.8	100.0†	11.5	<0.001
Ever any pesticide use, %	46.5†	10.7†	71.2†	<0.001
Glyphosate, %	19.8†	0.0	3.8	<0.001
2,4-D, %	23.3†	0.0†	9.6†	<0.001
Paraquat, %	9.3	3.6	25.0†	0.002
Chlorpyrifos, %	0.0	0.0	23.1†	<0.001
Cypermethrin, %	18.6†	3.6†	42.6†	<0.001

Values are mean±SD unless indicated otherwise.
*p Value for differences between groups: ANOVA for normally distributed continuous variables, Kruskal-Wallis for not normally distributed continuous variables, χ^2 test for categorical variables.
†Significantly different from the other two categories in post hoc tests.
‡Significant difference only between sugarcane cutters and construction workers.

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