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Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador – A cross-shift study of workers at risk of Mesoamerican nephropathy

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A R T I C L E I N F O

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A B S T R A C T

Background: An epidemic of progressive kidney failure afflicts sugarcane workers in Central America. Repeated high-intensity work in hot environments is a possible cause.
Objectives: To assess heat stress, dehydration, biomarkers of renal function and their possible associations. A secondary aim was to evaluate the prevalence of pre-shift renal damage and possible causal factors.
Methods: Sugarcane cutters ($N=189$, aged 18–49 years, 168 of them male) from three regions in El Salvador were examined before and after shift. Cross-shift changes in markers of dehydration and renal function were examined and associations with temperature, work time, region, and fluid intake were assessed. Pre-shift glomerular filtration rate was estimated (eGFR) from serum creatinine.
Results: The mean work-time was 4 (1.4–11) hours. Mean workday temperature was 34–36 °C before noon, and 39–42 °C at noon. The mean liquid intake during work was 0.8 L per hour. There were statistically significant changes across shift. The mean urine specific gravity, urine osmolality and creatinine increased, and urinary pH decreased. Serum creatinine, uric acid and urea nitrogen increased, while chloride and potassium decreased. Pre-shift serum uric acid levels were remarkably high and pre-shift eGFR was reduced (< 60 mL/min) in 23 male workers (14%).
Conclusions: The high prevalence of reduced eGFR, and the cross-shift changes are consistent with recurrent dehydration from strenuous work in a hot and humid environment as an important causal factor. The pathophysiology may include decreased renal blood flow, high demands on tubular reabsorption, and increased levels of uric acid.

Abbreviations: eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; MeN, mesoamerican nephropathy; WBGT, wet bulb globe temperature; HI, heat index; CPK, creatine phosphokinase; NGAL, neutrophil gelatinase-associated lipocalin; NSAIDS, non-steroid anti-inflammatory drugs; BMI, body mass index; OR, odds ratio; SG, specific gravity; RAAS, renin-angiotensin-aldosterone system

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1. Introduction

A devastating and lethal epidemic of chronic kidney disease (CKD) of undetermined origin has been present in the coastal lowlands of El Salvador at least since 1999 (Trabanino et al. 2002). The disease is not limited to El Salvador; it has been documented throughout many areas of the Pacific Ocean's coast, from southern Mexico to Costa Rica (Brooks et al., 2012; Weiner et al., 2013; Ramírez-Rubio et al., 2013b; Wesseling et al., 2014; Correa-Rotter et al., 2014). The disease has been labeled the Mesoamerican Nephropathy (MeN) and is prevalent mainly in male agricultural laborers, especially sugarcane cutters (Correa-Rotter et al. 2014). MeN is initially asymptomatic with limited or no proteinuria, but usually leads to end-stage renal disease. Common risk factors such as diabetes or hypertension are usually absent (Wesseling et al., 2014; Correa-Rotter et al., 2014). The limited data from kidney biposies show tubular atrophy, interstitial fibrosis, and global glomerulosclerosis, often with an ischemic component (Wijkstrom et al., 2013; López-Marín et al., 2014).

Many hypotheses have been proposed to explain the epidemiological, clinical and histopathological pattern of the disease, including pesticides, heavy metals, infections, or other regional environmental factors (Trabanino et al., 2002; Wesseling et al., 2014; Correa-Rotter et al., 2014; López-Marín et al., 2014; Gracia-Trabanino et al., 2005), self-medications (Wesseling et al., 2014; Ramírez-Rubio et al., 2013a), and heat exposure (Peraza et al., 2012; Brooks et al., 2012; Crowe et al., 2013; Wesseling et al., 2014; Correa-Rotter et al., 2014). Currently, repeated high-intensity work in a hot environment with dehydration is believed to be a key causal factor (Johnson et al., 2014), although the specific pathophysiology has not been clarified. Potential mechanisms suggested include subclinical rhabdomyolysis (Paula Santos et al. 2014), hyperosmolality-induced activation of the aldose reductase pathway in the kidney (Roncal Jimenez et al. 2014), vasopressin effects, and effects of hyperuricemia (Knochel and Dotin (1974)).

Little is known about the impact on the kidney of short-term physiological changes of intense work in very hot conditions. We therefore performed a cross-shift study in sugarcane cutters in El Salvador. The overall aim was to contribute to the knowledge about risk factors for MeN in sugarcane workers and potential preventive measures. The specific aims were to assess heat stress and dehydration, as well as biomarkers of renal function and their possible associations with heat stress. A secondary aim was to evaluate the prevalence of pre-shift renal damage and possible causal factors.

2. Methods

2.1. Setting and study design

The study, executed by the Agency for Agricultural Health and Development, AGDYSA, of El Salvador, was conducted in three groups of sugarcane cutters working in different regions in El Salvador: one located in a higher region at about 400 m above sea level, one at medium altitude at about 265 m altitude, and the third in the coastal region at sea level, all cutting cane for the same mill. The workers in the higher region were hired directly by the sugar mill, workers from the coast region were subcontracted, whereas the workers at medium altitude belonged to a cooperative. The a priori hypothesis was that workers in the coastal region would be more affected by heat stress.

The design is cross-sectional (in March/April 2014, at the end of the 6 months sugarcane cutting season), as well as a study of cross-shift changes. Each worker was examined on the same day before work (from about 05:30 AM) and after end of work (usually before noon, but in some cases not until 4 PM). The examinations were performed over 8 workdays, 12–32 workers per day.

2.2. Participants

In each of three regions, one sugarcane field was randomly selected among fields available according to the list of the sugar mill and its associated cooperatives. In each field there were groups of workers (cutting squads). The field supervisors provided lists of cutting squads and their members. In the coastal field, three cutting squads were selected randomly and in the higher altitude field two squads. In the medium altitude field there were only two cutting squads, and both were selected. From the lists of workers in these squads, workers were randomly selected, approached and checked against inclusion criteria until about 30 workers in each squad had been recruited. Inclusion criteria were age less than 50 years, and working as a full time cutter. In total 226 workers <50 years were eligible and approached, and 189 of these agreed to participate and were recruited into the study (84% participation).

All participants signed a written informed consent to participate in the study, in accordance with the Declaration of Helsinki. The study was approved by the National Committee of Ethics for Clinical Research of the Superior Council of Public Health, El Salvador. The participants were aware that temperature and water intake was an important part of the study.

2.3. Work environment

Workers generally wore long pants and long sleeves. Many wore sandals or plastic shoes, while some wore boots. A shin guard was used to protect from machete cuts—other protective gear, including hats, gloves, handkerchiefs, glasses, etc. were rare (Fig. 1). On seven of the eight days, the Wet Bulb Globe Temperature (WBGT) was recorded every half hour during the whole work day in the fields at 1.25 m above the ground using two 800036 WBGT (Sper Scientific, China) handheld devices simultaneously (for quality assurance), and the mean of the results of the two devices was used. Temperature and relative humidity were measured in the fields using a Lascar Electronics data logger (EL-USB-2-LCD equipment, LASCAR, China), and the data were used to calculate the Heat Index (HI) used by the US Occupational Safety and Health Administration (OSHA) (OSHA, 1999; OSHA, 2014). For temperature, WBGT, and HI the means were calculated from the start of work until noon. The workers brought their own water for the work-day, and in the higher field the mill also provided additional water via a water-truck, although many workers did not like the taste or found it to be too far away.

2.4. Medical examinations

Examinations were performed in tents located at the entrance to the sugarcane fields. Trained nurses and doctors from the AGDYSA team used a structured interview to ask workers about use of tobacco, alcohol, prescribed and non-prescribed medications (pictures of drug packages shown), previous work history, including pesticides commonly used in the region, symptoms, and liquid intake on that day. Body weight, blood pressure, pulse rate, and blood (venipuncture) and urine samples were collected before and after the work-day. Liquids for personal intake on the work-day were weighed before and after shift.

Body weight was measured with minimal clothing (underwear) using SECA electronic AD 769 scales (Seca, Birmingham, UK). Blood pressure was measured in the sitting position using a digital system (Omron HEM 7220, Omron Healthcare Inc., Bannockburn, IL, USA), which also recorded the pulse rate.

Fig. 1. Sugarcane cutting (in Nicaragua; Photos Ed Kashi).

The participants were asked to perform their work in the sugarcane fields as they would do on a typical day. At the end of the work-day, all medical examinations and blood and urine collection were repeated, and a post-shift questionnaire was applied about work characteristics during the day such as number of hours worked, liquid intake (amount and type, at home and during work), and amount of cane cut.

2.5. Blood and urine sampling, and biochemical analyses

Blood samples were collected in vacuum tubes, which were centrifuged in the field after 30 min for separation of serum, placed in coolers and transported to a local laboratory where they were aliquoted and analyzed (see below) on the same day. Aliquots were frozen (−20° C) and sent about six weeks later to the University of Colorado in Denver for some duplicate and extra analyses (see below). Urine was collected in sterile polypropylene tubes, and aliquots were collected into cryotubes with and without preservative; one portion was centrifuged in the field and examined in situ (see below) and the rest was transported in the coolers to the local laboratory. At the laboratory urines aliquots were frozen (-20° C) and sent to Denver together with the serum samples, and later to the University of Gothenburg.

Hemoglobin and hematocrit in whole blood were determined by standard routines at a local laboratory as were serum samples for glucose, creatinine, uric acid, sodium, potassium, chloride, magnesium, ionized calcium, and liver aminotransferase (alanine and aspartate) enzymes. Serum butyryl cholinesterase was measured using a cholinesterase (PTC) reagent set (kinetic procedure) from TECO DIAGNOSTICS (TC). In addition serum samples were analyzed at the laboratory in Denver for serum urea nitrogen, and osmolality (freezing point method; Micro-Osmometer), and for creatinine, uric acid, and sodium levels using the VetAce automated biochemistry machine (Alfa Wassermann, West Caldwell, NJ). Uric acid was determined using uricase, and sodium using an ion selective electrode. Creatinine was determined using an alkaline picrate rate method, and adjusted to standardized creatinine, calibrated against creatinine determined by isotope dilution mass spectrometry. Serum urea nitrogen (BUN) was determined using the ACE Alera clinical chemistry system (Alfa Wassermann, West Caldwell, NJ). Serum creatine phosphokinase (CPK) was analyzed in a random subgroup of 30 workers before and after shift using a human ELISA kit (BMASSAYS, Beijing, China). Estimated glomerular filtration rate (eGFR) per 1.73 m^2 of body surface area was calculated using the EPI-CKD formula based on serum creatinine.

Urine samples were analyzed semi-quantitatively in the field with dip-sticks for protein, blood, glucose, and specific gravity (Dirui, Changchun, China), as well as albumin (Cypress Diagnostics, Langdorp, Belgium). Sediments were examined for cells, casts, and crystals. The aliquots shipped to the Denver laboratory were analyzed for creatinine, sodium, total protein, and neutrophil gelatinase-associated lipocalin (NGAL). NGAL was analyzed using a commercial ELISA kit (Abcam, MA, USA), according to the manufacturer's instructions. Specific gravity was measured with a Ceti Digit 012 refractometer (Medline, Oxfordshire, UK), and pH with pH-sensitive electrode (Metrohm AG, Switzerland) at University of Gothenburg, Sweden.

2.6. Statistics

Differences between groups were tested with analysis of variance, *t*-test, Wilcoxon rank sum test (for variables not normally distributed), or Fisher's exact test (for categorical variables). Differences between pre- and post-shift characteristics were tested by paired *t*-tests or by Wilcoxon's signed rank test (for variables not normally distributed). Associations between variables were assessed by multiple linear regression (continuous variables) or logistic regression models (occurrence of reduced eGFR). When assessing the impact of environmental factors on cross-shift changes in blood pressure, blood, serum or urine biomarkers, the same model was used for all outcomes; predictors were WBGT, liquid intake per hour, work-time (in hours), and region. The rationale for this model was that temperature, work time and liquid intake were the main a priori factors believed to have an effect on hydration status and renal function. As WBGT was only measured seven out of 8 days, only 174 out of 189 participants were used in these models. Analyses were performed using SAS V.9.3 (SAS Institute, Cary, NC).

3. Results

Characteristics of the 189 sugarcane cutters are shown in Table 1. The mean age was 30 years, 89% were men, and about half of them never-smokers. Half of them worked as sugarcane cutters for at least five previous harvests. Few had a physician's diagnosis of diabetes ($N=2$) or hypertension ($N=4$). Musculoskeletal pain was relatively common, and many had used acetaminophen and NSAIDS, at least occasionally (Table 1). Differences between workers from the three regions were small.

3.1. Work conditions

Workers generally wore long pants and long sleeves. The mean temperature from start of work until noon varied between 33.7 and 36.0 °C during the seven measurement days and wet bulb

Table 1
Characteristics of the study population of 189 sugarcane cutters in three different sugarcane fields.

	All	Higher alt. (N=55)	Medium alt. (N=41)	Coast (N=93)
Sex, N men/women	168/21	52/3	38/3	78/15
Age, years (mean)	30 (18–49)	30 (18–49)	30 (18–49)	30 (18–49)
Body weight, pre-shift, kg (mean)	64 (45–108)	67 (45–108)	63(45–91)	62 (48–89)
Men	65 (45–108)	68 (45–108)	64 (45–91)	63 (48–89)
Women[a]	60 (48–79)			
Height, cm (mean)	164 (140–182)	167 (150–182)	164 (147–182)	164 (140–179)
BMI	24 (18–36)	24 (18–36)	24 (18–36)	23 (19–34)
Men	23 (18–36)	24 (18–36)	24 (18–38)	23 (18–33)
Women[a]	25			
Current smokers (%)	28	36	20	27
Ex-smokers (%)	23	15	27	27
Alcohol ≥ 7 drinks/week (%)	5	6	7	1
Literate (%)	79	76	78	82
Previous harvests, N (range)	9 (1–38)	7 (1–28)	12 (1–38)	8 (1–34)
Ever used pesticides (%)[b]	89	95	98	83
At home (%)	40	55	32	34
At present harvest (%)	16	27	12	12
Ever used (%)				
Glyphosate	76	84	83	67
Paraquat	82	91	93	73
2,4-D	71	87	76	60
Organophosphates	76	84	85	66
Carbamates	35	20	22	49
Triazines	73	91	85	58
Pyrethroids	59	76	78	39
Captan	2	2	2	2
Hypertension (N)	4	2	0	2
Diabetes (N)	2	1	1	0
Nephrolithiasis (N)	8	1	2	5
Use of NSAIDs (%)[c]	24	27	29	19
Use of acetaminophen (%)[c]	52	64	66	39
Use of aspirin (%)[c]	5	0	5	8
Use of diuretics (%)[c]	11	4	5	18

All items except body weight and height are self-reported.
[a] Data stratified by region not given due to few women.
[b] At work or at home.
[c] Ever used for at least one week.

globe temperature (WBGT) between 24.8 and 33.8. At noon temperature was 39–42 °C, and in the afternoon even higher (maximum 48 °C). The mean heat index (HI) was 98–111 °F, but higher if 15 °F was added, taking into account that work was performed in full sunshine (Fig. 2) (OSHA, 1999; OSHA, 2014). The average work time was about 4 hours, but varied substantially. The mean liquid intake was 0.3 L at breakfast and 3.3 L during work (mean 0.8 L per hour), and about 90% was water. The rest was mainly sweet drinks.

3.2. Pre-shift reduced renal function

Elevated pre-shift serum creatinine was common – in 20% of 168 men S-creatinine was > 1.2 mg/dL, and in one of 23 women > 0.9 mg/dL. In addition to a high prevalence of elevated serum creatinine, elevated serum uric acid was also common; 27% of the men had a level above 7.0 mg/dL. Twenty-three workers, all men (14%), had eGFR < 60 ml/min/1.73 m^2. If not temporary, this fulfills criteria for chronic kidney disease (National Kidney Foundation, 2002; KDIGO 2012). Three workers had severely reduced eGFR

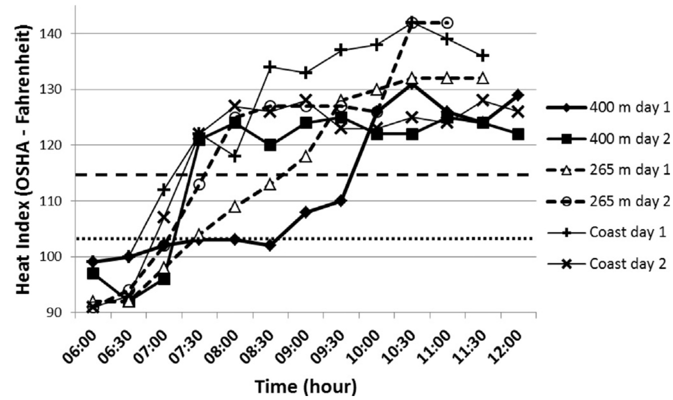

Fig. 2. Heat index (HI, in °F) calculated each 30 minutes at work in sugarcane fields from start of work until noon on six workdays in March-April 2014 in three different regions. 15 °F was added since work was performed in full sunshine (OSHA, 1999; OSHA, 2014). At a heat index above 103°F (dotted straight line) OSHA considers the workers' risk is “high” and at a heat index above 115 °F (dashed straight line) the risk is considered “very high to extreme”.

(< 30 mL/min), Table 2. Workers with reduced eGFR were slightly older (mean 34 years) but the prevalence of smoking, self-reported previous disease, and use of medications or alcohol was unremarkable. None had diabetes, and only one reported hypertension. A few had proteinuria, or suspected microalbuminuria (Table 3). As expected, mean levels of serum urea nitrogen ($P < 0.001$), serum uric acid ($P < 0.001$) and urinary neutrophil gelatinase-associated lipocalin (NGAL) ($P=0.02$) were higher than for other workers. Serum urea nitrogen was abnormal (> 23 mg/dL) in 13 cases, and uric acid (≥ 7 mg/dL) in 21 cases (91%). In a backwards logistic regression model, including age, BMI, smoking, kidney stones, hypertension, use of NSAIDS, diuretics, number of previous harvests, any use of pesticides, and region, the only significant predictors of reduced eGFR were age (OR 1.09 per year, 95% CI 1.02–1.16, $P=0.008$) and region (coastal versus the other two regions combined, OR 3.5, 95% CI 1.3–9.4, $P=0.01$). Self-reported use of carbamate pesticides was more common among the workers with reduced eGFR (reported by 74% versus 29% among remaining workers), and a predictor in the regression model if replacing “any use of pesticides”.

3.3. Cross-shift changes in hydration and cardiovascular parameters

Some workers lost weight during the workday, but some gained weight, and the mean change during the workday was close to zero (Table 4). However, the change was positively associated with the estimated liquid intake per day ($P < 0.001$). In a multivariable model including region and WBGT, one extra liter of fluid increased post-shift body weight with about 0.5 kg ($P=0.008$).

Urinary osmolality and urinary specific gravity (SG) increased substantially across shift, while serum osmolality did not change. The mean SG was 1.016 in pre-shift and 1.020 in post-shift samples (Fig. 3, Table S2). Post shift SG tended to be lower ($P=0.06$) with increased liquid intake per hour in a multivariable model including also region, work-time, and WBGT. Serum sodium increased slightly, while potassium and chloride fell significantly (Fig. 3, Table S1).

As expected, the pulse rate was higher post-shift than pre-shift. The mean blood pressure decreased, however ($P < 0.001$) (Fig. 3, Table S1). In a multiple linear regression model including region, WBGT, work time and liquid intake per hour, the decrease of diastolic blood pressure was smaller at higher WBGT ($P=0.007$).

Hemoglobin and hematocrit fell 3–5% (Fig. 3, Table S1). In a multivariable model including region, WBGT, work time, and

Table 2
Pre-shift serum creatinine and estimated glomerular filtration rate (eGFR) based on the CKD–EPI equation (Levey 2009).

	All	Higher alt. (N=55)	Medium alt. (N=41)	Coast (N=93)	P-value difference between regions
S-creat (mg/dL)	1.07 (0.6–4.1)	1.09 (0.62–3.9)	0.88 (0.62–2.26)	1.13 (0.65–4.11)	0.04
e-GFR mL/min/1.73 m^2	101 (17–135)	102 (19–131)	113 (38–135)	96 (17–131)	0.004
Reduced eGFR					
e-GFR <30 (N)	4	2	0	2	
$30<$e-GFR<60 (N)	19	4	2	13	
Total, N (%)	23 (12)	6 (11)	2 (5)	15 (16)	0.18

liquid intake per hour, the decrease of hemoglobin (P=0.01) and hematocrit ($P<0.001$) was larger in the coastal region. High WBGT was associated with a decrease of hemoglobin ($P<0.001$) but an increase in hematocrit (P=0.02).

3.4. Cross-shift changes in biomarkers related to renal function

There were significant cross-shift increases (about 10%) in serum creatinine, urea nitrogen, and uric acid. Prevalence of elevated S-creatinine increased from 20% pre-shift to 25% post-shift, indicating a drop in GFR. Serum uric acid was elevated (> 7 mg/dL in men and > 6 mg/dL in women) pre-shift in 26% of workers, and post-shift in 43%. Urinary NGAL decreased over shift. There was a marked increase of mean urinary creatinine from 1.1 to 1.9 g/L (Fig. 3, Table S2). The excretion of sodium decreased as did urinary pH. Urinary sediment showed elevated numbers of leukocytes in about 10% of the workers. Erythrocytes, granular casts, and leukocyte casts (> 2) were found in a few workers before or after shift; none had erythrocyte casts. Urate crystals were found in 16% of the workers pre-shift and in 22% in the more concentrated and acidic post-shift urine.

In a multivariable model including region, WBGT, work time, and liquid intake per hour, there was a significant association between WBGT and post-shift increase in S-creatinine (about 2% increase per degree of WBGT, $P<0.001$). There was also a significant impact of region (P=0.008), with a smaller increase in the coastal area. There was a borderline protective effect of liquid intake (0.06 mg/dL decrease of S-creatinine for each dL per hour of liquid intake, P=0.07). If cases with reduced eGFR were excluded, the effect of liquid intake became significant (P=0.03), but not the effect of region.

One third of the male workers (N=42) lost > 0.5 kg of body weight and were compared with male workers who lost less weight or gained weight (Table 5). Those who lost weight had a larger increase in serum osmolality, sodium and creatinine, as well as urinary creatinine.

The increase in S-creatinine was significantly larger ($P<0.001$) in the 23 workers with reduced eGFR in a multivariable model taking region, WBGT, liquid intake per hour, and work-time into account. This was also the case for the increase in serum uric acid (P=0.009), but not for serum urea nitrogen, which was essentially unchanged, while it increased for the other workers (P=0.03). Workers with reduced eGFR had lower urine specific gravity and osmolality than the other workers pre-shift (P=0.006) and post-shift ($P<0.001$). They had also lower urinary pH pre-shift (P=0.01) and post-shift (P=0.009).

If sex was added to models for cross-shift changes of biomarkers, results were similar (data not shown). The cross-shift decrease of systolic blood pressure and urinary pH was, however, larger among women.

Table 3
Characteristics in 23 cases of probable[a] chronic kidney disease. Estimated GFR based on preshift serum creatinine and the CKD–EPI equation (Levey et al. 2009).

	Age	eGFR[a]	S-crea[b] mg/dL	BUN[b,c] mg/dL	S-UA[b,d] mg/dL	BP[b] mm Hg	Hb g/L	U-prot[b] mg/L	U-Alb[be] > 30 mg/L	BMI	Smoking	Stones	HT	NSAIDS ever	Diuretics ever	Harvests N	Region
1	40	17	4.1	36	11.2	163/91	142	647	Yes	27	Never	No	No	No	No	8	Coast
2	37	19	3.9	38	13.6	140/80	147	778	No	20	Never	No	No	No	No	24	Higher
3	40	19	3.7	40	12.0	127/85	124	338	No	19	–	No	No	No	No	2	Higher
4	25	19	4.0	25	7.2	117/74	117	310	Yes	23	Never	No	No	No	No	10	Coast
5	49	32	2.3	45	12.3	126/68	145	160	No	18	Former	No	No	Yes	No	20	Higher
6	27	35	2.5	31	12.6	109/76	117	141	Yes	23	Current	No	No	No	No	3	Coast
7	25	36	2.4	35	8.7	132/76	142	329	No	23	Current	No	No	No	Yes[d]	2	Coast
8	48	37	2.0	25	9.6	134/66	111	154	No	20	Former	No	No	No	No	34	Coast
9	30	38	2.3	29	6.8	124/62	102	99	No	24	Current	No	No	No	No	4	Medium
10	43	38	2.1	21	12.3	120/70	146	111	Yes	27	Never	No	No	No	No	26	Coast
11	23	38	2.3	18	9.5	127/64	117	250	No	19	Current	No	No	No	No	7	Coast
12	46	40	2.0	18	8.8	142/64	140	36	No	23	Former	No	No	No	Yes[d]	5	Coast
13	27	41	2.1	20	10.5	169/82	129	55	No	21	Current	No	No	No	Yes[d]	5	Coast
14	38	42	2.0	26	8.9	114/64	129	176	No	27	Never	No	No	Yes	No	10	Higher
15	28	47	1.9	26	9.4	110/62	130	123	No	24	Never	No	No	No	No	20	Higher
16	28	48	1.9	20	8.0	110/62	133	257	No	29	Never	No	No	No	No	16	Coast
17	28	50	1.8	15	7.0	134/81	120	108	No	24	Current	No	No	Yes	No	12	Coast
18	29	52	1.7	28	7.7	113/57	121	74	No	26	Current	No	No	No	No	6	Higher
19	30	55	1.6	15	5.8	134/82	133	79	No	22	Never	No	No	No	No	6	Coast
20	36	56	1.6	15	8.5	131/62	133	128	No	24	Current	No	No	No	No	15	Medium
21	34	58	1.5	16	7.9	98/52	131	8	No	24	Former	Yes	Yes	No	No	22	Coast
22	38	58	1.5	22	9.8	138/78	129	15	Yes	26	Former	No	No	Yes	No	3	Coast
23	22	59	1.6	16	11.4	111/63	138	79	Yes	19	Never	No	No	No	No	4	Coast

[a] The diagnose of CKD can only be concluded when eGFR is reduced for > 3 months.
[b] in the morning before work.
[c] Serum urea nitrogen.
[d] Uric acid.
[e] only semi-quantitative, none had > 300 mg/L.

Table 4
Work conditions, fluid intake, and cross-shift changes in body weight, pulse rate, and blood pressure (Systolic=SBP, Diastolic=DBP) over a work-day in 189 sugarcane workers in three different sugarcane fields. Mean (range) or number (*N*) is given. Temperature data are based on measurements over 2 work-days in the high altitude region, 2 days in the medium altitude region, and 3 days in the coastal region.

	All	Higher alt. (N=55)	Medium alt. (N=41)	Coast (N=93)	*P*-value change overall	*P*-value difference between regions
Time start of work	6:27 (5:43–7:40)	6:34 (5:46–7:40)	6:17 (5:43–6:47)	6:28 (5:50–6:59)		
Time end of work	10:40 (7:40–16:45)	12:43 (7:40–16:45)	9:07 (7:45–15:30)	10:08 (8:30–15:40)		
Work hours (h)	4.13 (1.4–10.7)	6.1 (1.4–10.7)	2.8 (1.4–9.1)	3.7 (2.1–9.5)		< 0.001
Cane cut (tonnes/h)	0.9 (0.1–2.0)	0.8 (0.2–1.9)	1.2 (0.3–2.5)	0.8 (0.1–1.7)		< 0.001
Mean temperature[a](°C)		34.2	33.7	35.5		
Mean WBGT (°C)[a,b]		25.2	27.0	28.8		
Mean Heat Index[a,c]		98	102	104		
Liquid intake total (L)[d]	3.6 (0.25–10)	4.8 (1.2–10)	2.5 (0.3–5.1)	3.3 (0.25–6.8)		< 0.001
Water	3.1 (0–9)	4.2 (0.25–9)	2.2 (0.2–4.4)	2.9 (0–6.6)		< 0.001
Sweet drinks	0.4 (0–1.5)	0.5 (0–1.5)	0.2 (0–0.9)	0.4 (0–1.3)		< 0.001
Liquid intake, L/hour	0.8 (0–2.0)	0.7 (0.3–1.2)	0.8 (0–1.4)	0.9 (0.1–2.0)		0.05
Chewing sugarcane (*N*)	28	14	10	4		< 0.001
Body weight start (kg)	64.1(44.4–108)	67.3 (45.1–108)	64.6 (45.4–90.8)	62.0 (38.1–89)		0.01
Weight change (kg)	0.0 (–2.5 to 2.3)	0.1 (–1.4 to 1.8)[e]	–0.7 (–2.5 to 0.5)	0.2 (–2.5 to 2.3)	0.52	< 0.001
Pulse rate Pre	67 (40–107)	65 (42–97)	70 (49–100)	67 (40–107)		0.09
Pulse rate change	17 (–25 to 54)	20 (–9 to 54)	14 (–25 to 45)	16 (–14 to 52)	< 0.001	0.07
SBP Pre	124 (93–169)	120 (95–160)	116 (81–133)	126 (97–169)		0.03
SBP change	–8 (–42 to 35)	–5 (–32 to 22)	–10 (–35 to 17)	–9 (–42 to 35)	< 0.001	0.06
DBP Pre	70 (41–99)	68 (45–95)	70 (41–98)	72 (52–99)		0.20
DBP change	–5 (–32 to 57)	–4 (–32 to 16)	–2 (–24 to 57)	–6 (–29 to 16)	< 0.001	0.14

[a] Between 6 AM and noon.
[b] According to local laws 3 degrees should be added to these values if work was performed in full clothes.
[c] According to OSHA rules 15 °F should be added to these values when work is performed in the sun.
[d] From breakfast at home until end of work. Includes 0.3 L at breakfast and 3.3 L at work (means).
[e] Most of these workers had lunch at the work-place.

3.5. Other biomarkers

None of the workers showed cholinesterase inhibition before or after the work shift (data not shown), which was expected since they did not use organophosphates or carbamates on that day. Liver aminotransferase enzymes showed mainly normal levels. Levels of serum CPK (only a subgroup of 30 workers) were normal with no significant cross-shift change.

4. Discussion

This study of 189 sugarcane cutters from El Salvador showed a high prevalence of reduced kidney function. The pre-shift eGFR was reduced (< 60 mL/min) in 14% of male workers. Substantial cross-shift changes were noted for blood pressure as well as for kidney-related serum and urine biomarkers involved in maintaining water and electrolyte balance, and for serum uric acid. The association between the cross-shift increase in serum creatinine and temperature and liquid intake provides some empirical support to the hypothesis that kidney damage in MeN is caused by recurrent dehydration and strenuous work in a hot and humid environment. In addition, serum uric acid levels were much higher than expected (mean 6.5 mg/dL; higher than in general population).

The prevalence of reduced eGFR (14% in males) is consistent with findings in population-based cross-sectional studies in coastal Nicaragua and El Salvador (Torres et al., 2010; Sanoff et al., 2010; O'Donnell et al., 2011; Orantes et al., 2011; Peraza et al., 2012; Herrera et al., 2014), except for one study with a prevalence of 40% in men 20-49 years (Raines et al., 2014). The present study was performed exclusively in active sugarcane workers. Therefore one might expect a higher prevalence of reduced eGFR, but we included only individuals aged < 50 years, and healthy enough to work as a cane cutter. In a study of cane cutters in Nicaragua at late harvest, only 3/54 (6%) had reduced eGFR, but in that study workers with elevated S-creatinine were not hired at start of harvest (Laws et al., 2015).

Age, male sex, and coastal region (where temperature and humidity is higher) were risk factors for reduced eGFR, in agreement with previous studies. Cases of reduced eGFR occurred, however, also at somewhat higher altitudes (Table 3), and could not be explained by well-known risk factors such as diabetes, hypertension, or nephrotoxic medications. Previous use of carbamate insecticides was more common among the 23 workers with reduced eGFR, but we had no a priori hypothesis regarding this specific chemical group of pesticides. It should, however, be examined further. Interestingly, elevated serum uric acid levels were very common, even more common than in patients starting kidney replacement therapy (Suliman et al., 2006).

Work as a sugarcane cutter is hard and repetitive, particularly using a machete, and walking carrying the cut cane. Short breaks are taken, but still under the sun. It is likely that part of the work, engaging arms to a large extent, will be anaerobic, resulting in increased lactate formation (McArdle et al., 1991). Although the results showed some indications towards stronger effects in the coastal region, the differences were smaller than we had anticipated. The reason for this may be that within a group of workers from a specific region there are large differences in work load, water intake, and individual factors. In addition, as shown in Table 4, although the WBGT tended to be lower in the highest altitude region, the mean number of work hours was longer in this region.

The mean temperatures and WBGT were very high already in the morning. According to the US OSHA, there is need for rest about 50% of the time when WBGT exceeds 28 °C, and 75% of time when it exceeds 30 °C, to avoid increased core body temperature (OSHA, 1999). Workers did not take such breaks. If full sunshine is taken into account, the mean HI was 110–125 °F (Fig. 2), which requires aggressive protective measures (OSHA, 1999; OSHA, 2014). At these conditions the only way to counteract heat production and maintain core body temperature is by evaporation

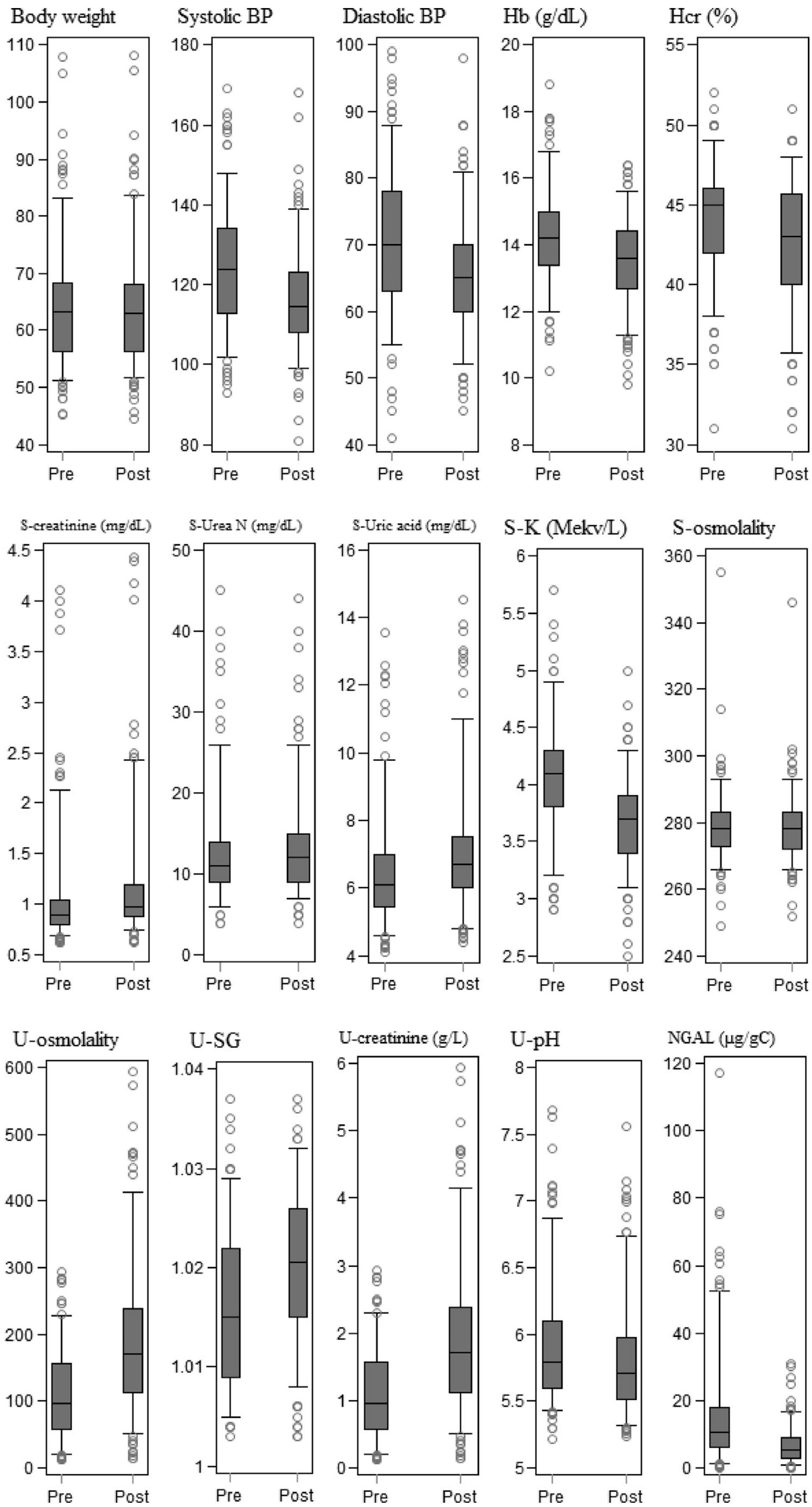

Fig. 3. A-C Cross-shift changes in selected outcomes in 189 sugarcane workers examined before and immediately after work. The boxes depict medians and 25th and 75th percentiles, and outliers are shown. All changes except for body weight and serum osmolality are statistically significant ($P < 0.001$).

Table 5
Characteristics and cross-shift changes in selected biomarkers in male workers with eGFR > 60 mL/min who lost > 0.5 kg bw, and workers who lost less weight or gained weight. For other biomarkers changes there were no significant differences between the two groups. For comparison also results for the 23 workers with eGFR < 60 mL/min are shown.

	Weight loss (> 0.5 kg) $N=47$	No weight loss $N=95$	*P*-value	eGFR < 60 mL/min $N=23$
Δ Weight (kg)	−1.0 (−2.5 to 0.6)	0.3 (-0.5–2.3)	–	0.2 (−2.5 to 1.4)
Age	30 (18–46)	28 (18–49)	0.07	34 (22–49)
Work hours (h)	3.7 (1.4–11)	4.7 (1.6–10)	0.001	3.7 (1.4–7.8)
Cane cut (tonnes/h)	1.1 (0.4–2.5)	0.9 (0.1–1.9)	0.04	0.9 (0.5–1.5)
Mean WBGT	27.5 (25–34)	27.0 (25–34)		29.2 (25–34)
Liquid intake (L/h)	0.8 (0–1.7)	0.8 (0.1–1.6)	NS	0.8 (0.1–2.0)
Δ Hematocrite	−4.0 (−0.6 to 4.0)	−1.5 (−5 to 1.0)	0.001	−2.3 (−11 to 1.0)
Δ S-osmolality (mosm/L)	2.0 (−16 to 19)	−1.2 (−20 to 23)	0.03	0.91 (−13 to 13)
Δ S-creatinine (mg/dL)	0.12 (−0.1 to 0.4)	0.09 (−0.3 to 0.7)	0.04	0.24 (−0.05 to 0.9)
Δ S-sodium (mmol/L)	2.9 (−2 to 8)	−0.2 (−10 to 5)	< 0.001	1.1 (−6 to 7)
Δ S-calcium (mmol/L)	0.33 (−0.52 to 0.98)	0.54 (−0.73 to 1.85)	0.04	0.34 (−0.43 to 1.1)
pH Pre	5.8 (5.4–6.7)	6.0 (5.2–7.7)	0.02	5.7 (5.3–6.5)
Δ U-pH	−0.12 (−1.0 to 1.0)	−0.08 (−1.9 to 1.3)	NS	−0.11 (−1.1 to 0.3)
Δ U-creatinine (g/L)	1.1 (−1.5 to 4.5)	0.74 (−1.0 to 3.2)	0.06	0.89 (0.03–2.5)

(sweating) (Lucas, 2013; McArdle et al., 1991). The heat loss by sweating is, however, decreased when air humidity is high, and by clothing.

The mean liquid intake (0.8 L per hour of work) seemed to be enough to maintain body weight and serum osmolality (Table 4, Table S1), but only at expense of strong demands on renal reabsorption of water by the kidney, and only for about half of the workers. Considering the strong cross-shift effects on renal physiology and the significant association between change in body weight and liquid intake, our interpretation is that liquid intake should be higher than 0.8 L per hour under these work conditions. Sweating during running typically results in loss of 0.5–2 L per hour, depending on speed and weather conditions (McArdle et al., 1991; Kratz et al., 2002; Junglee et al., 2013). In many workers part of the water intake occurred at the end of the work-shift, while ideally frequent water intake should be ascertained during work or breaks. Thus there may well have been some loss of body weight during work for most workers. Nevertheless, one third of workers lost > 0.5 kg of body weight, and showed also other signs of dehydration (Table 5). High water intake may, however, also require replacement of salts, in order to avoid hyponatremia.

Work in a hot environment will redistribute blood flow to muscles, and to skin in order to increase heat loss. This will decrease blood flow to visceral organs such as intestines and the kidneys. Hard work, even in normal temperature, will reduce renal blood flow by about 50% (Astrand et al., 2003). Sweating will cause loss of water, sodium and chloride. Together with redistribution of blood flow, this will cause a decrease in central blood volume, which we interpret as the cause of the decrease in post-shift blood pressure (Table 4). Since work conditions favored dehydration, we had expected hemoconcentration with increased hematocrit and hemoglobin, but found the opposite (Fig. 3, Table S2). Hemoconcentration occurs in parallel to loss of body weight during exercise in a hot environment, but hematocrit returns rapidly at recovery, also without hydration (Harrison et al., 1975). In these workers, who had access to water, there was probably an influx of interstitial fluid to plasma after work, as a physiologic response to the vasodilation in skeletal muscles and skin.

Loss of water by sweating and decrease of central blood volume will activate the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS), resulting in release of the antidiuretic hormone (vasopressin) and aldosterone. This causes a strong increase of renal tubular reabsorption of water, sodium, and urea, as indicated by the significant increase in U-osmolality, U-SG, and U-creatinine (Fig. 3, Table S2). The aldosterone effect saves sodium, as shown by the decrease in urinary sodium excretion rate (lower post-shift creatinine-adjusted U-Na). Aldosterone also increases the excretion of potassium (decreased S-K, Fig. 3). It is likely that the loss of sodium by sweating was not balanced by the decrease in urinary sodium, and the fact that serum sodium remained unchanged may be due to influx of sodium from extracellular fluid.

The increase (about 10%) in serum levels of creatinine, urea nitrogen, and uric acid is probably an effect of reduced GFR during work (due to decreased renal blood flow, and reduced filtration pressure). Increased breakdown of muscle creatine and purine could also contribute to this. The fall of serum glucose is expected due to glucose consumption at prolonged muscular effort.

We have found only one previous published study examining cross-shift changes in sugarcane cutters; in a small study of 27 cane cutters in Brazil, all had normal pre-shift S-creatinine, which increased by 0.2 mg/dL over an 8 h shift, in agreement with our results (Paula Santos et al., 2014).

The high prevalence of elevated serum uric acid (26% pre-shift and 43% post-shift) is remarkable. The effect of aldosterone and the lactate formation decrease urinary pH (Fig. 3, Table S2). Interestingly, urate crystals were found in a relatively large fraction of workers–pre-shift as well as post-shift. Concentration and acidification of urine in combination with increased serum levels of urate, may increase formation of urate crystals, a condition which has recently been proposed (Roncal-Jimenez, submitted manuscript) as a causative factor in MeN.

The present study provides the first detailed data showing the extent of heat stress in sugarcane workers in a region with high prevalence of MeN. The concentrated post-shift urine suggests a repeated heavy load on renal tubular reabsorption. This may be compatible with histopathological findings of tubular atrophy and interstitial fibrosis in cases of MeN (Wijkstrom et al., 2013; López-Marín et al. 2014). The redistribution of blood to muscles and skin indicates that renal blood flow is much reduced during work, as also supported by cross-shift increase of serum creatinine and urea nitrogen, indicating reduced GFR during work. This may be one factor behind glomerulosclerosis found in MeN kidney biopsies (Wijkstrom et al., 2013; Lopez-Marín et al., 2014). Another factor could be the increase of serum uric acid; animal studies have shown that hyperuricemia can cause renal arteriolar constriction, and eventually glomerular hypertension (Kang et al., 2002, Sanchez-Lozada et al., 2005). Acidification of urine, and relatively high prevalence of urate crystals in the sediments, support the hypothesis that urate microcrystals could be a causative factor in MeN. The finding that self-reported previous use of carbamates was more common among cases with reduced eGFR should be addressed in larger studies of MeN.

The detailed measurements of temperature, and the comprehensive physiological data and biomarkers examined before and after a work-shift make the present study unique. Cutting squads and workers were randomly selected, and no pre-employment screening of kidney function is performed in this area, which increases the validity. Interview data on previous work, medications, and liquid intake on the workday were detailed. The study also has several limitations. It would have been an advantage to examine cross-shift changes also in a control group working in hot climate with low physical work load, or a group with similar tasks but working in a less hot climate. The pre- and post-shift sampling was only performed on one day per worker. It is likely that the workers drank more than they usually do, since they were aware that temperature and water intake was an important part of the study. True serum osmolality may have been higher due to effects of freezing and thawing of samples (Bohnen et al., 1992), but the cross-shift change should be unbiased. Other limitations include the semi-quantitative measurements of urinary albumin, and the absence of more detailed data on the timing of use of medications (ever used for $>$ one week). We used the CKD-EPI equation which is preferable at near normal GFR (Tent et al., 2010; Murata et al., 2011), and creatinine concentrations were calibrated against creatinine determined with isotope dilution mass spectrometry. Regarding external validity it should be noted that work conditions may differ between countries, and even between mills within the same country.

Given that the main causal hypothesis is that repeated high-intensity work in a hot environment causes irreversible kidney damage, the high WBGT and the indications of a decrease of GFR during work suggest that work practices must be improved with more frequent breaks, access to shade during breaks, larger intake of water, and probably also salt. The mean intake of 0.8 L/h in the present study was enough for maintaining body weight, but not for protecting the kidneys from a heavy load on tubular reabsorption and from reduced glomerular filtration.

This study suggests that workers with pre-shift reduced eGFR seem to be more sensitive to further reduction of GFR, as their serum creatinine increased more across shift than it did in other workers. It is, however, desirable that the work environment should be good enough to allow workers with reduced kidney function to take part in sugarcane work, which is often the only job alternative.

In conclusion, the present study demonstrates the very hot environment for El Salvadoran sugarcane cutters, and substantial cross-shift changes indicating a heavy load on the kidney to counteract dehydration. There is a strong need for preventive measures, which may be as simple as the provision of water, rest, and shade.

Ethics review

All participants signed a written informed consent to participate in the study, which was approved by the National Committee of Ethics for Clinical Research of the Superior Council of Public Health, El Salvador.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2015.07.007.

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