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Inflammation-Related Factors Identified as Biomarkers of Dehydration and Subsequent Acute Kidney Injury in Agricultural Workers

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Abstract

Globally, there is increasing recognition that agricultural workers are at risk for chronic kidney disease of unknown etiology (CKD*u*). Recurrent heat exposure, physical exertion, dehydration, muscle damage, and inflammation are hypothesized to contribute to the development of CKD*u*, but the relative importance of these processes and the interactions among them remain unclear. Moreover, there is a need to identify biomarkers that could distinguish individuals who are at greatest risk for kidney damage to target preventative interventions for CKD*u*. In this study, we evaluated dehydration and markers of inflammation, muscle damage, and renal function in agricultural workers at a non-workday baseline assessment. Urine specific gravity and kidney function were measured before and after work shifts on three subsequent days, and heat index, core body temperature, and heart rate were monitored during the work shifts. A combination of direct comparisons and machine learning algorithms revealed that reduced levels of uromodulin and sodium in urine and increased levels of interleukin-6 and C-reactive protein in serum were indicative of dehydration at baseline, and that dehydration, high body mass index, reduced urine uromodulin, and increased serum interleukin-6, C-reactive protein, and lipopolysaccharide-binding protein at baseline were predictive of acute kidney injury on subsequent workdays. Our findings suggest a method for identifying agricultural workers at greatest risk for kidney injury and reveal potential mechanisms responsible for this process, including pathways overlapping in dehydration and kidney injury. These results will guide future studies confirming these mechanisms and introducing interventions to protect kidney health in this vulnerable population.

Keywords

dehydration, inflammation, acute kidney injury, chronic kidney injury of unknown etiology, classification tree, agricultural workers

According to the National Oceanic and Atmospheric Administration, the past 7 years (2014–2020) have been the hottest recorded in 140 years ("State of the Climate: Global Climate Report for July 2020," 2020). The health effects of rising environmental temperatures are of increasing concern. Heat-related illness (HRI) occurs when physiological responses to dissipate heat are insufficient. The mildest forms of HRI manifest with symptoms such as excessive sweating, moist and clammy skin, and fatigue, while the most severe form, heat stroke, involves multi-organ dysfunction and can be fatal (Hashim, 2010; Mutic et al., 2018). These reactions to acute heat stress are well-recognized, but less is known about physiologic consequences of chronic heat exposure.

Agricultural workers commonly experience occupational heat exposure, which can result in injuries, reduced productivity, disease, and death (National Institute for Occupational

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Safety and Health [NIOSH], 2016). Another concern that has emerged in the last decade is an epidemic of chronic kidney disease of unknown etiology (CKD*u*) among agricultural workers in countries including El Salvador, Nicaragua, and Mexico (Glaser et al., 2016). Although CKD*u* has not been widely documented in the United States, renal dysfunction has been found in immigrant agricultural workers working in Florida and California (Mix et al., 2018; Moyce et al., 2016). Unlike traditional chronic kidney disease, which is frequently associated with advanced age, diabetes, and hypertension, CKD*u* occurs in younger, otherwise healthy adults and is characterized by seemingly abrupt disease onset and rapid progression (Glaser et al., 2016). The etiology of CKD*u* is elusive and controversial; previous studies have suggested the involvement of factors including pesticide exposure (Valcke et al., 2017), drinking water quality (Wasana et al., 2016), and non-steroidal anti-inflammatory drug use (Senevirathna et al., 2012). An emerging hypothesis is that recurrent and strenuous manual labor in hot environments leads to chronic dehydration, inflammation, and ultimately kidney damage and disease (Hansson et al., 2020; Orantes-Navarro et al., 2017).

Dehydration can limit the body's ability to cope with heat stress, impairing thermoregulation responses and increasing the likelihood of elevated core body temperature, which threatens the muscular, cardiovascular, central nervous, and renal systems (Sato et al., 2019; Sawka et al., 1998). In the context of prolonged exercise, dehydration has been shown to reduce estimated glomerular filtration rate (eGFR) and increase indicators of kidney injury (Bongers et al., 2018). While the acute impacts of dehydration are considered reversible upon rehydration (Wegman et al., 2018), recurrent dehydration induces renal fibrosis and inflammation in rodent models (Hilliard et al., 2016; Roncal Jimenez et al., 2014). These changes mimic features observed in biopsies from humans with CKD*u* (Fischer et al., 2018). Whether chronic dehydration is indeed linked to inflammatory immune responses and kidney damage in humans requires further investigation.

Rhabdomyolysis is a potentially serious condition in which muscle breakdown allows intracellular components (e.g., creatine kinase, myoglobin) to enter the circulatory system where they can induce inflammation and potentially kidney injury (Hamel et al., 2015). The condition can be caused by the high core body temperature associated with heat stroke (Carter et al., 2005; Keltz et al., 2013), as well as by strenuous, repetitive exertion (Patel et al., 2009), but clinical rhabdomyolysis is not regularly documented in agricultural workers. It has been demonstrated, however, that muscle-damaging exercise augments acute kidney injury (AKI) associated with exertion and heat exposure (Junglee et al., 2013), and there is speculation that sub-clinical muscle breakdown could increase the risk of recurrent AKI and CKD*u* among agricultural workers (Diniz et al., 2020; Hansson et al., 2020).

Systemic inflammation is hypothesized to be a key mechanism of CKD*u* pathogenesis (Hansson et al., 2020). Mediators of physiological heat stress responses often exhibit immunomodulatory activity (Pockley, 2003). When heat stress is combined with strenuous physical exertion, as it is in agricultural work settings, it can induce intestinal barrier dysfunction, or "leaky gut," resulting in elevated levels of proinflammatory microbial lipopolysaccharides in circulation (Armstrong et al., 2018; Vargas & Marino, 2016). Muscle damage from prolonged exercise can also activate innate immune responses with systemic effects (Hamel et al., 2015; Kawanishi et al., 2016a, 2016b). Inflammation is a recognized feature of chronic kidney disease (Mihai et al., 2018), and higher levels of circulating proinflammatory cytokines are associated with a more rapid decline in kidney function (Amdur et al., 2016).

The interrelationships of heat exposure, exertion, core body temperature response, dehydration, muscle breakdown, inflammation, and renal function are a puzzle that must be solved to fully understand the health consequences of exposure to increasing temperatures and to develop strategies to protect individuals at risk. To contribute to the understanding of these complex interrelationships, we investigated biomarkers associated with dehydration and AKI in agricultural workers exposed to high environmental temperatures.

Materials and Methods

Participants

Thirty-two agricultural workers who self-identified as Hispanic were recruited in Homestead, Florida, a large agricultural community south of Miami, in July-August 2017. They were a convenience sample of subjects participating in a larger study of the relationship of heat exposure and core body temperature response, which was approved by the Emory Institutional Review Board (IRB00075192). Eligible workers were between the ages of 18 and 54 years, had been working in agriculture for at least 1 month, and reported no history of Type 1 diabetes or current pregnancy. Participants worked in crop agriculture, nurseries, or landscaping, and their occupational activities included planting, cultivating, cutting or trimming, harvesting, washing, packing, loading, and transporting plants, as well as weeding, applying pesticides, and cleaning their worksites. All experienced extreme heat typical of south Florida summers during their workdays. All participants completed a verbal informed consent process which was conducted in their choice of English or Spanish and documented by trained community health workers from our community partner, the Farmworker Association of Florida (FWAF).

Baseline Assessment

Participants underwent a baseline assessment on a non-workday. They completed a survey about demographic characteristics and work and health histories, including recent experience of HRI symptoms, which was defined as muscle cramps, nausea/vomiting, excessive sweating, confusion, dizziness, fainting, and/or headaches while working or doing activities outside in the past week. They were given a CorTemp® sensor pill (HQInc., Palmetto, FL) to ingest that evening with a meal. Blood (fingerstick, 500μL) and urine samples were collected, and plasma and serum were isolated from blood. Samples were flash frozen and stored

on dry ice for shipment to the laboratory, where they were stored at −80°C.

Workday Assessments

Before their work shift on each of three days following the baseline assessment (time period selected to balance the benefits of capturing day-to-day variation in participants' activities and heat exposure with the risks of losing participants due to excessive study demands or changes in employment), participants provided a sample of their first morning void of urine. Urine specific gravity (USG) was determined using a digital refractometer (Reichert™ TS Meter-D; Fisher Scientific, Pittsburgh, PA). A fingerstick blood sample was processed and stored for measurement of serum creatinine. The workers were equipped with a Polar® T31 heart rate monitor and a CorTemp® data recorder (HQInc., Palmetto) that recorded heart rate and core body temperature every 30 s during work hours. We assessed whether each individual's core body temperature, on at least two readings within one min, reached the NIOSH (2016) heat stress risk thresholds of 38°C (mild hyperthermia) or 38.5°C (moderate hyperthermia) during at least one workday. Only four participants met the latter criterion, so this variable was not used further. We also examined mean and maximum heart rate over the work shift and averaged these values for the workdays. After each work shift, monitors were removed, and USG was measured.

We obtained environmental temperatures and relative humidity every 15 min during the workdays from the Florida Automated Weather Network (FAWN) monitoring station in Homestead, FL (https://fawn.ifas.ufl.edu/; Lusher et al., 2008). Heat index was calculated using the National Weather Service algorithm and averaged over the work shift and then over the three workdays (Steadman, 1979a, 1979b).

Biomarkers and Dehydration and Renal Function Measures

To evaluate baseline dehydration, we classified USG values according to whether they were ≥ 1.020 or < 1.020, defining the former as dehydration based upon the hypohydration threshold guidelines of the American College of Sports Medicine (Sawka et al., 2007). To validate dehydration classification by USG, in a subset of participants, we also calculated serum osmolality using the equation deemed most accurate by Martín-Calderón et al. (2015; Supplemental Table 1, Supplemental Figure 1). In the full cohort, we also measured antidiuretic hormone (ADH) in urine and aldosterone in plasma. We measured myoglobin and creatine kinase in serum as indicators of muscle breakdown.

To evaluate renal function at baseline, we measured creatinine, cystatin-C, and uric acid in serum, and sodium, kidney injury molecule 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), creatinine, uromodulin, and beta-2 microglobulin (B2M) in urine. Only 6 of 32 samples had detectable B2M levels, so we did not analyze this variable further. Urine analyte concentrations were normalized to concentrations of urine creatinine. To evaluate workday renal function, we calculated pre- and post-shift values of eGFR using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation based on serum creatinine (Levey et al., 2014). We considered a participant to have AKI if the pre- to post-shift change in serum creatinine was ≥ 0.3 mg/dL or ≥ 1.5 times the pre-shift value based on the Kidney Disease: Improving Global Outcomes (KDIGO) criteria (Khwaja, 2012).

Inflammatory factors C-reactive protein (CRP), lipopolysaccharide binding protein (LBP), interleukin (IL)-1b, IL-6, C-X-C motif ligand 8 (CXCL8), IL-10, tumor necrosis factor (TNF), and interferon (IFN)-γ were measured in baseline serum using the MesoScale Discovery platform (MesoScale Diagnostics, Rockville, MD). Details are provided in Supplemental Table 2.

Statistical Analysis

We compared baseline blood and urine biomarker levels and demographic and health characteristics of participants who met the criteria for baseline dehydration and for AKI on any of the workdays with those who did not, using Wilcoxon rank sum tests for continuous variables and chi square tests for categorical variables. Logistic regression was used to control for potential confounders. Associations between levels of specific analytes and various participant lifestyle factors were evaluated using generalized linear models for continuous predictors and Wilcoxon rank sum tests for categorical predictors. Pearson correlations were used to assess linear relationships between pairs of variables. If any significant correlations appeared to be driven by a single high extreme value (true for TNF, NGAL, and myoglobin), this value was excluded and the correlation re-run.

We employed recursive partitioning to examine relationships between biomarkers and dehydration and AKI (Friedman, 1977). Classification tree analysis is a non-parametric method that aims to produce a model that best fits a dataset. Minimum node size was set to 5, and trees were pruned to minimize cross-validated error. In order to confirm the predictive value of the independent variables identified in previous analyses and to rank the value of the remaining variables, we employed random forest analysis (Breiman, 2001). The random forest method extends classification tree analysis to bootstrapped samples of independent variables from the dataset and accounts for interactions among independent variables and non-linear relationships between independent and dependent variables (Ishwaran et al., 2014). The number of variables randomly selected for each tree was set to 5, and the number of trees was set to 2,500 to minimize the error rate. Due to the small size of this cohort, we did not attempt to split the dataset into training and test sets for these analyses. Ongoing research projects in this population will be able to validate these predictive models. The independent variables considered for each dependent variable in recursive partitioning analyses are listed in Supplemental Table 2 (also including BMI and excluding B2M). For

Table 1. Participant Demographics and Health History.

Variables	Not Dehydrated ($N = 9$)	Dehydrated ($N = 23$)	p Value	No AKI ($N = 16$)	AKI ($N = 15$)	p Value
Age			0.967			0.937
- Mean (SD)	40.2 (11.6)	40.5 (10.7)		39.6 (12.4)	41.4 (9.4)	
- Min - Max	20.0 - 53.0	18.0 - 54.0		18.0 - 53.0	25.0 - 54.0	
Sex			0.682			0.283
- Male	3 (33.3%)	6 (26.1%)		6 (37.5%)	3 (20.0%)	
- Female	6 (66.7%)	17 (73.9%)		10 (62.5%)	12 (80.0%)	
BMI			0.056			0.006
- Mean (SD)	25.5 (3.9)	29.7 (6.4)		27.3 (7.3)	30.2 (4.3)	
- Min–Max	19.1 - 31.3	19.4 - 51.7		19.1 - 51.7	22.5 - 41.8	
Waist: Hip Ratio			0.869			0.358
- Mean (SD)	0.9 (0.1)	0.9 (0.1)		0.9 (0.1)	0.9 (0.1)	
- Min–Max	0.8 - 1.0	0.8 - 1.1		0.8 - 1.1	0.8 - 1.0	
History of Hypertension*	1 (11.1%)	13 (59.1%)	0.015	5 (33.3%)	8 (53.3%)	0.269
History of Diabetes/ Abnormal Glucose	0 (0.0%)	12 (52.2%)	0.006	4 (25.0%)	8 (53.3%)	0.106
At least 3 HRI symptoms last week*	8 (88.9%)	11 (50.0%)	0.044	10 (62.5%)	8 (57.1%)	0.765

Note. *One subject did not provide a response.

analyses of AKI, we also considered average heat index over the shifts worked, average and maximum heart rate over the shifts worked, whether core body temperature was ever $\geq 38°C$ during a workday, baseline USG, average USG post-workdays, and the average change in USG pre- to post- workday over the three workdays. A seed (20201005) was set for random number generation.

All analyses were performed using R (v 4.0.2; R Core Team, 2018) in the RStudio (RStudio Team, 2017) integrated development environment (v 1.3.1093) with the following packages: tidyverse (Wickham et al., 2019), psych (Revelle, 2020), desctable (Wack, Boukobza, & Xie, 2020), arsenal (Heinzen et al., 2020), sjlabelled (Lüdecke, 2020), RColorBrewer (Neuwirth, 2014), ggpubr (Kassambara, 2020), Hmisc (Harrell Jr. & Dupont, 2020), corrplot (Wei & Simko, 2017), rpart (Therneau et al., 2010), partykit (Hothorn & Zeileis, 2015), rpart.plot (Milborrow, 2016), randomForestSRC (Ishwaran & Kogalur, 2014), and ggRandomForests (Ehrlinger, 2016). P values < 0.05 were considered significant.

Results

Demographics and Health Histories

Demographics and health characteristics of the participants are detailed in Table 1. While over 2/3 of participants were female, sex, age, and waist-to-hip ratio did not differ significantly between participants who were dehydrated at the baseline assessment and those who were not or between participants who exhibited indications of AKI after a workday and those who did not. Differences in BMI by hydration status did not reach the threshold for significance but were trending ($p = 0.056$), while participants with AKI had higher BMIs than participants without ($p = 0.006$). Participants who were dehydrated at baseline were more likely to report a history of hypertension ($p = 0.015$) and diabetes/abnormal glucose levels ($p = 0.006$) and were less likely to have experienced multiple HRI symptoms in the past week ($p = 0.044$). There were no differences in histories of hypertension, diabetes, or recent HRI symptoms between participants with AKI and those without. When evaluating other factors that could contribute to HRI and/or dehydration (Supplemental Table 3), the primary finding was that participants dehydrated at baseline had last worked more recently than adequately hydrated participants.

AKI Association With Baseline USG

Average heat index, high core body temperature, and mean and maximum heart rate on workdays were not significantly different in participants who met criteria for AKI compared with those who did not (Supplemental Table 4). On the other hand, USG values at baseline were significantly higher ($p = 0.029$) among individuals with AKI in the subsequent three days of work. The average USG after those work shifts also trended higher among individuals with AKI, though the difference was not statistically significant ($p = 0.06$; Figure 1). There were no significant differences in average post-workday USG by baseline dehydration status ($p = 0.21$); however, very high average post-workday USG values (>1.030) were found only among participants dehydrated at baseline (Supplemental Figure 2).

Baseline Urine and Serum Biomarkers

Dehydrated participants had significantly lower levels of urine uromodulin ($p = 0.0025$) and sodium ($p = 0.014$) and significantly higher levels of serum IL-6 ($p = 0.024$) and CRP ($p = 0.011$) compared to euhydrated participants (Figure 2(a), Supplemental Table 5). Accounting for history of hypertension attenuated the differences in IL-6 ($p = 0.052$), CRP ($p = 0.107$), and sodium ($p = 0.070$), and accounting for experience of three or more HRI symptoms in the previous week attenuated differences in CRP ($p = 0.062$). Differences in uromodulin

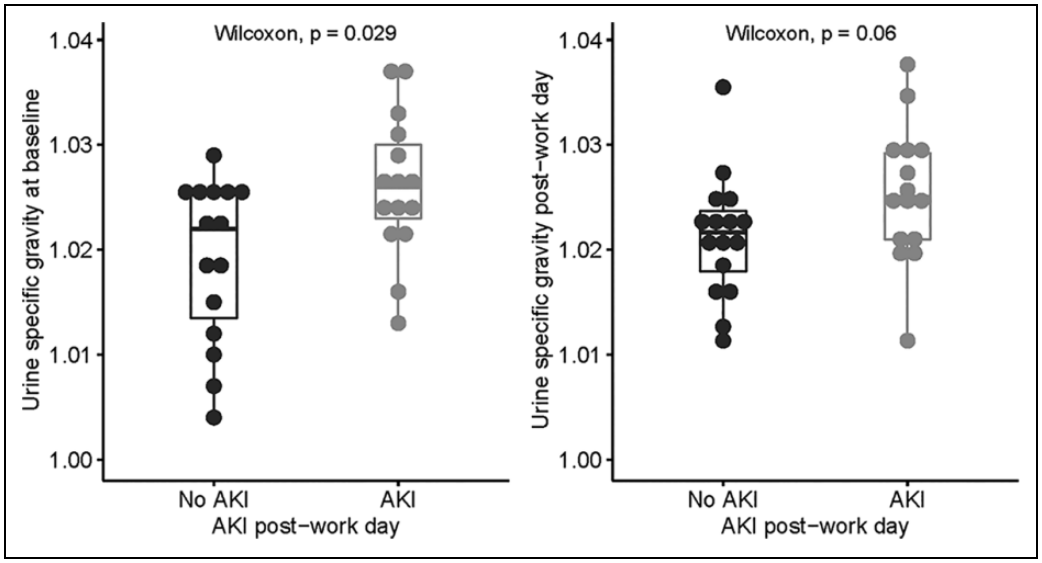

Figure 1. Participants with AKI had higher urine specific gravity at baseline. Urine specific gravity compared by Wilcoxon rank sum test at the baseline assessment and post-workday averaged over three days in participants who met the criteria for acute kidney injury on at least one of the three workdays and those who did not.

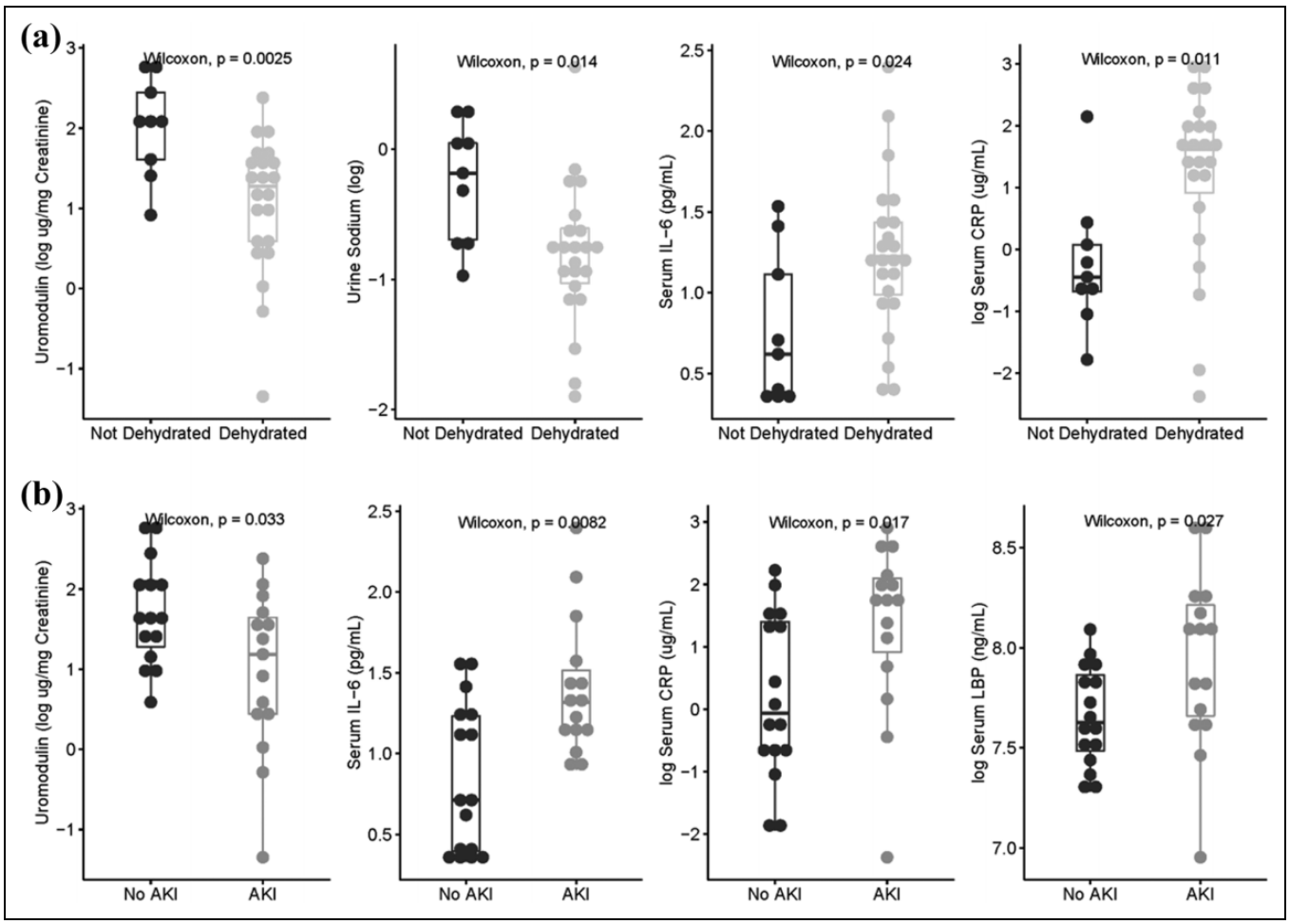

Figure 2. Biomarkers significantly elevated in participants with baseline dehydration or post-workday AKI. Urine and serum analytes that differed significantly by Wilcoxon rank sum test in (a) participants dehydrated or euhydrated at the baseline assessment and (b) participants who met the criteria for acute kidney injury on at least one of the three workdays and those who did not.

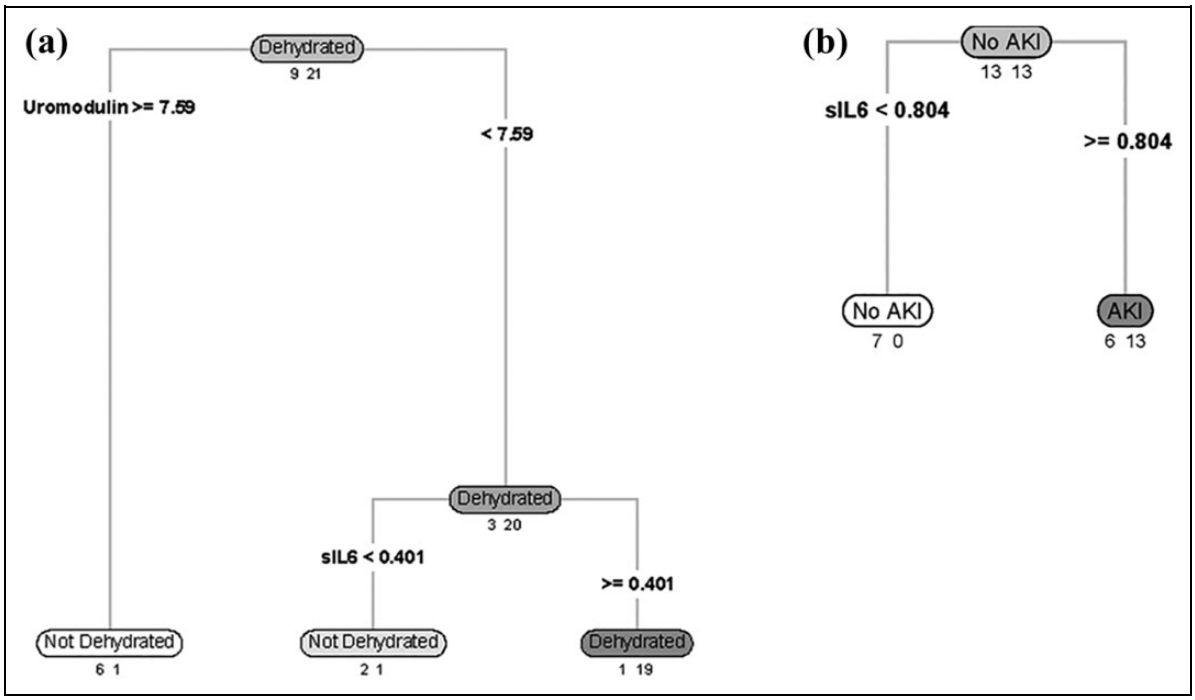

Figure 3. Classification trees for baseline dehydration and post-workday AKI. Classification trees to distinguish (a) participants dehydrated or euhydrated at the baseline assessment and (b) participants who met the criteria for acute kidney injury on at least one of the three workdays and those who did not. Numbers below node labels reflect the number of participants without (left) and with (right) dehydration or AKI. Serum IL-6 concentrations in pg/mL; urine uromodulin concentration in μg/mg creatinine.

remained significant regardless of health history (Supplemental Table 6A). The impact of controlling for diabetes/abnormal glucose levels could not be assessed as no euhydrated subjects reported having this history.

Levels of serum IL-6 ($p = 0.009$), CRP ($p = 0.017$), and LBP ($p = 0.027$) at baseline were significantly higher in participants who subsequently developed AKI while levels of urine uromodulin were significantly lower ($p = 0.033$ Figure 2(b), Supplemental Table 5). The difference in IL-6 levels remained significant ($p = 0.022$) after accounting for baseline USG or BMI, but differences in uromodulin, CRP, and LBP were attenuated (Supplemental Table 6B).

Recursive Partitioning Analysis

Two baseline serum and urine markers were used to classify participants' baseline dehydration status—urine uromodulin and serum IL-6 (Figure 3(a)). All but one subject with uromodulin levels of at least 7.59 μg/mg creatinine were adequately hydrated; 95% of subjects with uromodulin levels below this threshold and serum IL-6 levels of at least 0.401 pg/mL were dehydrated. Subjects with lower uromodulin but IL-6 levels below this threshold were more likely to be adequately hydrated. In keeping with the results of the individual biomarker comparisons, random forest analysis indicated that the most important variables for prediction of dehydration at baseline were urine uromodulin, serum CRP, serum IL-6, and urine sodium (Figure 4(a)). BMI and serum cystatin-C and CXCL8 were also marginally useful for classification; the other variables were not.

To distinguish participants who met the criteria for AKI after a workday from those who did not, a classification tree was built utilizing baseline blood and urine biomarkers and USG, as well as BMI and workday measures of heat exposure, exertion, and dehydration. Only serum IL-6 was retained to classify participants (Figure 3(b)). All participants with AKI had serum IL-6 levels of at least 0.804 pg/mL; however, 46% of participants without AKI indications also had IL-6 levels above this threshold. Serum IL-6 was the variable ranked most important for prediction of AKI by random forest analysis. Behind it were BMI, USG at baseline, serum CRP and LBP, urine uromodulin, serum uric acid and IL-10, the heat index, and serum IFNγ (Figure 4(b)). Efforts to identify connections between the key analytes linked to dehydration and AKI—urine uromodulin and serum IL-6—and specific lifestyle factors and work activities yielded few significant associations (Supplemental Table 7, Supplemental Figure 3).

Biomarker Correlation Analysis

To further explore the relationships among variables in this study, correlations between variable pairs of interest were examined. Participants working when the average heat index was higher experienced a greater increase in USG from pre- to post-workday ($r = 0.44$, $p = 0.013$), and subjects with higher levels of urine NGAL at baseline had a higher mean heart rate

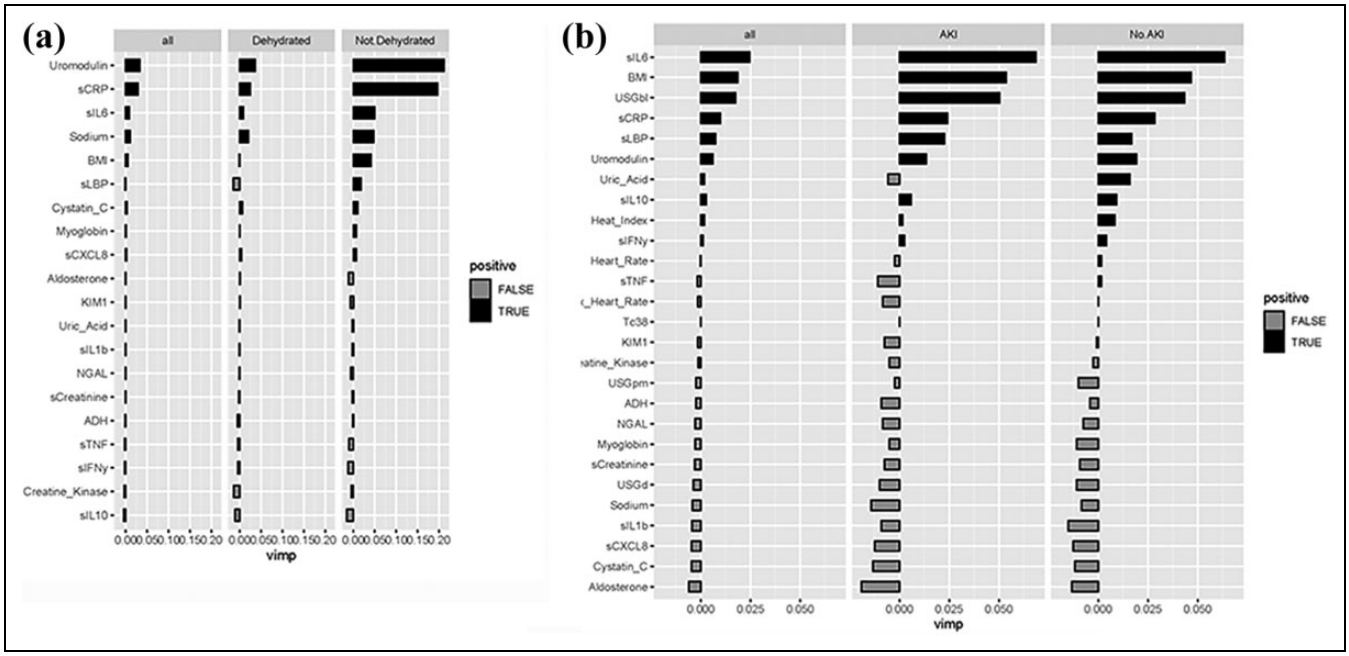

Figure 4. Variable importance for classifying baseline dehydration and post-workday AKI. Variable importance (vimp) determined by random forest analysis for (a) participants dehydrated or euhydrated at the baseline assessment and (b) participants who met the criteria for acute kidney injury on at least one of the three workdays and those who did not. Positive vimp (>0) indicates that a variable was useful for distinguishing the different categories of participants.

in the subsequent workdays ($r = 0.44$, $p = 0.018$; Figure 5(a)). Levels of myoglobin and cystatin-C in serum were significantly correlated ($r = 0.63$, $p = 0.00017$; Figure 5(b)), and there was evidence of interactions among circulating bacterial products, systemic inflammation, and kidney dysfunction (Figure 5(c)). Levels of LBP in serum were strongly correlated with levels of CRP ($r = 0.7$, $p = 0.0000089$) as well as with IL-6 ($r = 0.44$, $p = 0.011$). Levels of CRP and IL-6 were also correlated with one another ($r = 0.4$, $p = 0.025$). Higher concentrations of IL-6 in serum were associated with lower levels of uromodulin ($r = -0.48$, $p = 0.006$) and with more negative changes in eGFR pre- to post-workday ($r = -0.43$, $p = 0.015$). Significant relationships between select serum cytokines and serum indicators of kidney function were also found; TNF correlated with cystatin-C ($r = 0.36$, $p = 0.048$), IL-1β with uric acid ($r = 0.38$, $p = 0.034$), and IFNγ with creatinine ($r = 0.49$, $p = 0.0055$; Supplemental Figure 3).

Discussion

In this study, we identified specific inflammatory and renal function markers that were associated with baseline dehydration and were predictive of the occurrence of AKI on subsequent workdays in a cohort of U.S. agricultural workers, a workforce mostly immigrant from Mexico and Central America. Participants who were dehydrated at the baseline assessment had significantly lower levels of uromodulin and sodium in urine. Uromodulin is one of the most abundant proteins in urine, and its full range of functions is still being investigated (Micanovic et al., 2020). It is known to regulate sodium reabsorption (Pruijm et al., 2016), supporting the observation of corresponding changes in both uromodulin and sodium levels. Relatively little research has been published on the effects of dehydration on uromodulin regulation, but it has been reported that its excreted levels correlate with urine volume and decrease in humans under conditions of water restriction (Lynn et al., 1982; Pruijm et al., 2016). The effects of chronic dehydration and uromodulin reduction warrant further investigation, particularly as evidence is accumulating that uromodulin protects against kidney injury (Micanovic et al., 2020). Higher levels of uromodulin are associated with lower long-term risk of declines in kidney function (Garimella et al., 2015; Garimella, Katz, et al., 2017) and with lower immediate risk of AKI following cardiac surgery (Bennett et al., 2018; Garimella, Jaber, et al., 2017). Our findings indicate that higher uromodulin levels may also protect against AKI associated with agricultural work in hot conditions, as baseline uromodulin levels were significantly lower among participants who subsequently developed AKI.

A potential mechanism by which uromodulin may minimize kidney damage is through its role as an anti-inflammatory immune regulator (Micanovic et al., 2020). Uromodulin influences the recruitment and function of myeloid cells in the kidney (Micanovic et al., 2015; Micanovic et al., 2018) and also acts as a decoy receptor for urinary inflammatory cytokines, limiting their activity (Liu et al., 2012). This anti-inflammatory effect is not limited to the renal environment; we found a significant inverse correlation between levels of

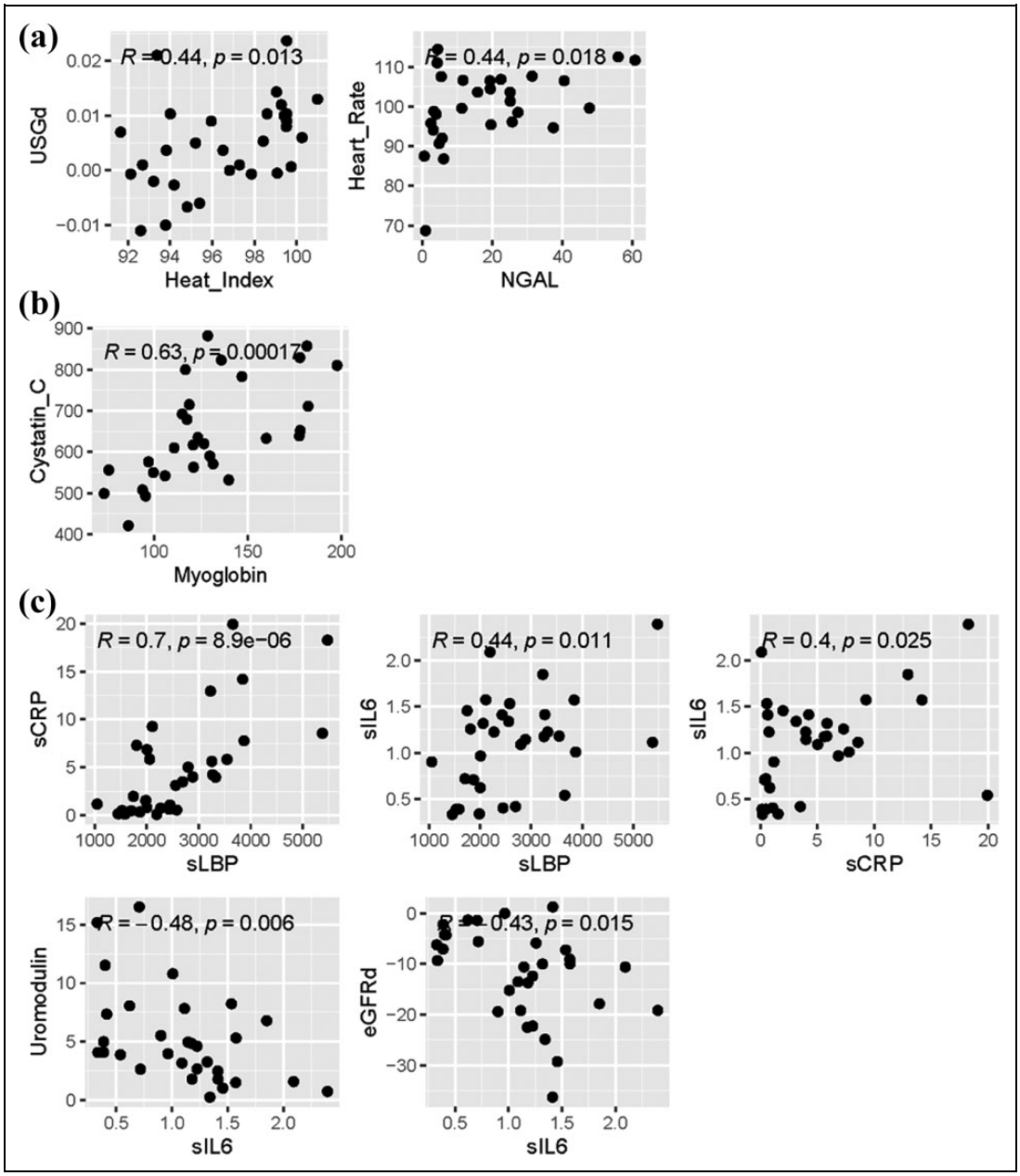

Figure 5. Significant correlations among the different variables. Scatterplots of variables with significant Pearson correlations related to (a) average workday heat index and heart rate, (b) muscle breakdown and kidney function, and (c) inflammatory markers and kidney function.

uromodulin in urine and IL-6 in serum which aligns with findings from uromodulin knock-out experiments in mice (Liu et al., 2012) and correlations between uromodulin and circulating inflammatory cytokine levels in humans (Garimella, Katz, et al., 2017; Jian et al., 2015). In addition to uromodulin, dehydration at baseline was associated with higher levels of IL-6 and CRP in serum, and in our classification tree, uromodulin and IL-6 were the markers retained to distinguish dehydrated subjects. The precise nature of inflammatory reactions to dehydration, their duration, and their effects remain largely unknown. Our findings suggest potential mediators of an inflammatory response associated with dehydration which may prove more damaging to the kidneys in individuals producing lower levels of protective uromodulin.

Differences in the levels of IL-6 and CRP in dehydrated vs. euhydrated participants no longer reached the threshold for significance, however, after accounting for differences in health histories including HRI and hypertension. Participants dehydrated at the baseline assessment were less likely than euhydrated ones to report having had multiple HRI symptoms in the week prior to the assessment. This association was not well-explained by any of the lifestyle factors, beverage choices, or past week work activities assessed in this study. It is possible that experiencing HRI symptoms prompted participants to

increase their fluid intake in the next few days, minimizing dehydration-associated inflammation, though this is only speculative since fluid intake was not measured in this study. Dehydrated participants were also more likely to report a history of hypertension or diabetes/abnormal glucose levels. Both diabetes and hypertension can increase the risk of dehydration and are associated with inflammation (Laragh, 1985; Savoia & Schiffrin, 2007). Information on medications participants might have been taking to treat either of these conditions was not collected in this study, so any impact of these medications on urine concentration was also not evaluated. Additionally, while not statistically significant, the mean BMI was higher among dehydrated than euhydrated participants. It is important to recognize the potential for dehydration to compound health risks in agricultural workers with chronic conditions, and future studies with more participants should also distinguish whether dehydration is associated with increases in inflammatory factors independent of existing comorbidities and their treatments.

Intriguingly, levels of IL-6 and CRP, along with LBP, in serum at the baseline assessment were significantly higher in individuals who exhibited indications of AKI after work on at least one of the next three days. IL-6 was also the only variable retained in the classification tree analysis for AKI, and it was the variable determined to be most important in the random forest analysis, which also identified CRP and LBP as important predictors. IL-6, CRP, and LBP concentrations were also intercorrelated in participants in this study. This suggests the involvement of a physiological cascade involving the intestine, which is receiving increasing attention in the context of exertion and heat (Armstrong et al., 2018; Vargas & Marino, 2016). The lumen of the human intestine is occupied by trillions of microbes. Normally, the intestinal epithelial barrier remains relatively impermeable to these microbes and their products, but conditions including heat stress, strenuous exercise, dehydration, and gastrointestinal inflammation can weaken the gut barrier. This allows inflammatory microbial antigens such as lipopolysaccharide to escape the gut and circulate in the body (Armstrong et al., 2018; Vargas & Marino, 2016). These antigens can trigger powerful inflammatory responses involving the release of cytokines such as IL-6 and CRP (Guy et al., 2017). This cascade is thought to be at least partially responsible for exhaustion, diminished performance, and HRI (Hansson et al., 2020; Leon & Bouchama, 2015).

The association between high levels of IL-6 and CRP and both dehydration and AKI raises the possibility that prior dehydration could increase susceptibility to immune-mediated kidney damage. Indeed, we found that subjects who met the criteria for AKI on a workday had significantly higher USG values at baseline compared with subjects without AKI, and baseline USG was ranked highly in importance for AKI prediction in the random forest analysis. Furthermore, correcting for baseline USG accounted for the associations between AKI and uromodulin, CRP, and LBP, emphasizing the importance of dehydration in this process. Though individuals who were dehydrated at baseline were not consistently dehydrated after their work shifts, we did observe that subjects with the highest average USG values after workdays were also dehydrated at baseline, suggesting that at least a subset of participants were frequently dehydrated. Furthermore, we found that participants who had worked more recently prior to the baseline assessment were more likely to be dehydrated than those who had more time off before the assessment, indicating insufficient recovery of hydration levels after a workday. This finding relating baseline dehydration and workday AKI presents an opportunity for simple educational interventions that could impact AKI risk. The importance of monitoring hydration based on urine color was discussed with participants in this study as part of post-participation education, and this is also included in community trainings on HRI given by FWAF. Our findings suggest that emphasizing the importance of adequate hydration before work shifts and on non-workdays could be a beneficial addition to educational programs for agricultural workers.

In addition to differences in baseline USG, participants who developed AKI had significantly higher BMIs than those who did not, and associations between AKI and uromodulin, CRP, and LBP were no longer significant after accounting for BMI. The majority of the participants in this study were categorized as overweight (BMI 25 to <30 kg/m^2), a condition which is known to promote systemic inflammation (Ellulu et al., 2017) and to increase vulnerability to HRI (Kenny et al., 2010) and to chronic kidney disease (Cignarelli & Lamacchia, 2007). The prevalence of overweight and obesity among agricultural workers in this study and its connection with AKI is concerning and highlights an area of need.

Notably, we found that the differences in IL-6 levels between subjects with and without AKI remained significant after accounting for baseline USG or BMI, indicating that these factors alone cannot explain the elevated levels of this inflammatory cytokine prior to AKI. Examination of relationships with other lifestyle factors and work activities revealed only that participants with higher IL-6 levels drank fewer sports drinks at work and reported spending more time planting or potting in the week prior to the baseline assessment. Increases in cytokine release immediately after exercise or heat exposure can normalize quickly, within 24 hr (Guy et al., 2017; Ostrowski et al., 1998), so increased levels of IL-6 as well as CRP and LBP on a non-workday are unlikely to reflect an acute increase related to strenuous occupational activity in the heat. Rather, sustained high levels of inflammatory mediators on non-workdays may be influenced by other factors not captured in this study including diet (Armstrong et al., 2018; Shapiro et al., 2014) or environmental exposures such as pesticides (Gangemi et al., 2016). They could also reflect a potentially harmful sustained response to chronic heat stress. Repeated heat exposure and regular exercise, while eliciting acute changes in intestinal permeability and inflammation, can also produce adaptive responses that protect against HRI in subsequent exposures (Armstrong et al., 2018; Hashim, 2010; Starkie et al., 2003). In fact, IL-6, in addition to its inflammatory effects, can also contribute to the resolution of inflammation (Starkie et al., 2003; Vargas & Marino, 2016). Nonetheless, in our cohort, IL-6 levels did not appear to be protective with

respect to kidney injury; higher baseline IL-6 levels were associated with reduced kidney function both at baseline and on the following workdays. Whether IL-6 is directly involved in kidney damage or merely correlates warrants further investigation.

Most of the variables in this study were not found to be useful in predicting which subjects would exhibit indications of AKI. As has been previously described for CKD*u* (Glaser et al., 2016), history of diabetes and hypertension did not differ among subjects with and without AKI, and, interestingly, neither did the average heat index, the incidence of high core body temperature values, nor the mean nor maximum heart rate during work shifts. While these values do reflect averages over three days and as such may lose some precision in their associations, this finding suggests that ambient temperature, body temperature, and exertion are not the best predictors of who will develop AKI. The random forest analysis, however, did identify heat index as useful in distinguishing subjects with and without AKI. One reason that heat index may have predictive value is because of its impact on hydration, as we observed that heat index was positively correlated with the change in USG from pre- to post-work shift. We also did not find strong associations between indicators of muscle breakdown and dehydration or AKI, though myoglobin and cystatin-C in serum were significantly correlated.

In addition to those already discussed, limitations of this study included its relatively small size which precluded subset analyses, the division of the data set into training and testing sets for the recursive partitioning analyses, and the examination of certain variables of interest such as sex. Over 2/3 of our participants were female, and the work activities, behaviors, and vulnerability to AKI may differ by sex in important ways. Larger cohorts will be needed to confirm the findings of this exploratory study and to evaluate the contribution of additional variables. Due to the sample size and exploratory nature of this study, we also did not correct for multiple hypothesis testing. This increases the risk for identification of false-positives, though the use of multiple approaches to identify analytes associated with baseline dehydration and post-workday AKI increases confidence in those key findings. Other limitations may exist due to the relationship between urine concentration and indicators of kidney function. We attempted to correct for this by normalizing to urine creatinine. There is also the possibility of minute impacts of analyte concentration on USG, which was used to determine dehydration. There are other methods to identify dehydration such as estimating fluid loss by a change in body weight, but these were not found to be feasible for our study (Mac et al., 2017).

In conclusion, this study demonstrated that select immune mediators and markers of renal function differed significantly in dehydrated and euhydrated agricultural workers on a non-workday, that baseline dehydration was a factor related to subsequent development of AKI, and that high serum levels of two immune-related molecules—IL-6 and CRP—and low levels of urine uromodulin were found in participants who were dehydrated and in those who experienced AKI after a workday. The overlap in these findings presents a focus for future studies seeking to understand and prevent chronic kidney disease in this important, vulnerable population.

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Supplemental Material

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