**Kidney function in sugarcane cutters in Nicaragua – a longitudinal study of workers at risk of Mesoamerican nephropathy**

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**Abbreviations:** BMI = Body mass index. CKD = Chronic kidney disease. eGFR = estimated glomerular filtration rate. KIM-1 = Kidney injury molecule 1. MeN = Mesoamerican nephropathy. NGAL = neutrophil gelatinase-associated lipocalin. Hsp72 = Heat shock protein 72 kD. NSAIDS = Non-steroid anti-inflammatory drugs. RAAS = renin-angiotensin-aldosterone system.

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**Abstract**

**Background.** Chronic kidney disease is common among sugar cane workers in Central America. The main risk factor seems to be repeated high-intensity work in hot environments. Several cross-sectional studies have been performed but few longitudinal studies.

**Objectives.** The aim of the study was to examine whether kidney function changes over a few months of work during the harvest period.

**Methods:** A group of male sugarcane cutters in Nicaragua (N=29, aged 17 – 38 years) was examined with renal biomarkers before and after shift on the first day at the start of harvest, on the sixth day during acclimatization, and then in mid-harvest 9 weeks later. A reference group (N=25, mainly office workers) was examined with the same biomarkers at start of harvest, and then at end of harvest 5 months later.

**Results:** The pre-shift renal function decreased significantly during 9 weeks of work in the cane cutters. Mean serum creatinine increased (20%), mean estimated glomerular filtration rate decreased (9%, 10 mL/min), serum urea N (BUN) increased (41%), and mean urinary neutrophil gelatinase-associated lipocalin (NGAL) increased (four times). The cane cutters also developed cross-shift increases in these biomarkers, in particular serum creatinine and BUN, and in urinary uric acid. The longitudinal decrease in eGFR tended to be associated with the cross-shift increase in serum creatinine.

**Conclusions:** There was a remarkable decrease of glomerular kidney function, after only 9 weeks of harvest. The cross-shift increase in serum creatinine may be caused by dehydration (pre-renal dysfunction), and when repeated on a daily basis this may cause permanently reduced GFR.

**1. Introduction**

The recognition of Mesoamerican Nephropathy (MeN) - also labelled Chronic Kidney Disease of non-traditional origin (CKDnt) - as an epidemic in Central America, has led to the publication of a number of reports examining risk factors and causal hypotheses (Wesseling et al. 2014, Correa-Rotter et al. 2014). Experimental studies have also been performed (Roncal-Jimenez et al. 2014).

To date the findings have shown that the epidemic primarily affects males working in heavy manual labor in hot environments, mainly living in the coastal lowlands. There is generally no history of diabetes or hypertension and no substantial proteinuria. Kidney biopsies have shown a tubulointerstitial pattern with tubular atrophy and interstitial fibrosis, but also global glomerulosclerosis, often with an ischemic component (Wijkström et al. 2013, López-Marín et al. 2014).

The main hypothesis to account for the disease is heat stress with repetitive episodes of dehydration (Peraza et al. 2012, Brooks et al. 2012, Wesseling et al. 2014, Correa-Rotter et al. 2014, García-Trabanino et al. 2015). Suggested pathophysiologic mechanisms driven by strenuous work and heat stress include subclinical rhabdomyolysis (Paula Santos et al. 2014), effects of hyperuricemia and hyperuricosuria (Knochel et al. 1974, Johnson et al. 2015, Roncal-Jimenez et al. 2015a, 2015b), hyperosmolality-induced activation of the aldose reductase-fructokinase pathway in the kidney, and vasopressin effects (Roncal Jimenez et al. 2014, Roncal-Jimenez et al. 2015a, 2015b). It has also been proposed that the disease is multifactorial, and could include additional factors such as self-medication with nonsteroidal anti-inflammatory drugs, exposure to heavy metals or pesticides/agrochemicals, infections, or genetic factors (Correa-Rotter et al. 2014, Herrera et al. 2014, Laws et al. 2015a, 2015b, Ramirez-Rubio et al. 2015, Wesseling et al. 2015).

Most of the epidemiological studies are cross-sectional population based surveys. Two studies have examined cross-shift changes in biomarkers of hydration and kidney function, over a workday in Brazil (Paula Santos et al. 2014), and El Salvador (García-Trabanino et al. 2015), and two studies have performed a follow-up of kidney function among sugarcane cutters over the course of a harvest season, in Brazil (Paula Santos et al. 2014) and in Nicaragua (Laws et al. 2015a, 2015b). While the two studies on cross-shift changes both show a decrease in renal function over a cane cutting shift, the two longitudinal studies were not in agreement, and the question of whether the pre-shift glomerular function changes over a harvest period of several months still remains unclear. The aim of the present study was to assess longitudinal changes of kidney function over a harvest period in sugarcane cutters as well as in a reference group. We examined pre- and post-shift kidney function in sugarcane cutters at the start of harvest, on day 1 and day 6 to assess acclimatization effects, and at mid-harvest two months later. A reference group of non-cane cutters was examined at start and end of the harvest.

**2. Methods**

**2.1 Setting and study design**

The study was conducted in 2012-2013 in a convenience sample of 29 sugarcane cutters from León and Chinandega municipalities in the northern Nicaraguan Pacific region, and a reference group (N=25, mainly office workers) from the same area. The sugarcane cutters were examined “pre-shift” in the morning between 3 and 5 am and “post-shift” between 4 and 7 pm at their homes on the first day of the harvest in November 2012. The pre-shift examination on the first day (called Cut1) was considered to be the baseline. The examinations (pre- and post-shift) were repeated after 5 days of work (Cut2), and then 9 weeks later in January 2013 (in the mid-harvest period; Cut3). The first (“pre-shift” 7 – 9 am and “post-shift” 4 – 6 pm) examination of the reference group was at their work places in November 2012 (Ref1), while the repeated examination was performed at the end of the harvest season in May 2013 (Ref2), again at their workplaces. Originally, a fourth examination of the cane cutters was planned for the end of the harvest in May 2013, together with the reference group, but due to removal of participating workers from their jobs in February 2013, this could not be realized, apart from a small number (n=7) of post-shift urine samples.

**2.2 Participants**

Community leaders of six villages in the municipalities of León and Chinandega provided lists of men who planned to work as sugarcane cutters. Information meetings were held with these workers in their communities. An invitation to provide blood and urine for a screening test was made to those who were confirmed to be enrolled as sugarcane cutters at plantations of the mill in the study area. The aim was to recruit young healthy men. In total 92 sugarcane cutters <40 years of age participated in the screening test (blood tests for glucose, creatinine, uric acid, lipids, cell count, as well as a urine test with dip-stick and examination of sediment). These analyses were performed in the laboratory of the Medical School of UNAN-Léon. In 45 of them serum creatinine was ≥1.1 mg/dL, the strict pre-set exclusion criterion. Another 15 had abnormal results in at least one of the other tests, or a history of diabetes or hypertension. Thus only 32 workers met the inclusion criteria (<40 years of age, serum creatinine ≤1.0 mg/dL and all other lab tests within the reference values; in addition no known diabetes, hypertension or kidney disease). Three men decided not to participate, leaving 29 subjects for the study.

A reference group without known diabetes, hypertension or kidney disease was recruited at the town halls near the sugarcane plantations. The group included mostly office workers, but also five persons with a predominantly outdoor job, albeit without major physical effort.

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 Ethical Review Board of UNAN-León, Nicaragua, and the Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico.

The work conditions were similar to those described previously for sugar cane cutting in this region (Crowe et al. 2015, García-Trabanino et al. 2015).

**2.3 Medical examinations**

Blood pressure was measured by a technician with a calibrated digital sphygmomanometer (Omron BP710N, Omron Healthcare Inc., Bannockburn, USA) with the participant seated after resting for at least 10 minutes. Body weight was measured with a calibrated Seca 803 digital flat mobile scale (Seca, Birmingham, UK) with minimal clothing and height with a foldable stadiometer (Seca, Birmingham, UK). Certified technicians collected blood samples in three vacuum tubes (Becton, Dickinson & Co., USA), one tube with anticoagulant for blood cell count and two tubes with clot activator and gel for serum separation. All samples were placed on ice and transported immediately to the laboratory at the Research Center on Health, Work and Environment (CISTA) at UNAN-León, where they were centrifuged at 3500 RPM for 10 minutes at room temperature and the serum was separated into four labeled cryovials and stored at -80oC.

Each participant delivered a spot urine sample (50 cc) in a sterile polypropylene container (Becton, Dickinson & Co., USA), which was aliquoted into two vacuum tubes with and without (two tubes) preservative immediately at the participant’s home, placed in an icebox (4 oC) and then transported to the laboratory at the Research Center on Health, Work and Environment (CISTA) at UNAN-León, where aliquots were frozen at -80oC. Serum and urine aliquots were later sent to the Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubiran, Mexico (about seven months after collection), and urine aliquots to the University of Colorado Denver (within a month).

Baseline data were recorded by trained interviewers using a questionnaire recording data on age, education, smoking, alcohol, and some other background factors, as well as health (medically diagnosed diseases and nephrotoxic medications), and work history.

**2.4 Biochemical analyses**

Sodium, potassium, calcium, uric acid, urea nitrogen (“BUN”), phosphate, and creatinine in serum were measured in Mexico City with an autoanalyzer (UniCel DxC 600, Beckman Coultier).Creatinine was calibrated against creatinine determined by isotope dilution mass spectrometry. Urine neutrophil gelatinase-associated lipocalin (uNGAL) and Kidney Injury Molecule 1 (uKim-1) levels were analyzed using commercially available enzyme-linked immune absorbent assay (ELISA) kits; uNGAL from BioPorto Diagnostics and uKim-1 from BioAssay Works. All procedures were performed according to manufacturers’ instructions. For urinary heat shock protein 72 kD (Hsp72) detection by Western blot, 10 µL of each urine sample was loaded and resolved by 8.5% SDS-PAGE electrophoresis and electroblotted, as previously described (Barrera-Chimal et al. 2011). Membranes were then blocked with 5% blotting-grade non-fat dry milk and incubated in 0.1% blotting-grade non-fat dry milk with monoclonal Hsp72 antibody, 1:5000 (ENZO Life Science). Then, the detection of Hsp72 in urine was performed with goat anti-mouse antibody (1:5000 dilution) overnight at 4 °C (Santa Cruz Biotechnology). Proteins were detected with an enhanced chemiluminescence kit (Immobilon TM Western Chemiluminescent HRP substrate, Millipore) and autoradiography, following the manufacturer’s recommendations. All Western blot analyses were performed within the linear range of protein loads and antibody use. The bands were scanned for densitometric analysis (E3 Bioctem Imaging System UVP, Upland CA) and densitometry was performed using Vision Works Software UVP.

Dipstick analyses of urine were also performed in connection with urine sampling using a Bayer Clinitek 50 Urine Chemistry Analyzer with Multistix 10SG reagent strips (Siemens Diagnostics, United States) for semi-quantitative measurements of proteinuria (at levels of ≥30 to <300 mg/dL and ≥300 mg/dL, glucosuria (+ at ≥100 mg/dL), urinary specific gravity, pH, blood, nitrite, leukocytes, bilirubin, ketones and urobilinogen.

Studies performed at the University of Colorado included measurement of urine creatinine (Vet Ace analyzer), urine osmolarity (with Advance Micro Osmometer Model 3300) and urine pH (using a pH meter). Urinary fructose was measured using the EnzyChrom Fructose Assay Kit (BioAssay Systems, Hayward, CA) and corrected for urinary creatinine. Urine uric acid was measured using the QuantiChrom TM Uric Acid kit assay (BioAssay systems) and included measurements of soluble uric acid and the uric acid in the pellet (the latter following correction of the pH to 7).

To normalize for differences in urinary flow rate, each urinary biomarker was adjusted for the urinary creatinine concentration. Estimated glomerular filtration rate (eGFR) per1.73m2 of body surface area was calculated using the EPI-CKD formula based on serum creatinine (Levey et al. 2009).

**2.5 Data analyses**

Several variables were not normally distributed. Differences between groups were tested with Wilcoxon rank sum test or Fisher's exact test (for categorical variables). Differences between pre- and post-shift results were tested by Wilcoxon's signed rank test. Associations between variables were evaluated by the Pearson correlation coefficient (rp).

For the key renal biomarker differences in sugarcane cutters between baseline (Cut1) and follow-up 9 weeks later (Cut3), as well as differences in referents between baseline (Ref1) and follow-up 5 months later (Ref2), were assessed by a mixed effects model. Skewed variables were log-transformed. In these models, with separate covariance matrices for cutters and referents, subject was a random factor and group (cutters and referents), day (first and last), and time (pre- and post-shift) were fixed effects. The model included the fixed effects and a three-way interaction term in order to assess the effect of group and day separately for pre- and post-shift results.

All analyses were repeated after exclusion of one sugarcane cutter who had a low eGFR already at baseline (sensitivity analysis). P-values <0.05 were considered statistically significant. Data analyses were performed using SAS 9.4.

**3. Results**

Characteristics of the 29 sugarcane cutters and the 25 referents are shown in Table 1. The mean age of the cane cutters was 25 years. The referents were somewhat older, had slightly higher BMI, smoked slightly less, and used alcohol more often. In the interviews, one participant in each group reported hypertension, but both of them had normal blood pressure at examination and they did not take antihypertensive medications. Six cutters reported ever use of NSAIDS >3 months versus one referent (P=0.11). In spite of pre-screening, one sugarcane cutter had a low eGFR at baseline, as determined post data collection at the laboratory in Mexico City.

**3.1 Cross-shift changes**

For the sugarcane cutters, body weight, heart rate, blood pressure, and results for serum and urine biomarkers over a work-day at start of harvest (first and sixth work-day), and 9 weeks later are shown in Table 2A. There were several cross-shift changes. Body weight decreased somewhat on the first day, but not on the other days. Serum creatinine and serum urea N increased over shift on all three sampling days. This was the case also if excluding the cutter with a low eGFR at baseline. The cross-shift changes of serum creatinine and serum urea N tended to be more marked in the six workers reporting use of NSAIDS (P=0.053 for cross-shift change of serum urea N at Cut1). The cross-shift changes in the referent group were less pronounced (Table 2B). Uric acid in urine increased over shift in the sugar cane workers, but not in the reference group. There was also a tendency (P=0.052) towards a cross-shift change in number of cutters with Hsp72 above the detection limit at Cut1.

**3.2 Longitudinal changes**

Table 2 also shows changes over time for all individuals. Over the nine-week period (first half of the harvest) the sugarcane workers decreased in body weight, heart rate and blood pressure pre-shift (Table 2A). In the reference group, re-examined after 5 months, body weight increased substantially, heart rate was unchanged, while blood pressure decreased (Table 2B). For the evaluation of longitudinal changes in key kidney function biomarkers we restricted the analysis to individuals who took part in examinations both at baseline and at end of follow-up (Table 3). Serum creatinine increased substantially in the sugarcane cutters over the nine-week period, pre-shift means from 0.98 mg/dL to 1.18 mg/dL, and serum urea N even more. Urinary NGAL also increased substantially, as did urinary uric acid, although mainly post-shift. Pre and post-shift serum phosphate and pre-shift serum potassium decreased among cane cutters. Although there were some changes in the reference group, the signs of deteriorated renal function were more pronounced in the sugarcane cutters. The mean estimated GFR decreased by 10 mL/min after only 9 weeks (reflecting the increase in serum creatinine), and in addition to one sugarcane cutter with reduced (<60 mL/min) eGFR at start of harvest, two more cutters had reduced eGFR after nine weeks (Cut3). Some changes occurred already in the first week (see Table 2A): blood pressure decreased, urinary NGAL and KIM-1 increased, and there was a non-significant tendency towards an increase in serum creatinine. The sensitivity analysis excluding a cutter with low baseline eGFR showed that all statistically significant longitudinal changes in kidney function shown in Table 3 remained significant (data not shown).

There was a tendency towards an association between the cross-shift change of serum creatinine in the first week and the longitudinal change of eGFR over 9 weeks (rp = -0.40, P=0.06 for association between cross-shift change of serum creatinine at Cut1 and long-term change of eGFR over 9 weeks, and rp=-0.42, P=0.06 for association between the mean of the cross-shift changes of serum creatinine at Cut1 and Cut2, and change of eGFR over 9 weeks).

**3.3 Differences between cane cutters and referents**

While there were no significant group differences in most indicators of renal function at baseline (comparison of Cut1 and Ref1 in Table 3), at follow-up cane cutters had significantly higher serum creatinine (pre- and post-shift), serum urea N (pre- and post-shift), and urinary NGAL (pre- and post-shift), and lower eGFR than referents (comparison of Cut3 and Ref2 in Table 3). Post-shift urinary uric acid was higher in cane cutters both at baseline and at follow-up. Cane cutters and referents had similar pre-shift levels of serum potassium but at end of follow-up the post-shift potassium levels were significantly lower in cane cutters than in referents. Serum phosphate showed a different pattern. Pre-shift phosphate was higher in cane cutters than in referents, while post-shift levels at follow-up were not significantly different (Table 3). The statistically significant group differences remained after exclusion of a cutter with low baseline eGFR with one exception; the P-value for eGFR comparing Cut3 with Ref 2 changed from 0.03 to 0.06.

**3.4 Additional Analyses**

As mentioned in the Methods section, we only had seven post-shift urine samples from sugar cane cutters at the end of harvest (“Cut4” in May 2013). However, noteworthy is that those samples showed very high urinary uric acid values, with total urinary uric acid levels varying from 786 to 2413 mg/g creatinine (unadjusted uric acid 82 – 204 mg/dL), with a median of 1664 mg/g creatinine (unadjusted 134 mg/dL). All of these subjects were found to have urate crystals (dihydrate) in their urine. Interestingly, the days of collection represented some of the hottest days of the year for this region.

**4. Discussion**

In the present study a group of sugarcane cutters was examined on the first day at start of harvest, after 6 days, and again after 9 weeks. The most remarkable finding was a clear and significant decrease in pre-shift renal function after 9 weeks of work, with a 16% increase in mean serum creatinine, a 40% increase in serum urea N, 10% decrease in estimated GFR and two new cases of reduced eGFR (<60 mL/min).

While the rise in post-shift serum creatinine could reflect dehydration with a loss of extracellular volume (prerenal dysfunction), this is not likely for the pre-shift samples. We cannot exclude a slight increase in muscle mass over the nine-week period, but their mean body weight in fact decreased. The substantial increase in pre-shift NGAL also supports a deleterious effect of sugarcane work on kidney function. Therefore, the rise in serum creatinine and the decrease of eGFR in pre-shift samples likely reflect true renal injury, and are less likely to reflect alterations in hydration status, diet, or changes in muscle mass.

There were also some changes in markers of kidney function in the reference group, although more modest. We have no obvious explanation for the increase in serum creatinine in the referents over 5 months, but one possibility is that there is a seasonal effect on serum creatinine in this region with slightly higher levels when ambient temperature is higher, as has been suggested in some previous studies (Dalpino et al. 2005, Masugata et al. 2011). The mean body weight increased (mean 1.6 kg) in the referents, but it is not likely that this reflected any increase of muscle mass. Their serum urea N did not change but NGAL increased, but less than in the cane cutters.

We are aware of only two longitudinal studies, with a follow-up of workers over a harvest season, in Brazil (Paula Santos et al. 2014) and in Nicaragua (Laws et al. 2015a, 2015b). The Brazilian study showed no increase in serum creatinine in 28 cane cutters over an eight months harvest season, while the study of 51 cane cutters in Nicaragua found a mean increase in pre-shift serum creatinine of 0.07 mg/dL (8%), and a drop in eGFR of 3 mL/min over five months (Laws et al. 2015a). In a larger combined group of sugarcane field workers the decrease in eGFR was significantly larger than in a group of non-field workers. This study also found that NGAL, IL-8, and NAG increased more over the harvest season in field workers compared to non-field workers (Laws et al. 2015b). Our results show the same general pattern as in the study by Laws et al., but the increase in serum creatinine and the decrease in eGFR were larger in the present study in spite of a shorter follow-up period. Hsp72 has been suggested to be a good biomarker for predicting and detecting acute kidney injury (Morales-Buenrostro et al. 2014). In an experimental rat model of AKI, Hsp72 is a reliable biomarker for stratifying different degrees of tubular injury and recovery, as well as for monitoring a renoprotective intervention (Barrera-Chimal et. al 2011), and kidney levels of Hsp72 increase in mice exposed to heat (Islam et al. 2013). Therefore, the tendency towards a cross-shift increase of Hsp72 is biologically plausible, but the evaluation of this biomarker in the present study is hampered by the limited number of sugarcane cutters with detectable Hsp72 levels.

Another interesting result in the present study was the fact that pre-shift serum creatinine seemed to increase (although not statistically significant) already in the first six days of the harvest work. This could possibly indicate that this is a sensitive period, before the workers have been acclimatized to the hard work in a hot environment. In line with this, the cross-shift increase of serum creatinine was largest on the first day, when there was also a significant weight loss, and the cross-shift increase in serum creatinine could reflect a loss of extracellular volume (prerenal dysfunction). The significant increase in KIM-1 in the first week may support this hypothesis, although we cannot explain why KIM-1 returned to normal after 9 weeks.

The aim was to select young healthy men for the study, and therefore individuals with serum creatinine ≥1.0 mg/dL at a screening session were excluded. In spite of this, shortly after the screening, at the baseline sampling, several individuals had serum creatinine higher than the screening cut-off value. The reason for this may be the fact that the analyses were performed at two different laboratories, and/or temporal variability in serum creatinine levels.

The cross-shift decrease of serum potassium in the cutters was of the same size as recently found in the aforementioned study in El Salvador (García-Trabanino et al. 2015), and is likely the result of activation of the renin-angiotensin-aldosterone system (RAAS), which increases the excretion of potassium. It is unclear why the cane cutters had higher pre-shift (but not post-shift) serum phosphate than the referents. Possibly dietary habits differ over time and between cane cutters and referents.

Cross-shift changes of serum creatinine and serum urea N were in agreement with a recent larger study of 189 sugarcane workers in El Salvador (García-Trabanino et al. 2015). In that study there were also cross-shift changes in electrolytes and stronger effects on cardiovascular function. In the study in El Salvador, the cane cutters were examined in the field, immediately after shift. In the present study the post-shift examination of the cutters was performed in their homes, several hours after the work had ceased. Since the workers had then been able to rest, eat and drink, the present study is less optimal for studying cross-shift changes than the study by García-Trabanino et al. (2015). The significant increase in urinary NGAL during the work-day supports renal injury, although there is a report that a rise in serum NGAL could simply reflect dehydration (Nejat et al. 2012). Also the Brazilian study by Paula Santos et al. (2014) found a substantial cross-shift increase in serum creatinine. They found a cross-shift increase of serum creatinine >0.3 mg/dL in 5 out of 28 cane cutters at the end of the harvest season, i.e. compatible with acute kidney injury (AKI) (KDIGO 2012). In the present study 3 out of 22 cane cutters at mid-harvest had an increase of >0.3 mg/dL. AKI is relatively common in hospitalized patients, often in association with renal ischemia (Bucaloiu et al. 2012). Because it is usually reversible, it was previously not assumed to be a risk factor for chronic kidney disease (CKD). Long-term follow-up of patients with episodes of AKI have, however, shown that these patients run an increased risk of CKD (Bucaloiu et al. 2012). This has also been demonstrated in experimental studies of ischemic AKI in rats (Barrera-Chimal et al. 2011, Rodríguez-Romo et al. in press). Since repeated episodes of AKI seem to occur in sugar cane cutters, it is a reasonable hypothesis that this in the long-term increases the risk of CKD. The association between the cross-shift increase of serum creatinine and the decrease of eGFR over nine weeks in the cutters lends further support to this hypothesis. To the best of our knowledge no previous study examined cross-shift changes and development of kidney function over a longer period. Unfortunately the group size is small, and therefore we cannot exclude that this finding is due to chance. Interestingly, already in 1970, a small case series of South African miners with AKI from heat stroke showed development of CKD with interstitial fibrosis and tubular atrophy at follow-up after 8-21 months (Kew et al. 1970).

Recently elevated urinary concentrations of uric acid have been proposed to contribute to the development of MeN, either through direct effects of soluble uric acid or due to actions of urate crystals on tubular epithelium (Roncal-Jimenez et al. 2015a, 2015b). The present study confirmed increases of urinary uric acid levels over the working day in the sugarcane cutters (Table 2A), while this was not the case in the reference group (Table 2B). Interestingly, in the seven cutters with urine samples collected post-shift during very hot days in May at the end of the harvest the urinary uric acid levels were very high, and much higher than in the referents sampled in May. This might be due to relatively greater dehydration at that time.

A main limitation of the present study is the modest number of subjects. Other limitations include different timing of the follow-up between the two groups, with the follow-up of the sugarcane cutters at 9 weeks (in January 2013), while the follow-up of the referents occurred at the end of harvest in May 2013. While the study was supposed to include a follow-up in May for the sugarcane workers, 50% of the enrolled cane cutters were fired at the middle of the harvest because they were participating in the study. Second, we also had to collect blood and urine samples from cane cutters at their home instead of examining them on site immediately after they ended the work-shift. This allowed the workers to rehydrate themselves if they had become dehydrated in the field. A third limitation is the fact that the cane cutters had on average a lower socioeconomic status than the reference group. They had fewer years of schooling than the referents and they were shorter and had a lower body weight. These differences at baseline should, however, not be important for the analyses of cross-shift or longitudinal changes. A final limitation is that analyses were performed on frozen samples, and long-term storage can decrease concentrations of KIM-1 and NGAL (Nauta et al. 2012). Despite these limitations, the longitudinal increase in serum creatinine, serum urea N, and urinary NGAL resulted in substantial and statistically significant differences between cane cutters and referents at follow-up. In addition, there were group differences in serum levels of potassium and phosphate. Post-shift urinary uric acid levels were higher in the cane cutters both at start of harvest and at follow-up.

An explanation is warranted about the situation surrounding the firing of the study participants in this region where there are practically no alternative employment opportunities. The workers were not fired directly by the sugar company; the task was delegated to the subcontractors who had hired the workers. The Nicaraguan partners of our research team, with support from the authorities of their university, immediately demanded that the company reinstate these workers, but without success. The principal investigator of a research group working with the company flew down to Nicaragua to mediate with the company. The workers were rehired six weeks later, during which time the project provided them with financial support. The fired workers were not willing to continue their participation in the study. Most of the other workers followed suit. Protecting the identity of workers who participate in a study remains a major concern in planning and executing studies in this region. In a broader context, mounting scientific evidence of a link between sugarcane cutting and Mesoamerican nephropathy, as well as press attention to working conditions and labor rights, is impacting the company’s attitude towards improvements.

In conclusion, we found a remarkable decrease of glomerular kidney function, after only 9 weeks of harvest. If glomerular filtration decreases by 10% only after half a harvest season, it is not surprising that chronic kidney disease with severely reduced glomerular filtration is common in this sugarcane area. The cross-shift increase in serum creatinine is probably caused by dehydration (pre-renal dysfunction), which when repeated on a daily basis may cause permanently reduced GFR, and the present study provides some support for this hypothesis. There is a strong need for preventive measures, including the provision of water, rest, and shade.

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**Disclosures**: The authors disclose no conflicts of interest related to this study. Dr Johnson does have patents and patent applications related to blocking fructose and uric acid metabolism, is on the Scientific Board for Amway and XORT therapeutics, has lectured at Danone symposia, and is a member of Colorado Research Partners that is developing inhibitors for fructose metabolism. Dr. Bobadilla does have patents and patent applications related to Hsp72 as a biomarker of acute kidney injury.

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Table 1. Characteristics of the study population of sugarcane cutters and the reference group in Nicaragua, all men, at start of harvest.

Mean, median (range) are shown for continuous variables, % for smoking habits, and numbers (N) for medical conditions.

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Cutters (N=29) Reference group (N=25) P-value

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Age, years 25, 24 (17 – 38) 30, 31 (19 – 38) <0.001

Body weight, pre-shift 64, 60 (50 – 89) 75, 74 (52 – 103) <0.001

Height, cm 165, 166 (152 – 173) 169, 169 (159 – 178) 0.002

BMI, mean 24, 23 (18 – 31) 26, 27 (18 – 34) 0.01

Current smokers, %**a)** 45 28 0.26

Ex-smokers, % 10 16 0.69

Current use of alcohol, % 41 72 0.03

Years of schooling 3, 3 (0 – 10) 13, 16 (0 – 16) <0.001

Hypertension, N**b)** 1 1

Diabetes, N**b)** 0 0

Nephrolithiasis, N**b)** 1 0

NSAIDs, N**c)** 6 1

Nephrotoxic antibiotics, N**d)** 0 1

S-creatinine (mg/dL), pre-shift**e)** 0.96, 0.85 (0.65 – 2.4) 0.83, 0.81 (0.45 – 1.31) 0.07

eGFR mL/min/1.73 m2 **f)** 111, 118 (37 – 141) 116, 120 (75 – 151) 0.49

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a) mean number of cigarettes smoked per day was 7 in cane cutters and 4 in referents; b) ever suffered (self-reported); c) used for >3 months; d) gentamycin (at least one week in the past year); e) workers were pre-screened for S-creatinine before harvest, and none had S-creatinine >1.0 mg/dL by that time; f) one cutter had reduced eGFR (<60 mL min/1.73 m2).

Table 2A. Body weight, pulse rate, blood pressure, and results for serum and urine biomarkers over a work-day at start of harvest (first and 6th day) and 9 weeks later in 29 sugarcane cutters. Median (10 – 90-percentiles) or number (N) is given. P-values (Wilcoxon’s signed rank test) for cross-shift changes are only presented if ≤0.05. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Day Cut1 P-value Cut2 P-value Cut3 P-value

Nov 2012 cross-shift Nov 2012 cross-shift Jan 2013 cross-shift first work-day change sixth work-day change change N=29 N=29 N=28 N=26 N=28 N=25

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Body weight Pre (kg) 59.5 (57 – 80) 59.5 (57 – 80) 58.4 (55 – 72)

Body weight change -0.4 (-1.4 – 0.7) 0.02 0.2 (-1.0 – 1.3) 0.3 (-0.8 – 1.3)

Pulse rate Pre (per min) 63 (50 – 84) 61 (50 – 74) 58 (45 – 68)

Pulse rate change 10 (-15 – 27) 0.005 19 (2 – 30) <0.001 11 (2 – 25) <0.001

Systolic BP Pre (mm Hg) 130 (117 – 134) 122 (106 – 138) 118 (103 – 127)

Systolic BP change -7 (-23 – 21) 0.05 1 (-18 – 19) 2 (-11 – 10)

Diastolic BP Pre (mm Hg) 83 (73 – 92) 81 (61 – 95) 80 (64 – 86)

Diastolic BP change - 2 (-19 – 8) 0.04 -1 (-13 – 10) -3 (-12 – 3) 0.01

***Serum biomarkers***

S-creatinine Pre (mg/dL) 0.85 (0.67 – 1.2) 0.94 (0.67 – 1.3) 0.95 (0.77 – 1.6)

S-creatinine change 0.12 (-0.04 – 0.21) <0.001 0.06 (-0.01 – 0.15) <0.001 0.08 (-0.12 – 0.35) 0.02

S-urea N Pre (mg/dL) 10.4 (7.5 – 17) 10.5 (6.7 – 16) 15.9 (8.9 – 20)

S-urea N change 1.4 (-1.4 – 4.3) 0.002 0.9 (-1.6 – 3.7) 0.01 1.2 (-3.2 –3.5) 0.08

S-uric acid Pre (mg/dL) 5.7 (4.3 – 7.5) 5.2 (3.6 – 7.1) 5.5 (4.5 – 7.2)

S-uric acid change -0.1 (-0.7 – 0.5) 0.2 (-0.2 – 0.7) 0.003 0.2 (-0.7 – 1.0)

S-glucose Pre (mg/dL) 70 (60 – 86) 77 (65 – 84) 83 (76 – 93)

S-Na (mmol/L) 141 (138 – 144) 140 (138 – 142) 140 (138 – 142)

S-Na change 0.1 (-2.2 – 2.5) 0.2 (-1.5 – 1.7) -0.5 (-2.1 – 2.1)

S-K (mmol/L) 4.3 (3.6 – 5.2) 4.3 (3.6 – 4.7) 4.0 (3.4 – 4.8)

S-K change -0.5 (-1.2 – 0.3) -0.1 (-0.5 – 0.5) -0.2 (-0.9 – 0.5)

S-Ca (total) ( mg/dL) 9.6 (9.1 – 10.2) 9.3 (8.7 – 9.8) 9.5 (9.0 – 10.1)

S-Ca (total) change -0.10 (-0.5 – 0.6) 0.15 (-0.3 – 0.5) 0.05 (-1.2 – 0.5)

S-phosphate (mg/dL) 4.6 (3.7 – 5.1) 4.4 (3.5 – 5.2) 4.2 (3.4 – 4.9)

S-phosphate change -0.2 (-0.9 – 0.6) 0 (-0.8 – 0.6) -0.4 (-1.1 – 0.5) 0.005

***Urine biomarkers***

pH Pre 6 (5.5 – 7) 6 (5.5 – 7) 6 (5.5 – 7)

pH change 0.5 (-0.5 – 0.5) 0.02 0 (-0.5 – 1) 0 (-0.5 – 1)

NGAL Pre (µg/gCr) 9.3 (2 – 48) 18 (4 – 89) 17 (6 – 285)

NGAL change 1.4 (-31 – 29) 2.8 (-30 – 146) 6.3 (-18 – 118)

KIM-1 (ng/gCr) 4.7 (0.5 – 35) 11 (0.8 – 98) 4.6 (0.6 – 50)

KIM-1 change -0.2 (-17 – 34) 0.8 (-38 – 22) 2.8 (-3 – 23)

Hsp Pre (N>LOD) 1 3 0

Hsp Post (N>LOD) 7 3 3

Creatinine (g/L) 0.86 (0.3 – 2.0) 0.51 (0.3 – 1.7) 0.80 ( 0.3 – 1.5)

Creatinine change 0.04 (-1 – 1.3) 0.12 (-1 – 1.4) -0.01 (-0.06 – 0.03)

Osmolality 652 (356 – 923) 471 (332 – 836) 525 (350 – 877)

Osmolality change 50 (-179 – 277) 46 (-375 – 393) -1 (282 – 125)

U-fructose Pre (µmol/gCr) 129 (64 – 458) 175 (80 – 312) 343 (119 – 686)

U-fructose change 15 (-201 – 312) 103 (-90 – 513) <0.001 114 (-157 – 583) 0.02

U-uric acid Pre (mg/gCr) 442 (304 – 653) 474 (332 – 810) 498 (310 –1337)

U-uric acid change (mg/gCr) 74 (-127 – 256) 0.02 104 (-49 – 263) 0.01 25 (-80 – 536)

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Table 2B. Body weight, pulse rate, and blood pressure over a work-day and 5 months later in a reference group of 25 individuals. Median (10 – 90-percentiles) or number (N) is given. P-values (Wilcoxon’s signed rank test) for cross-shift changes are only presented if ≤0.05. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Day Ref1 Ref2

Nov 2012 P-value May 2013 P-value

cross-shift cross-shift

change change

N=25 N=25 N=25 N=25

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Body weight Pre (kg) 74 (56 – 89) 80 (57 – 90)

Body weight change 0.0 (0 – 1.5) 1.6 (-2 – 4.5) 0.002

Pulse rate Pre (per min) 76 (63 – 85) 77 (65 – 82)

Pulse rate change 4 (-4 – 15) 0.004 4 (-8 – 19)

Systolic BP Pre (mm Hg) 119 (97 – 135) 111 (99 – 131)

Systolic BP change 3 (-7 – 15) 2 (-8 – 17)

Diastolic BP Pre (mm Hg) 81 (64 – 90) 72 (53 – 83)

Diastolic BP change -3 (-15 – 12) 3 (-6 - 18)

***Serum biomarkers***

S-creatinine Pre (mg/dL) 0.81 (0.66 – 1.1) 0.85 (0.71 – 1.1)

S-creatinine change 0.08 (-0.02 – 0.29) <0.001 0.04 (-0.08 – 0.20)

S-urea N Pre (mg/dL) 9.6 (7.2 – 14) 9.8 (6.3 – 13)

S-urea N change 1.2 (-2.1 – 5.7) 0.02 1.0 (-0.9 – 3.7) 0.002

S-uric acid Pre (mg/dL) 5.5 (4.0 – 8.1) 5.7 (4.1 – 7.4)

S-uric acid change 0.0 (-0.7 – 0.7) -0.3 (-0.6 – 0.7)

S-glucose Pre (mg/dL) 81 (68 – 95)

S-Na (mmol/L) 140 (137 – 143) 140 (138 – 142)

S-Na change 0.8 (-2 – 3) 0.8 (-2 – 4)

S-K (mmol/L) 4.4 (3.8 – 5.0) 4.2 (3.8 – 4.7)

S-K change -0.3 (-1.0 – 0.3) 0.1 (-0.4 – 0.4)

S-Ca (total) (mg/dL) 9.1 (8.7 – 9.6) 9.2 (8.7 – 9.5)

S-Ca (total) change 0.1 (-0.4 – 0.7) 0.0 (-0.3 – 0.4)

S-phosphate (mg/dL) 3.5 (2.9 – 4.2) 3.5 (2.6 – 3.8)

S-phosphate change 0.7 (- 0.7 – 1.4) 0.6 (0.0 – 1.6)

***Urine biomarkers***

pH Pre 6 (5 – 7) 6.5 (5.5 – 7.5)

pH change 0 (-1 – 1) 0 (-0.5 – 0.5)

NGAL Pre (µg/gCr) 6.4 (0.9 – 22) 8.0 (2.0 – 46)

NGAL change (µg/gCr) 0.1 (-13 – 14) -0.3 (-23 – 27)

KIM-1 (ng/gCr) 3.1 (0.96 – 11) 3.5 (0.6 – 54)

KIM-1 change -0.6 (-3.6 – 12) -0.2 (-12 – 22)

Hsp corr Pre (N>LOD) 4 4

Hsp corr Post (N>LOD) 4 3

Creatinine (g/L) 1.5 (0.4 – 2.0) 1.8 (0.5 – 3.8)

Creatinine change 0.37 (-0.3 – 1.9) 0.01 0.01 (-0.9 – 1.5)

Osmolality 837 (356 – 1023) 731 (220 – 1060)

Osmolality change 90 (-272 – 320) 77 (-394 – 616)

U-fructose Pre (µmol/gCr) 80 (63 – 191) 164 (69 – 1030)

U-fructose change 116 (2 – 295) <0.001 131 (-617 – 550)

U-uric acid total Pre (mg/gCr) 363 (171 – 549) 429 (299 – 667)

U-uric acid change (mg/gCr) -6 (-167 – 150) 6 (-121 – 186)

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Table 3. Longitudinal changes in pre-shift and post-shift levels (mean, median (range)) of selected indicators of renal function (9 weeks in sugarcane cutters and 5 months in referents) in those individuals (23 sugarcane cutters and 25 referents) who took part in both examinations. P-values (mixed effect model) for changes between testing days and for differences between groups are given.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Cutters, N=23 | | P for change  Cut3 vs. Cut1 | Referents, N=25 | | P for change Ref2 vs. Ref1 | P for group diff Cut1 vs. Ref1 | P for group diff Cut3 vs. Ref2 |
| Cut1 | Cut3 | Ref1 | Ref2 |
| S-creatinine Pre, mg/dL | 0.98, 0.91  (0.65 – 2.4) | 1.18, 0.95  (0.69 – 3.6) | 0.002 | 0.83, 0.81  (0.45 – 1.3) | 0.88, 0.85  (0.63 – 1.3) | 0.02 | 0.07 | 0.009 |
| S-creatinine Post | 1.06, 0.99  (0.78 – 2.5) | 1.26, 1.10  0.73 – 3.3) | 0.002 | 0.93, 0.86  (0.67 – 1.4) | 0.94, 0.90  (0.70 – 1.5) | 0.74 | 0.15 | 0.003 |
| eGFR Pre, mL/min/1.73 m2 | 109, 111  (37 – 141) | 99, 108  (22 – 134) 1 | 0.02 | 116, 116  (75 – 151) | 112, 117  (78 – 138) | 0.07 | 0.35 | 0.03 |
| S-Urea N Pre, mg/dL | 11.3, 10.4  (5.9 – 19) | 15.9, 15.9  (6.9 – 46) | <0.001 | 10.3, 9.6  (5.2 – 18) | 9.7, 9.8  (4.3 – 15) | 0.29 | 0.34 | <0.001 |
| S-Urea N Post | 13.0, 12.5  (7.4 – 23) | 16.3, 14.3  (7.2 – 34) | 0.007 | 11.8, 11.6  (7.1 – 23) | 10.8, 11.0  (6.7 – 15) | 0.11 | 0.38 | <0.001 |
| S-K Pre, mmol/L | 4.4, 4.3  (3.6 – 5.3) | 4.0, 4.0  (2.8 – 5.3) | 0.002 | 4.4, 4.4  (3.8 – 5.0) | 4.1, 4.2  (3.3 – 5.5) | 0.01 | 0.91 | 0.42 |
| S-K Post | 3.9, 4.0  (3.2 – 4.7) | 3.8, 3.8  (3.1 – 5.6) | 0.44 | 4.1, 4.1  (3.2 – 5.4) | 4.2, 4.3  (3.6 – 5.1) | 0.11 | 0.29 | 0.007 |
| S-phosphate Pre, mg/dL | 4.5, 4.6  (2.9 – 5.6) | 4.2, 4.2  (2.6 – 5.3) | 0.02 | 3.5, 3.5  (2.3 – 4.6) | 3.3, 3.5  (2.2 – 4.0) | 0.24 | <0.001 | <0.001 |
| S-phosphate Post | 4.4, 4.4  (3.1 – 4.7) | 3.8, 3.9  (2.5 – 5.0) | <0.001 | 4.0, 4.0  (2.6 – 5.6) | 4.0, 3.8  (2.8 – 5.0) | 1.0 | 0.02 | 0.22 |
| U-NGAL Pre, µg/gCr | 18, 10  (0.2 – 57) | 72, 17  (3.8 – 351) | 0.003 | 8.6, 6.4  (0.04 – 23) | 23.7, 8.0  (0.3 – 214) | 0.02 | 0.10 | 0.02 |
| U-NGAL Post | 15, 11  (1.0 – 87) | 106, 32  (1.7 – 960) | <0.001 | 8.4, 4.4  (0.09 – 28) | 26.7, 5.6  (0.2 – 230) | 0.02 | 0.06 | 0.001 |
| U-uric acid Pre,2 mg/gC | 472, 433  (240 – 1160) | 647, 498  (160 – 1540) | 0.12 | 367, 368  (46 – 610) | 454, 423  (280 – 680) | 0.11 | 0.08 | 0.18 |
| U-uric acid Post2 | 530, 450  (64 – 950) | 725, 550  (330 – 2030) | 0.04 | 369, 354  (8.3 – 700) | 463, 450  (223 – 700) | 0.01 | 0.002 | 0.04 |

1Three individuals, all cutters, had eGFR <60 mL/min. 2 22 cutters and 24 referents

**Heat stress, dehydration, and kidney function in sugarcane cutters in Nicaragua – a longitudinal study of workers at risk of Mesoamerican nephropathy**

Catharina Wesseling, Aurora Aragón, Marvin González, Ilana Weiss, Jason Glaser, Norma A. Bobadilla, Carlos Roncal-Jiménez, Ricardo Correa-Rotter, Richard J Johnson, and Lars Barregard

**Highlights**

* We examined sugarcane cutters at start of harvest and 9 weeks later
* Pre-shift renal function decreased substantially after 9 weeks of harvest work
* Estimated glomerular filtration rate decreased by 10 mL/min
* Serum urea N (BUN) and urinary NGAL also increased substantially
* Repeated dehydration is a likely cause of long-term decrease in renal function