HHS Public Access

Author manuscript

Environ Int. Author manuscript; available in PMC 2023 November 10.

Published in final edited form as:

Environ Int. 2023 October ; 180: 108206. doi:10.1016/j.envint.2023.108206.

The impact of heat exposures on biomarkers of AKI and plasma metabolome among agricultural and non-agricultural workers

Roxana C. Chicas[a,*], **Yilin Wang**[b], **E. Jennifer Weil**[c], **Lisa Elon**[b], **Nezahualcoyotl Xiuhtecutli**[a,d], **Madelyn C. Houser**[a], **Dean P. Jones**[e], **Jeff M. Sands**[c], **Vicki Hertzberg**[a], **Linda McCauley**[a], **Donghai Liang**[b]

[a] Nell Hodgson Woodruff School of Nursing, Emory University, Atlanta, GA, USA

[b] Rollins School of Public Health, Gangarosa Department of Environmental Health, Emory University, Atlanta, GA, USA

[c] Division of Renal Medicine, Department of Medicine, Emory University, Atlanta, GA, USA

[d] Farmworker Association of Florida, Apopka, FL, USA

[e] Department of Medicine, Emory University, Atlanta, GA, USA

Abstract

Background: Agricultural workers are consistently exposed to elevated heat exposures and vulnerable to acute kidney injury. The underlying pathophysiology and detailed molecular mechanisms of AKI among agricultural workers, and the disproportionate burden of HRI and heat stress exposure are not well understood, especially at the level of cellular metabolism.

Objective: The aim of this study was to examine the impact of heat exposures on renal biomarkers and on the human metabolome via untargeted high-resolution metabolomics among agricultural and non-agricultural workers.

Methods: Blood and urine samples were collected pre- and post-work shift from 63 agricultural workers and 27 non– agricultural workers. We evaluated pre- and post-work shift renal biomarkers

* Corresponding author. rchicas@emory.edu (R.C. Chicas).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Considerations & Disclosure

The Institutional Review Board at Emory University provided approval (IRB00075192) for the study, and all participants provided informed consent.

CRediT authorship contribution statement

Roxana C. Chicas: Writing – original draft, Investigation, Conceptualization, Writing – review & editing. **Yilin Wang:** Formal analysis, Visualization, Methodology, Writing – review & editing. **E. Jennifer Weil:** Conceptualization, Methodology, Writing – review & editing. **Lisa Elon:** Formal analysis, Data curation, Methodology, Writing – review & editing. **Nezahualcoyotl Xiuhtecutli:** Investigation, Methodology, Writing – review & editing. **Madelyn C. Houser:** Conceptualization, Methodology, Writing – review & editing. **Dean P. Jones:** Investigation, Supervision, Methodology, Writing – review & editing. **Jeff M. Sands:** Supervision, Funding acquisition, Methodology, Writing – review & editing. **Vicki Hertzberg:** Supervision, Funding acquisition, Conceptualization, Methodology, Writing – review & editing. **Linda McCauley:** Supervision, Funding acquisition, Conceptualization, Methodology, Writing – review & editing. **Donghai Liang:** Writing – original draft, Formal analysis, Data curation, Visualization, Conceptualization, Writing – review & editing.

and completed untargeted metabolomics using high-resolution mass spectrometry with liquid chromatography. Metabolome-wide association studies (MWAS) models identified the metabolic features differentially expressed between agricultural workers and non-agricultural workers.

Results: Median values of pre-shift creatinine and osteopontin ($p < 0.05$) were higher for agricultural workers than non-agricultural workers. Metabolic pathway enrichment analyses revealed 27 diverse pathways differed between agricultural workers and non-agricultural workers ($p < 0.05$) including TCA cycle and urea cycle, carbohydrate metabolism, histidine metabolism and evidence for altered microbiome shikimate pathway.

Conclusion: This is the first investigation on the metabolic pathways that are affected among agricultural workers who are exposed to heat compared to non-heat exposed workers. This study shows extensive responses of central metabolic systems to heat exposures that impact human health.

Keywords

Metabolomics; Renal function; Environmental heat; Agricultural workers

1. Introduction

Recent investigations in the United States (U.S.) and around the globe have shown a link between heat exposure, heat-related illness (HRI), dehydration, and renal dysfunction among agricultural workers (Flouris et al., 2018; López-Gálvez et al., 2021; Mix et al., 2018; Moyce et al., 2017). A climatic chamber study with 13 healthy adults also found an increase risk of developing acute kidney injury (AKI) during physical work in the heat and dehydration (Chapman et al., 2020). An epidemic of chronic kidney disease of unknown etiology (CKDu) among agricultural workers has been noted globally (Johnson et al., 2019; Keogh et al., 2022), and in the U.S., there are indications of HRI and acute kidney injury (AKI) plaguing agricultural workers, even when they are considered fully acclimatized to working in a hot climate (Mix et al., 2018; Moyce et al., 2017). There have also been reports of renal dysfunction in non-agricultural occupations where workers are exposed to high ambient heat (Shi et al., 2022), such as bricklayers (Gallo-Ruiz et al., 2019), construction workers (Al-Bouwarthan et al., 2020), and sea salt workers (Luangwilai et al., 2022).

The etiology of CKDu and AKI among agricultural workers remains unknown; however, several risk factors have been identified, such as exposure to high ambient heat and increased metabolic heat due to physical exertion, chronic dehydration, pesticides, and consumption of sugar sweetened drinks, among other factors (Johnson et al., 2019). In a study in California's Central Valley, 11.8% of agricultural workers had impaired renal function based on pre- to post-work shift increases in creatinine of at least 0.3 mg/dL or 1.5 times the pre-shift value (Kidney Disease: Improving Global Outcomes (KDIGO) criteria (Khwaja, 2012) for AKI (Moyce et al., 2016). This same research group also showed an association of heat strain and AKI (Moyce et al., 2017). In Florida, a study of 192 agricultural workers found that on at least one of three workdays, 33% of workers developed AKI, and the odds of AKI increased 47% for each $5^{\circ}F$ increase in heat index (Mix et al., 2018). The type of agriculture, age, gender, and self-reported chronic

health conditions did not have any significant effects (Mix et al., 2018). The underlying pathophysiology and detailed molecular mechanisms of AKI among agricultural workers, and the disproportionate burden of HRI and heat stress exposure are not well understood (Aguilar-Ramirez, 2020; Garcia-Trabanino et al., 2015; Glaser et al., 2016; Kupferman et al., 2018; Mix et al., 2018; Moyce et al., 2016), especially at the level of cellular metabolism.

With the advent of high-throughput sequencing and analytical technology, various omics approaches have shown great promise in providing critical information detailing intricacy and perturbations at multiple molecular levels. Specifically, metabolomics, the study of metabolic features associated with exogenous exposure and endogenous processes, has emerged as an innovative analytical platform linking complex environmental exposure to internal dose and biological responses (Gaskins et al., 2021; Hood et al., 2022; Hwang et al., 2022; Li et al., 2022; Liang et al., 2018, 2019, 2022; Tang et al., 2022). Hence, metabolomics analysis may offer novel insights into the mechanistic underpinnings of physiological conditions associated with heat stress exposure and AKI. However, no metabolomics study has been published examining the impact of heat exposures on the human metabolome among agricultural workers, who are consistently exposed to elevated heat exposures and vulnerable to AKI.

To address these critical knowledge gaps, we conducted this study to examine the impact of heat exposures on biomarkers of AKI and on the human metabolome via untargeted high-resolution metabolomics among agricultural and non-agricultural workers. We aimed to identify metabolic perturbations associated with differential levels of heat exposure and explore whether these molecular signatures were indicative of renal alterations between agricultural and non-agricultural workers.

2. Methods

We conducted a pilot study embedded in a larger epidemiological study of heat exposure and symptoms of heat stress and AKI among agricultural workers employed in agricultural counties in Florida (CDC/NIOSH Girasoles #R01OH010657 and CDC/NIOS U54OH011230) (Mix et al., 2018). After obtaining IRB approval from the Emory University Institutional Review Board (#IRB00075192), trained community health workers from the Farmworker Association of Florida (FWAF) used community outreach strategies to recruit a convenience sample of agricultural workers (exposed to heat) and non-agricultural workers (non-heat exposed – control group). A total of 87 participants were monitored on one workday in Homestead, Florida during the study period in the Summer of 2018. Agricultural workers were exposed to hot environmental temperatures as part of their usual employment in fernery, nursery, crop, and landscape operations. Non-agricultural workers were participants that worked indoors in an air-conditioned environment and were generally not exposed to hot environmental temperatures during work hours. Non-agricultural workers participants were office workers, house and office cleaners, or childcare workers.

Agricultural and non-agricultural had the same eligibility criteria. The study was limited to workers who were 18–54 years of age because age differences can add additional

confounding in metabolomics studies, and older adults can respond differently to heat stress due to changes in immune function, cardiovascular compensation, and temperature regulation with age. To avoid confounding related to acclimatization, workers had to have been working in their respective occupation for at least 4 weeks.

Workers were excluded if they self-reported being pregnant or receiving treatment for hypertension, type 1 or type 2 diabetes. Community health workers explained study procedures, obtained informed consent, and collected data in the participant's primary language. Each participant received $50 in gift cards.

2.1. Baseline assessment

During the baseline assessment, the community health workers administered surveys to obtain socio-demographic variables and to characterize the occupational environment of these workers. Baseline anthropometrics, such as height, weight, and body fat percentage by impedance (Omcron Fat Loss monitor HBF-306CN) were also obtained.

2.2. Workday assessments

Pre- and post their work shift, participants arrived at the FWAF office and provided urine and blood samples. Certified nurses trained in the study protocol collected blood via a fingerstick (Unistik 2 Extra Safety Lancets). Blood for serum preparation was collected in red top microtainers with no additive (BD Microtainer®), then incubated at room temperature for 30–60 min. Blood for plasma was collected in lavender top microtainers (K2EDTA additive | BD Microtainer®) and mixed by inverting 10 time and then incubated at room temperature for 30–60 min. Both serum and plasma samples were centrifuged for 10 min at 1,500×g. Supernatant were immediately aliquoted to cryotubes (VWR® Micro Centrifuge Tube). Urine, plasma, and serum samples were flash frozen and stored on dry ice for shipment to the laboratory, where they were stored at −80 °C until analysis. The workers were equipped with a Polar® T31 heart rate monitor that recorded heart rate every 30 s during work hours.

Dry bulb and wet bulb temperature data during the study workdays were retrieved from the Homestead weather station of the Florida Automated Weather Network (FAWN) and used to calculate a daily maximum estimated wet bulb globe temperature (WBGT) for each participant workday based on their self-reported starting and ending work hours. The WBGT is an estimate based on a formula validated under Florida conditions to have a 95% confidence interval of (±2°C-true WBGT) (Bernard and Barrow, 2013). FAWN is a 42-station weather data collection network spanning mainland Florida for agricultural purposes.

2.3. Biomarkers of hydration

A Reichert TS Meter-D automatic digital refractometer (Fisher Scientific, Pittsburgh, PA) was used to measure pre-shift and post-shift urine specific gravity (USG). We categorized USG into three groups: normal level of hydration as <1.020; clinical dehydration as ≥1.020; and extreme dehydration as >1.030 (Sawka et al., 2007).

2.4. Biomarkers of renal function and injury

eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (Levey et al., 2007) equation based on the creatinine value obtained from an iSTAT Blood Analyzer (Abbott Point of Care, Inc, East Windsor NJ). Prior to use, the iSTAT Blood Analyzer passed the electronic simulator quality control test to check on the ability of the analyzer to take accurate and sensitive measurements. eGFR was truncated at 90 mL/min/1.73 m^2 to reflect clinical practice of ≥ 90 mL/min/1.73 m^2 as normal kidney function and < 90 mL/min/1.73 m^2 as reduced kidney function. AKI over the work shift was defined using the Kidney Disease Improving Global Outcomes (KDIGO) criteria (Kellum and Lameire, 2013). AKI was considered to be present if creatinine values increased from pre-shift to post-shift by at least 0.3 mg/dL or increased 1.5 times (Kellum and Lameire, 2013).

Beta-2 microglobulin (B2M), cystatin C, neutrophil gelatinase-associated lipocalin (NGAL/Lipocalin-2), osteopontin (OPN) and uromodulin (UMOD) were measured in pre- and post-workday urine samples using the QuickPlex instrument (MesoScale Diagnostics, LLC; Rockville, MD) in the Emory Multiplexed Immunoassay Core (EMIC). The EMIC lab uses MesoScale Diagnostics QuickPlex electro-chemiluminescence instrumentation, which has < 10% variability within and between plates. All testing was done in duplicate. Kidney injury molecule – 1 (KIM-1), creatinine, and uric acid biomarker testing of pre- and post-workday urine was completed at the School of Nursing biobehavioral laboratory. Creatinine-normed values are reported for urinary biomarkers. Creatinine-normed values are preferred for spot urine samples as they correct for changes in urine concentration due to urinary concentration or dilution related to fluid intake.

2.5. High-resolution metabolomics profiling

Pre- and post-shift plasma samples were analyzed in batches of 20 using high-resolution liquid chromatography coupled with mass spectrometry (HR-LCMS, Thermo Scientific™ Q- Exactive™ HF) at the Emory Clinical Biomarker laboratory via established protocol (Go et al., 2015; Liu et al., 2020). Plasma samples were treated with acetonitrile (2:1, v/v), spiked with internal standard mix, and centrifuged at 14,000×g for 5 min at 4 °C to remove proteins. To enhance the coverage of metabolic feature detection, the analysis was performed using both polar and nonpolar analytical columns and analysis modes: hydrophilic interaction liquid chromatography (HILIC) with positive electrospray ionization (ESI) and C18 hydrophobic reversed-phase chromatography with negative ESI. Samples were analyzed in triplicate to evaluate reproducibility for each metabolic feature in each sample, and samples were randomized to minimize batch effects. Rigorous standard operating procedures were implemented and provided detection of >20,000 m/z features (Go et al., 2015; Liu et al., 2020).

2.6. Metabolomics feature extraction

Detected signals (referred to as metabolic features) were extracted using apLCMS (Yu et al., 2009) with modifications by xMSanalyzer (Uppal et al., 2013), which performed peak detection, mass-to-charge ratio (m/z), retention time (RT) alignment, feature quantification, and data quality filtering. Data pre-processing steps included median summarization of technical replicates, filtering of features based on missing values cut-offs (overall 30%

and group-wise 80%) and relative standard deviation across all samples to remove features with a constant signal across all samples. Log base 2 transformation was used to reduce heteroscedasticity and normalize the data. Quantile normalization of samples was performed to minimize between sample variability.

2.7. Statistical analysis

For the primary statistical analysis, we conducted a series of metabolome-wide association studies (MWAS) models. Specifically, to identify the metabolic features differentially expressed between agricultural workers and non-agricultural workers, four sets of generalized linear models were constructed for pre- and post-shift periods among features detected in the HILIC and C18 chromatography columns, respectively. The pre-shift generalized linear model was constructed using the following form:

$$\log_2 Y_{ij} = \beta_0 + \beta_{1j} Agri\_Term_i + \gamma_{1j} Age_i + \gamma_{2j} Sex_i + \gamma_{3j} BMI_i + \varepsilon_{ij} \quad (1)$$

A similar generalized linear model was constructed for post-shift using the following form:

$$\log_2 Y_{ij} = \beta_0 + \beta_{1j} Agri\_Term_i + \gamma_{1j} Age_i + \gamma_{2j} Sex_i + \gamma_{3j} BMI_i + \gamma_{4j} Work\_Duration_i + \varepsilon_{ij} \quad (2)$$

where $log_2 Y_{ij}$ refers to the log base 2 intensity of metabolic feature j for participant i, β_0 is the intercept, and $Agri\_Term_i$ is a categorical variable representing whether participant i is an agricultural worker. We included covariates in the model to control for potential confounding factors and covariates, including age, sex (categorical), and body mass index (BMI_i). In the post-shift model, we included work duration as a covariate. ε_{ij} represents residual random error.

In addition to the main models, we also conducted four sets of models to evaluate the impact of heat exposure from the previous day and current day on the pre- and post-shift metabolic profiles among agricultural workers (N = 63) for features detected in the HILIC and C18 chromatography columns, respectively. The generalized linear model constructed for pre-shift metabolic profiles and previous day heat exposure uses the following form:

$$\log_2 Y_{ij} = \beta_0 + \beta_{1j} WBGT\_SUN\_B4WD\_max_i + \gamma_{1j} Age_i + \gamma_{2j} Sex_i + \gamma_{3j} BMI_i + \gamma_{4j} Work\_Duration_i + \varepsilon_{ij} \quad (3)$$

where $\log_2 Y_{ij}$ refers to the log base 2 intensity of metabolic feature j for participant i, β_0 is the intercept, and $WBGT\_SUN\_B4WD\_max_i$ is the maximum WBGT for participant i on the previous day. The same covariates previously described were included in this model.

As for the model constructed for post-shift metabolic profiles and current day heat exposure, we use the following form:

$$\log_2 Y_{ij} = \beta_0 + \beta_{1j} WBGT\_SUN\_max_i + \gamma_{2j} Age_i + \gamma_{2j} Sex_i + \gamma_{3j} BMI_i + \gamma_{4j} Work\_Duration_i + \varepsilon_{ij} \quad (4)$$

where $WBGT\_SUN\_max_i$ is the maximum outdoor WBGT on the participant i workday. Separate models were conducted for each column (HILIC positive ESI and C18 negative ESI). We implemented the Benjamini-Hochberg procedure to correct for multiple comparison. All analyses were completed in R (version 4.2.1).

2.8. Metabolite pathway enrichment analysis

To predict the functional activity of metabolic features, we conducted pathway enrichment analysis and metabolite annotation. Here we use mummichog (v. 2.0), a novel bioinformatics platform to predict functional biological activities of metabolites without prior identification. Mummichog analyses were conducted separately for significant features from each of the MWAS statistical models in the HILIC and C18 columns. We used two strategies to select eligible metabolic features for pathway analysis: raw p-value at 0.05 and multiple testing-corrected p-value at 0.05 using the Benjamini-Hochberg method for multiple comparison correction. To minimize the chance of false positive discovery, we excluded pathways identified by mummichog analyses with adjusted p-value>0.05 and those with<4 selected features matched in pathway enrichment. We also conducted a sensitivity analysis by using 0.5th and 1st percentile of raw p-values to perform pathway enrichment and examine whether the significant pathways would be largely different under different raw p-values.

2.9. Metabolite annotation and confirmation

Metabolic features significantly differing between agricultural workers and non-agricultural workers, and/or associated with heat exposures were annotated by matching accurate mass m/z value for adducts commonly formed to the METLIN, ChemSpider, Human Metabolome Database (HMDB), and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases, using a mass error threshold of 10 ppm. To minimize further false positive matches, each matched feature was screened on their retention time, isotope patterns, and spectrum peak quality by examining the extracted ion chromatographs (EICs). Finally, a select number of annotated metabolites were confirmed with level one evidence (Morrison et al., 2007) by comparing their m/z, retention time and ion dissociation patterns to analytical standards in an in-house library that contains a list of exogenous or endogenous metabolites analyzed under identical experimental conditions.

3. Results

3.1. Participants

Workday data were collected from 63 agricultural workers and 27 non– agricultural workers (Table 1). Compared to the controls, the agricultural worker sample was on average 9 years older (p = 0.003), had more males (35% vs.17%), a higher percentage reported Mexican nationality (56% vs. 30%, p = 0.04), and had a higher BMI (29.3 vs. 26.6, p = 0.02).

3.2. Work conditions

Forty percent of agricultural workers worked in nurseries, and 83% were paid by the hour. Controls worked in a variety of non-agricultural settings, with 58% paid by the hour. Agricultural work hours were somewhat longer (8.5 vs. 7.5 hs; 25th percentile, 7.9 vs. 6 hrs), and average heart rate during work was higher (95 vs. 86 bpm; p = 0.02).

3.3. Hydration

On average, agricultural workers drank nearly twice the volume of beverages as did control workers (76 vs. 38 oz; $p < 0.001$). Median sugary beverage consumption over the course of the workday was substantially higher among agricultural workers (12 oz vs. 0 oz; $p = 0.003$). Over half of the workers in both groups were dehydrated (USG ≥ 1.020) before work, and nearly three-quarters were dehydrated after work.

3.4. Biomarkers of renal function and injury

As shown in Table 2, significant differences observed between groups were for pre-work comparisons, with higher median values of pre-shift creatinine and osteopontin ($p < 0.05$) for agricultural workers than the control group.

3.5. MWAS Results

After data quality assurance and quality control, 9,746 and 6,689 metabolic features were detected in HILIC positive ESI and C18 negative ESI, respectively, and were included for the MWAS analyses. The number of metabolic features statistically different between agricultural workers and non-agricultural workers are shown in Table 3. Specifically, 394 features in the HILIC positive ESI and 284 features in the C18 negative ESI significantly differed between agricultural workers and non-agricultural workers in the pre-shift MWAS model, while 336 features in the HILIC positive ESI and 197 features in the C18 negative ESI were differentially expressed between agricultural workers and non-agricultural workers in the post-shift MWAS model after adjusting for covariates (p-value < 0.05).

To examine the impact of heat exposures on the metabolome, we stratified the analysis to only include the 63 agricultural workers. The MWAS models reveal that 636 features in the HILIC positive ESI and 666 features detected in the C18 negative ESI were significantly associated with the maximum WBGT of the previous day after adjusting for covariates (p-value < 0.05). Meanwhile, 526 features in the HILIC positive ESI and 468 features detected in the C18 negative ESI were significantly associated with the maximum WBGT of the current day among the agricultural workers after adjusting for covariates (p-value < 0.05).

Pathway Analysis.—Metabolic pathway enrichment analyses revealed 27 significant metabolic pathways differentially perturbed between agricultural workers and non-agricultural workers (adjusted p-values < 0.05, Fig. 1). Specifically, 6 and 11 differential metabolic pathways were found in the pre-shift period among the features detected in the HILIC positive ESI and C18 negative ESI, respectively, while 10 and 10 differential metabolic pathways were found in the post-shift period among the features detected in the HILIC positive ESI and C18 negative ESI, respectively. Moreover, 9 metabolic pathways were consistently found to differ between agricultural workers and non-agricultural workers in both pre- and post-shift periods across features detected in both HILIC and C18 columns. These differentially perturbed pathways were closely linked to renal dysfunction (i.e., urea cycle metabolism, tricarboxylic acid (TCA) cycle), energy production (i.e., butanoate metabolism, glutamate metabolism), and oxidative stress and systemic inflammation (i.e., cytochrome P450 metabolism, glycerophospholipid metabolism, lysine metabolism).

As shown in Fig. 2, when the analysis included only the agricultural workers, 5 and 16 unique pathways were found to be significantly associated with the maximum WBG temperature of the previous day in the HILIC positive ESI and C18 negative ESI, respectively. More metabolic pathways emerged that were associated with the maximum WBGT of the current day, with 20 and 38 significant pathways found in the HILIC positive ESI and C18 negative ESI, respectively. Among the 47 metabolic pathways found to be associated with WBGT (adjusted p-values < 0.05, Fig. 2), 34% of them (N = 16), including the top 5 renal dysfunction, inflammation, and oxidative stress related pathways (arginine and proline metabolism, butanoate metabolism, glycerophospholipid metabolism, lysine metabolism, and urea cycle metabolism), were consistently found in both the MWAS model using previous day temperature and pre-shift metabolomics profiling and the MWAS model using current day temperature and post-shift metabolomics profiling. More interestingly, a lot more unique and differentially perturbed metabolic pathways (27 vs 4) were found in the latter MWAS model examining current day temperature and post-shift metabolomics profiling, with most of these differentially perturbed pathways closely linked to acute, systemic inflammation (alanine and aspartate metabolism, aspartate and asparagine metabolism), oxidative stress (glycosphingolipid biosynthesis and metabolism, gam-linoleic acid metabolism), and DNA damage and repair (pyrimidine metabolism, purine metabolism).

High similarities and strong consistencies were found when comparing the pathway enrichment findings of the models using agricultural working status and WBGT as exposure variables. Specifically, 20 out of the 27 metabolic pathways (74%) associated with agricultural working status were also found to be associated with the WBGT. These overlapping pathways include all the top pathways closely involved in renal dysfunction (i.e., tricarboxylic acid (TCA) cycle, urea cycle metabolism), energy production (i.e., butanoate metabolism, glutamate metabolism), and oxidative stress (i.e., glycerophospholipid metabolism, lysine metabolism). Additionally, 27 more unique metabolic pathways were found to be associated with WBGT, including pathways involved in acute inflammation (e.g., alanine and aspartate metabolism, asparagine metabolism, leukotriene metabolism), DNA damage and repair (e.g., pyrimidine metabolism, purine metabolism), and oxidative stress (glycosphingolipid biosynthesis and metabolism).

3.6. Chemical confirmation

We confirmed 18 unique metabolites that were differentially expressed between agricultural workers and non-agricultural workers with level 1 evidence (Table 4). The features whose m/z (±10 ppm difference) and retention time (±10 s) matched the authentic compounds analyzed under identical experimental conditions were assigned with Level 1 confidence. Similar to the pathway enrichment findings, most of the confirmed metabolites, including citrulline, uracil, and urocanate, have been closely linked to renal dysfunction, kidney energy production, oxidative stress and systemic inflammation. Meanwhile, we confirmed the chemical identities of 19 metabolites significantly associated with the maximum WBGT of the previous day in the pre-shift metabolic profiles (Table 5), and 33 unique metabolites significantly associated with the maximum WBGT of the current day in the post-shift metabolic profiles (Table 6). Notably, unlike the similarities observed in the pathway

levels, much fewer overlapping metabolites were found between the MWAS model using previous day temperature and pre-shift metabolomics profiling and the MWAS model using current day temperature and post-shift metabolomics profiling. Specifically, only one confirmed metabolite, methyl indole-3-acetate, a catabolite of tryptophan converted by the gut microbiota, were identified in both MWAS models. Although most of the metabolites were different, their corresponding enriched pathways remained similar across the pre-shift and post-shift metabolomics profiling datasets (Tables 5 and 6). Similarly, no identical confirmed metabolites were found across models using agricultural working status and WBGT as exposure variables (Tables 4–6). However, the pathways where these differential metabolites are enriched remained consistent across various MWAS models. For instance, methylhistidine, which was found to be associated with agricultural working status, and methylhistamine, which was found to be associated with WGBT, are both closely involved in histidine and tyrosine metabolism.

4. Discussion

To our knowledge this is the first investigation on the metabolic pathways that are affected among agricultural workers who are exposed to heat compared to non-heat exposed workers. The differentially perturbed pathways identified were closely linked to renal dysfunction (i.e., urea cycle metabolism, TCA cycle), energy production (i.e., butanoate metabolism, glutamate metabolism), and oxidative stress and systemic inflammation (i.e., cytochrome P450 metabolism, glycerophospholipid metabolism, lysine metabolism). One possible interpretation of a portion of these molecular signatures is that precursors of gluconeogenesis are building up systemically and signal dysfunction of the proximal tubule of the kidney. The liver is generally thought of as the major site of gluconeogenesis; however, about 40% of endogenous gluconeogenesis occurs in the kidney proximal tubule (Legouis et al., 2022).

Our research shows the importance of the proximal tubule in health outcomes of agricultural workers. Although the cause of AKI and CKDu among agricultural workers has not been clearly identified, it is generally understood that repetitive kidney tubular injury can lead to tubulointerstitial nephritis and may progress towards CKDu (Sanchez Polo et al., 2020). A few kidney biopsy studies have shown histopathological confirmation of tubulointerstitial nephritis in CKDu (Gunawardena et al., 2021).

Although agricultural workers drank twice the fluids during the day compared to the control group (76 vs 38 oz), both had similar proportion of workers dehydrated at the end of the workday. It may be that the control group was not as thirsty as the agricultural workers or did not have time to drink fluids during their work shift. Perhaps, if the non-agricultural (non-heat exposed control group) had drunk similar quantity of fluids as the agricultural group throughout the workday, the proportion of workers dehydrated at the end of the workday would be less.

Among the most pronounced and consistent findings from the MWAS statistical modeling and metabolic pathway enrichment analyses were that both the comparison of agriculture workers and non-agricultural workers and the analyses of association with ambient

temperature identified perturbations in several key biological pathways, including nitrogen elimination, and histidine metabolism. Specially, we confirmed the identities of several metabolites from the nitrogen elimination pathway including arginine, citrulline, and uracil, which have been closely linked to renal dysfunction, kidney energy production, oxidative stress, and systemic inflammation (Baylis, 2006; Siboto et al., 2022; Tain et al., 2010). Recurrent heat exposures in the agricultural setting can impact the activity of these pathways, which may lead to renal dysfunction.

We also identified various metabolites enriched in the histidine metabolism pathway, including histamine, methylhistamine, 3-methylhistidine, and urocanate. Histidine is an essential amino acid that is metabolized in the body through several pathways, including the histidine-histamine pathway, which is primarily active in the kidneys. Previous literature has shown that during heat exposure, the body undergoes various physiological changes, including an increase in body temperature, blood flow, and sweating. These changes can alter histidine metabolism, leading to an increased breakdown of histidine and the accumulation of histamine, a biogenic amine that regulates various physiological processes (Hayakawa et al., 2012). Meanwhile, studies have also shown that in individuals with renal dysfunction, there may be alterations in the histidine-histamine pathway, resulting in decreased histamine synthesis and increased levels of histidine in the blood. This can lead to an accumulation of histidine and its metabolites, which may contribute to the development of various health conditions, including kidney disease, hypertension, and cardiovascular disease (Vera-Aviles et al., 2018; Watanabe et al., 2008). Specifically, in an animal study, alterations to histidine metabolism were observed in the group of rats with chronic kidney disease (Zhang et al., 2015). In a study of 325 patients with CKD, low plasma levels of histidine were associated with protein-energy wasting, inflammation, and oxidative stress (Watanabe et al., 2008). Consistently in our study, following elevated heat exposure, we observed perturbations in levels of key metabolites in the histidine-histamine pathway, indicative of the potential impact of heat exposure on renal dysfunction. However, additional research is needed to understand the relationship between histidine metabolism and kidney function.

We also observed a strong association between heat exposures and perturbations in the shikimate metabolic pathway, which reflects an impact on the function of resident microbes rather than on human cells. This series of reactions is an important metabolic pathway in microorganisms and some plants that leads to the biosynthesis of various aromatic compounds, including the amino acids phenylalanine, tyrosine, and tryptophan. Studies have shown that heat exposure can lead to the inhibition of the shikimate pathway in plants, resulting in decreased biosynthesis of aromatic compounds (Fucile et al., 2011). This can have various effects on plant growth and development, including reduced biomass production, altered gene expression, and decreased photosynthesis. In microorganisms, alterations in shikimate pathway activity have been attributed to exposure to glyphosate (primary ingredient in the herbicide Roundup), which alters the gut microbiome and can cause injury to the kidneys (Mesnage et al., 2021). It is well established that exposure to high ambient temperatures impacts immune activity and can damage the intestinal epithelial barrier and mucosa, all of which are likely to impact the composition and activity of intestinal microbial populations; indeed, numerous studies have shown that

heat stress alters microbiota composition in livestock and various animal models (Wen et al., 2021). Remarkably, the effects of heat exposure on the human microbiome remain largely uncharacterized, and our finding regarding shikimate metabolism highlights the need for detailed investigation of links between heat exposure, herbicide/pesticide exposure microbiome activity, and kidney diseases in humans.

Collectively, in this first MWAS study investigating the impact of heat exposures among agricultural workers, we observed perturbations in metabolites and pathways closely linked to renal dysfunction, energy production, and oxidative stress and systemic inflammation, providing novel insights on the molecular mechanisms underlying the impact of heat exposures on human health. Despite these promising findings, limitations inherent in omics-based analyses and the relatively small sample size in this pilot study deserve specific attention. Given the nature of the cross-sectional study, we were not able to draw any causal inferences regarding heat exposure and renal dysfunction. Using regional weather station data rather than work site data to estimated heat exposure was another limitation. Future studies will be needed to examine associations between levels of the renal biomarkers in plasma and AKI designations and between metabolic pathways and renal biomarkers. Although we controlled for several important confounding factors, including age, sex, BMI, working hours, additional covariates including nutritional and dietary variables were not considered in this study and non-fasting status may introduce measurement variation. Adjusting for more variables, however, might cause issues of over-adjustment. Additionally, we used pool standards and internal references in the metabolic profiling and followed a comprehensive metabolomics workflow to minimize the potential impact of non-fasting status, which has been shown to successfully analyze many non-fasting samples previously (Chang et al., 2022; Go et al., 2015; Taibl et al., 2023; Tchen et al., 2022). In highly multidimensional analyses like MWAS, false positives occur due to multiple comparisons and type I errors. The FDR analyses showed that very few metabolites are likely to be useful biomarkers. However, the pathway enrichment analysis uses permutation testing to protect against false discovery, and these analyses consistently showed pathway effects. Due to the limited statistical power and the exploratory nature of pilot studies, we only applied adjustments for multiple testing in the pathway enrichment analysis. Nevertheless, we did conduct sensitivity analyses using more stringent p-value thresholds for feature selection and observed consistent findings. Additionally, due to insufficient statistical power resulting from the relatively small sample size, we were not able to combine both the pre-shift and post-shift metabolomics profiling data using mixed effect models. Future hypothesis testing approaches with larger sample size, longitudinal design, and diverse populations are warranted to replicate and validate our findings.

5. Conclusion

This untargeted metabolomic and renal biomarker study found perturbations in metabolites and metabolic pathways among agricultural workers who are exposed to heat compared to non-agricultural workers generally not exposed to heat. These included molecular signatures related to dysfunctional gluconeogenesis potentially occurring due to repetitive and sustained proximal tubule injury as well as changes in histidine metabolism and in the

activity of resident microbes. This study produced novel insights on the molecular responses to heat exposures that impact human health.

Funding

This work was supported by The Southeastern Coastal Center for Agricultural Health and Safety (CDC/NIOSH# U54OH011230), The National Institute for Occupational Safety and Health (R01OH011782), and National Institute of Nursing Research (K23NR020356).

Data availability

Data will be made available on request.

References

Aguilar-Ramirez D, Raña-Custodio A, Villa A, Rubilar X, Olvera N, Escobar A, Johnson RJ, Sanchez-Lozada L, Obrador GT, Madero M, 2020. Decreased kidney function and agricultural work: a cross-sectional study in middle-aged adults from Tierra Blanca, Mexico. Nephrol Dial Transplant 36 (6), 1030–1038. 10.1093/ndt/gfaa041.

Al-Bouwarthan M, Quinn MM, Kriebel D, Wegman DH, 2020. Risk of Kidney Injury among Construction Workers Exposed to Heat Stress: A Longitudinal Study from Saudi Arabia. Int. J. Environ. Res. Public Health 17 (11). 10.3390/ijerph17113775.

Baylis C, 2006. Arginine, arginine analogs and nitric oxide production in chronic kidney disease. Nat. Clin. Pract. Nephrol. 2 (4), 209–220. 10.1038/ncpneph0143. [PubMed: 16932427]

Bernard TE, Barrow CA, 2013. Empirical approach to outdoor WBGT from meteorological data and performance of two different instrument designs. Ind. Health 51 (1), 79–85. 10.2486/indhealth.2012-0160. [PubMed: 23385431]

Chang CJ, Barr DB, Ryan PB, et al. , 2022. Per- and polyfluoroalkyl substance (PFAS) exposure, maternal metabolomic perturbation, and fetal growth in African American women: A meet-in-the-middle approach. Environ. Int. 158, 106964 10.1016/j.envint.2021.106964. [PubMed: 34735953]

Chapman CL, Johnson BD, Vargas NT, Hostler D, Parker MD, Schlader ZJ, 2020. Both hyperthermia and dehydration during physical work in the heat contribute to the risk of acute kidney injury. J. Appl. Physiol. (Bethesda, Md: 1985) 128 (4), 715–728. 10.1152/japplphysiol.00787.2019. [PubMed: 32078468]

Flouris AD, Dinas PC, Ioannou LG, et al. , 2018. Workers' health and productivity under occupational heat strain: a systematic review and meta-analysis. Lancet Planet Health. 2 (12), e521–e531. 10.1016/s2542-5196(18)30237-7. [PubMed: 30526938]

Fucile G, Garcia C, Carlsson J, Sunnerhagen M, Christendat D, 2011. Structural and biochemical investigation of two Arabidopsis shikimate kinases: the heat-inducible isoform is thermostable. Protein Sci. 20 (7), 1125–1136. 10.1002/pro.640. [PubMed: 21520319]

Gallo-Ruiz L, Sennett CM, Sánchez-Delgado M, et al. , 2019. Prevalence and Risk Factors for CKD Among Brickmaking Workers in La Paz Centro, Nicaragua. Am. J. Kidney Dis. 74 (2), 239–247. 10.1053/j.ajkd.2019.01.017. [PubMed: 30826087]

Garcia-Trabanino R, Jarquin E, Wesseling C, et al. , 2015. Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador–A cross-shift study of workers at risk of Mesoamerican nephropathy. Environ. Res. 142, 746–755. 10.1016/j.envres.2015.07.007. [PubMed: 26209462]

Gaskins AJ, Tang Z, Hood RB, et al. , 2021. Periconception air pollution, metabolomic biomarkers, and fertility among women undergoing assisted reproduction. Environ. Int. 155, 106666. [PubMed: 34116378]

Glaser J, Lemery J, Rajagopalan B, et al. , 2016. Climate Change and the Emergent Epidemic of CKD from Heat Stress in Rural Communities: The Case for Heat Stress Nephropathy. Clin J Am Soc Nephrol 11 (8), 1472–1483. 10.2215/cjn.13841215. [PubMed: 27151892]

Go Y-M, Walker DI, Liang Y, et al. , 2015. Reference standardization for mass spectrometry and high-resolution metabolomics applications to exposome research. Toxicol. Sci. 148 (2), 531–543. [PubMed: 26358001]

Gunawardena S, Dayaratne M, Wijesinghe H, Wijewickrama E, 2021. A Systematic Review of Renal Pathology in Chronic Kidney Disease of Uncertain Etiology. Kidney Int Rep 6 (6), 1711–1728. 10.1016/j.ekir.2021.03.898. [PubMed: 34169213]

Hayakawa T, Yoshida Y, Yasui M, et al. , 2012. Heat-induced gelation of myosin in a low ionic strength solution containing L-histidine. Meat Sci. 90 (1), 77–80. 10.1016/j.meatsci.2011.06.002. [PubMed: 21719207]

Hood RB, Liang D, Chiu Y-H, et al. , 2022. Pesticide residue intake from fruits and vegetables and alterations in the serum metabolome of women undergoing infertility treatment. Environ. Int. 160, 107061. [PubMed: 34959198]

Hwang S, Hood RB, Hauser R, et al. , 2022. Using follicular fluid metabolomics to investigate the association between air pollution and oocyte quality. Environ. Int. 169, 107552. [PubMed: 36191487]

Johnson RJ, Sánchez-Lozada LG, Newman LS, et al. , 2019. Climate Change and the Kidney. Ann. Nutr. Metab. 74 (Suppl 3), 38–44. 10.1159/000500344. [PubMed: 31203298]

Kellum JA, Lameire N, 2013. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). Crit. Care 17 (1), 204. 10.1186/cc11454. [PubMed: 23394211]

Keogh SA, Leibler JH, Sennett Decker CM, et al. , 2022. High prevalence of chronic kidney disease of unknown etiology among workers in the Mesoamerican Nephropathy Occupational Study. BMC Nephrol. 23 (1), 238. 10.1186/s12882-022-02861-0. [PubMed: 35794550]

Khwaja A, 2012. KDIGO clinical practice guidelines for acute kidney injury. Nephron Clin. Pract. 120 (4), c179–c184. [PubMed: 22890468]

Kupferman J, Ramírez-Rubio O, Amador JJ, et al. , 2018. Acute Kidney Injury in Sugarcane Workers at Risk for Mesoamerican Nephropathy. Am. J. Kidney Dis. 72 (4), 475–482. 10.1053/j.ajkd.2018.04.014. [PubMed: 30042041]

Legouis D, Faivre A, Cippà PE, de Seigneux S, 2022. Renal gluconeogenesis: an underestimated role of the kidney in systemic glucose metabolism. Nephrol Dial Transplant 37 (8), 1417–1425. 10.1093/ndt/gfaa302. [PubMed: 33247734]

Levey AS, Atkins R, Coresh J, et al. , 2007. Chronic kidney disease as a global public health problem: approaches and initiatives - a position statement from Kidney Disease Improving Global Outcomes. Kidney Int. 72 (3), 247–259. 10.1038/sj.ki.5002343. [PubMed: 17568785]

Li Z, Sarnat JA, Liu KH, et al. , 2022. Evaluation of the Use of Saliva Metabolome as a Surrogate of Blood Metabolome in Assessing Internal Exposures to Traffic-Related Air Pollution. Environ. Sci. Tech.

Liang D, Moutinho JL, Golan R, et al. , 2018. Use of high-resolution metabolomics for the identification of metabolic signals associated with traffic-related air pollution. Environ. Int. 120, 145–154. [PubMed: 30092452]

Liang D, Ladva CN, Golan R, et al. , 2019. Perturbations of the arginine metabolome following exposures to traffic-related air pollution in a panel of commuters with and without asthma. Environ. Int. 127, 503–513. [PubMed: 30981021]

Liang D, Batross J, Fiedler N, et al. , 2022. Metabolome-wide association study of the relationship between chlorpyrifos exposure and first trimester serum metabolite levels in pregnant Thai farmworkers. Environ. Res. 215, 114319. [PubMed: 36108722]

Liu KH, Nellis M, Uppal K, et al. , 2020. Reference standardization for quantification and harmonization of large-scale metabolomics. Anal. Chem. 92 (13), 8836–8844. [PubMed: 32490663]

López-Gálvez N, Wagoner R, Canales RA, et al. , 2021. Longitudinal assessment of kidney function in migrant farm workers. Environ. Res. 202, 111686 10.1016/j.envres.2021.111686. [PubMed: 34273367]

Luangwilai T, Robson MG, Siriwong W, 2022. Investigation of kidney function changes in sea salt workers during harvest season in Thailand. Rocz Panstw Zakl Hig 73 (1), 121–130. 10.32394/rpzh.2022.0201. [PubMed: 35322964]

Mesnage R, Teixeira M, Mandrioli D, et al. , 2021. Use of Shotgun Metagenomics and Metabolomics to Evaluate the Impact of Glyphosate or Roundup MON 52276 on the Gut Microbiota and Serum Metabolome of Sprague-Dawley Rats. Environ Health Perspect. 129 (1), 17005. 10.1289/ehp6990. [PubMed: 33502259]

Mix J, Elon L, Thein Mac VV, et al. , 2018. Hydration Status, Kidney Function, and Kidney Injury in Florida Agricultural Workers. J Occup Environ Med. 60 (5), e253–e260. 10.1097/jom.0000000000001261. [PubMed: 29271837]

Morrison N, Bearden D, Bundy JG, et al. , 2007. Standard reporting requirements for biological samples in metabolomics experiments: environmental context. Metabolomics 3, 203–210.

Moyce S, Joseph J, Tancredi D, Mitchell D, Schenker M, 2016. Cumulative incidence of acute kidney injury in California's agricultural workers. J. Occup. Environ. Med. 58 (4), 391–397. [PubMed: 27058480]

Moyce S, Mitchell D, Armitage T, Tancredi D, Joseph J, Schenker M, 2017. Heat strain, volume depletion and kidney function in California agricultural workers. Occup. Environ. Med. 74 (6), 402–409. 10.1136/oemed-2016-103848. [PubMed: 28093502]

Sanchez Polo V, Garcia-Trabanino R, Rodriguez G, Madero M, 2020. Mesoamerican Nephropathy (MeN): What We Know so Far. Int J Nephrol Renovasc Dis. 13, 261–272. 10.2147/ijnrd.S270709. [PubMed: 33116757]

Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS, 2007. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc 39 (2), 377–390. 10.1249/mss.0b013e31802ca597. [PubMed: 17277604]

Shi DS, Weaver VM, Hodgson MJ, Tustin AW, 2022. Hospitalised heat-related acute kidney injury in indoor and outdoor workers in the USA. Occup. Environ. Med. 79 (3), 184–191. 10.1136/oemed-2021-107933. [PubMed: 34750240]

Siboto A, Akinnuga AM, Khumalo B, et al. , 2022. Ameliorative Effects of a Rhenium (V) Compound with Uracil-Derived Ligand Markers Associated with Hyperglycaemia-Induced Renal Dysfunction in Diet-Induced Prediabetic Rats. Int. J. Mol. Sci. 23 (23) 10.3390/ijms232315400.

Taibl KR, Dunlop AL, Barr DB, et al. , 2023. Newborn metabolomic signatures of maternal per- and polyfluoroalkyl substance exposure and reduced length of gestation. Nat. Commun. 14(1), 3120. 10.1038/s41467-023-38710-3. [PubMed: 37253729]

Tain YL, Hsieh CS, Lin IC, Chen CC, Sheen JM, Huang LT, 2010. Effects of maternal L-citrulline supplementation on renal function and blood pressure in offspring exposed to maternal caloric restriction: the impact of nitric oxide pathway. Nitric Oxide 23 (1), 34–41. 10.1016/j.niox.2010.03.005. [PubMed: 20371384]

Tang Z, Sarnat JA, Weber RJ, et al. , 2022. The Oxidative Potential of Fine Particulate Matter and Biological Perturbations in Human Plasma and Saliva Metabolome. Environ. Sci. Tech. 56 (11), 7350–7361.

Tchen R, Tan Y, Boyd Barr D, et al. , 2022. Use of high-resolution metabolomics to assess the biological perturbations associated with maternal exposure to Bisphenol A and Bisphenol F among pregnant African American women. Environ. Int. 169, 107530 10.1016/j.envint.2022.107530. [PubMed: 36148711]

Uppal K, Soltow QA, Strobel FH, et al. , 2013. xMSanalyzer: automated pipeline for improved feature detection and downstream analysis of large-scale, non-targeted metabolomics data. BMC Bioinf. 14 (1), 1–12.

Vera-Aviles M, Vantana E, Kardinasari E, Koh NL, Latunde-Dada GO, 2018. Protective Role of Histidine Supplementation Against Oxidative Stress Damage in the Management of Anemia of Chronic Kidney Disease. Pharmaceuticals (Basel) 11 (4). 10.3390/ph11040111.

Watanabe M, Suliman ME, Qureshi AR, et al. , 2008. Consequences of low plasma histidine in chronic kidney disease patients: associations with inflammation, oxidative stress, and mortality. Am. J. Clin. Nutr. 87 (6), 1860–1866. 10.1093/ajcn/87.6.1860. [PubMed: 18541578]

Wen C, Wei S, Zong X, Wang Y, Jin M, 2021. Microbiota-gut-brain axis and nutritional strategy under heat stress. Anim Nutr. 7 (4), 1329–1336. 10.1016/j.aninu.2021.09.008. [PubMed: 34786505]

Yu T, Park Y, Johnson JM, Jones DP, 2009. apLCMS—adaptive processing of high-resolution LC/MS data. Bioinformatics 25 (15), 1930–1936. [PubMed: 19414529]

Zhang Z-H, Wei F, Vaziri ND, Cheng X-L, Bai X, Lin R-C, Zhao Y-Y, 2015. Metabolomics insights into chronic kidney disease and modulatory effect of rhubarb against tubulointerstitial fibrosis. Sci Rep 5 (1), 14472. 10.1038/srep14472. [PubMed: 26412413]

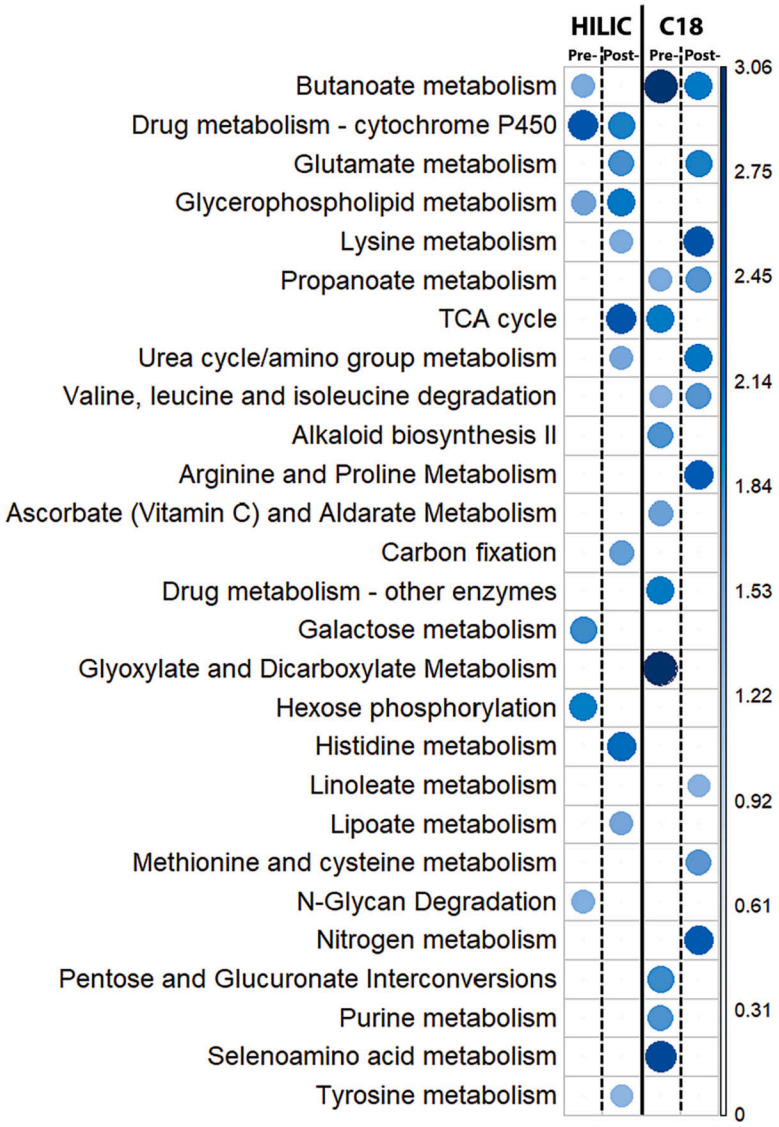

Fig. 1.

Bubble plot for the association between each metabolic pathway and significant features that were associated with work type (agricultural work / non-agricultural work). The size and color of the bubbles represent the pathway significant level based on −log10 (P-value).

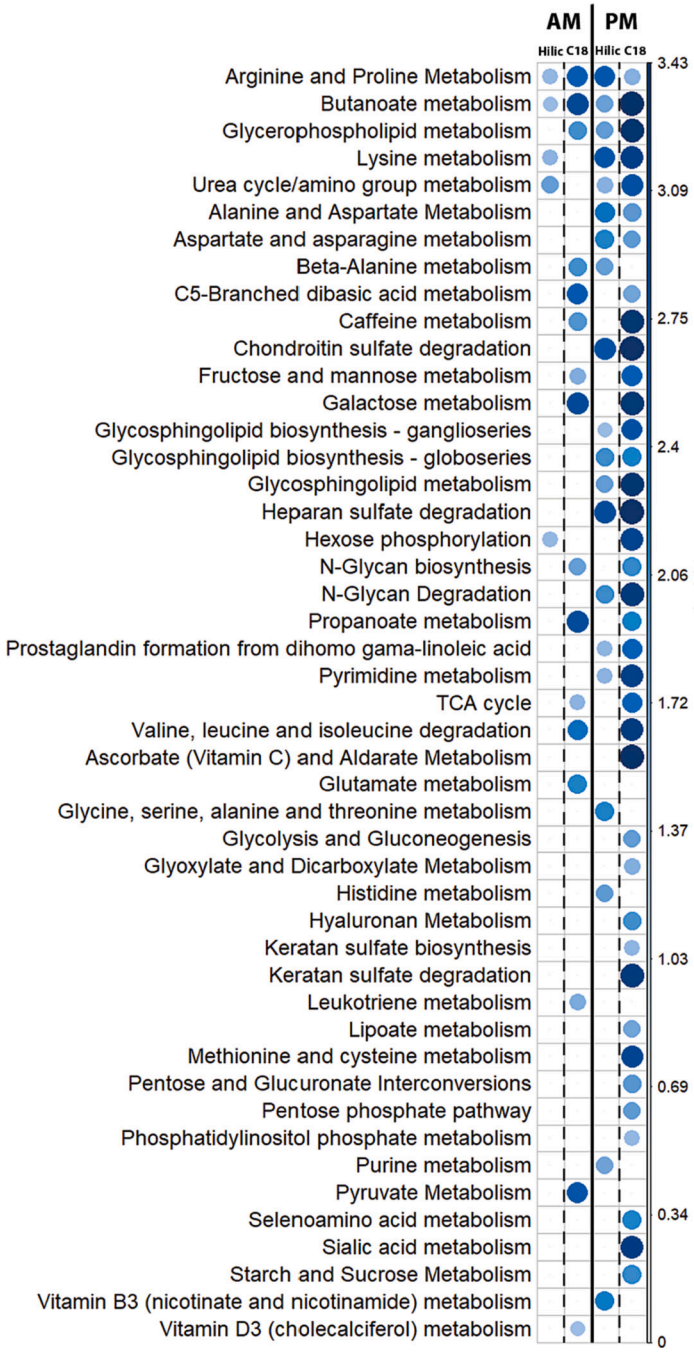

Fig. 2.

Bubble plot for the association between each metabolic pathway and significant features that were associated with Wet-Bulb Globe temperature of workday. The size and color of the bubbles represent the pathway significant level based on −log10 (P-value).

Table 1

Demographic and Work-related Characteristics of Study Participants.

	N	Agriculture N = 63[1]	Non-Agriculture N = 24[1]	p-value[2]
Demographics				
Age, yrs	87	43 (36, 49)	34 (26, 44)	0.003
Male	87	35% 22	17% 4	0.10
Mexican	86	56% 35	30% 7	0.04
BMI	85	29.3 (26.8, 31.3)	26.6 (24.4, 29.5)	0.02
Work-related Characteristics				
Years Working in agric	83	13 (3, 18)	0 (0, 0)	
Workday Duration, hrs	87	8.5 (7.9, 10.0)	7.5 (6.0, 9.1)	0.06
Heart rate, avg bpm during workday [3]	71	95 (89, 106)	86(76, 99)	0.02
Wet Bulb Glob Temp, max on current day	63	28.7 (27.9, 30.4)		
Wet Bulb Glob Temp, max on previous day	63	29.4 (28.4, 30.5)		
Beverage consumption during workday [4]**, oz**	73	76 (56, 108)	38 (22, 64)	<0.001
Sugary drink consumption during workday [4]**, oz**	73	12 (0, 20)	0 (0, 9)	0.003
Dehydrated[5]**at beginning of workday**	86	60% 37	54% 13	0.8
Dehydrated at end of workday	81	69% 41	73% 16	0.8
Acute Kidney Injury	74	15% 8	5% 1	0.4
eGFR < 90 at beginning of workday	84	5% 3	5% 1	<0.9
eGFR < 90 at end of workday	75	19% 10	29% 6	0.4

[1] Median (IQR); % n

[2] Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

[3] Missing heart rate data due to non-compliance (1) or technical issues/equipment malfunction (15)

[4] Missing beverage consumption due refusals (3) or not queried (11)

[5] Urine specific gravity >=1.020.

Environ Int. Author manuscript; available in PMC 2023 November 10.

Table 2

Creatinine-normed Urine Biomarkers.

Characteristic[1]	Agriculture, N = 63[2]	Non-Agriculture, N = 24[2]	p-value[3]
Creatinine, AM	1.181 (0.906, 1.778)	0.858 (0.507, 1.154)	**0.004**
Creatinine, PM	1.520 (0.777, 2.016)	1.170 (0.989, 1.485)	0.149
Creatinine, PM-AM	0.236 (−0.363, 0.810)	0.303 (−0.100, 0.660)	0.619
Albumin, AM	2.390 (1.451, 3.633)	1.955 (1.342, 4.430)	0.748
Albumin, PM	3.032 (2.087, 5.950)	2.335 (1.532, 4.259)	0.178
Albumin, PM-AM	0.112 (−0.755, 1.236)	− 0.038 (−0.854, 0.509)	0.467
Beta2 microglobulin, AM	0.106 (0.072, 0.156)	0.090 (0.070, 0.110)	0.231
Beta2 microglobulin, PM	0.079 (0.052, 0.117)	0.071 (0.054, 0.089)	0.245
Beta2 microglobulin, PM-AM	−0.019 (−0.061, 0.004)	− 0.020 (−0.059, 0.004)	0.987
CYSTATIN-C, AM	0.048 (0.031, 0.068)	0.040 (0.030, 0.067)	0.544
CYSTATIN-C, PM	0.049 (0.035, 0.065)	0.040 (0.035, 0.048)	0.090
CYSTATIN-C, PM-AM	−0.002 (−0.013, 0.012)	−0.001 (−0.024, 0.013)	0.806
EGF, AM	0.016 (0.010, 0.020)	0.015 (0.012, 0.018)	0.756
EGF, PM	0.016 (0.011, 0.020)	0.015 (0.011, 0.019)	0.682
EGF, PM-AM	0.001 (−0.003, 0.002)	− 0.001 (−0.002, 0.002)	0.921
KIM-1, AM	0.767 (0.497, 1.197)	0.701 (0.422, 0.958)	0.252
KIM-1, PM	0.555 (0.371, 0.780)	0.641 (0.396, 0.856)	0.435
KIM-1, PM-AM	−0.241 (−0.497, −0.033)	− 0.104 (−0.282, 0.125)	0.078
NGAL, AM	0.044 (0.025, 0.079)	0.044 (0.021, 0.142)	0.931
NGAL, PM	0.047 (0.027, 0.112)	0.050 (0.030, 0.106)	0.785
NGAL, PM-AM	−0.008 (−0.023, 0.008)	0.000 (−0.018, 0.009)	0.814
Osteopontin, AM	0.743 (0.590, 1.009)	0.539 (0.213, 0.801)	**0.033**
Osteopontin, PM	0.779 (0.608, 1.069)	0.725 (0.372, 0.874)	0.097
Osteopontin, PM-AM	−0.024 (−0.260, 0.314)	0.010 (−0.236, 0.225)	0.937
Uric acid, AM	0.460 (0.383, 0.661)	0.587 (0.376, 0.747)	0.492
Uric acid, PM	0.519 (0.401, 0.802)	0.511 (0.395, 0.578)	0.297
Uric acid, PM-AM	0.024 (−0.153, 0.241)	−0.032 (−0.185, 0.149)	0.159
Uromodulin, AM	12.976 (6.749, 21.550)	16.265 (8.092, 25.811)	0.336

Characteristic[1]	Agriculture, N = 63[2]	Non-Agriculture, N = 24[2]	p-value[3]
Uromodulin, PM	8.798 (4.099, 20.343)	12.217 (6.249, 19.754)	0.482
Uromodulin, PM-AM	−2.995 (−7.753, 3.877)	−0.616 (−20.510, 8.243)	0.789

[1] KIM-1 units = ng/mg creatinine; uric acid units = mg/mg creatinine; all others are μg/mg creatinine. Characteristics labeled 'AM' were collected at the pre-work visit, "PM" at the post-work visit, and "PM-AM" represents the post- minus pre-work difference.

[2] Median (IQR); For Agriculture, there were 5 missing AM and PM-AM values for each characteristic and 1 missing value for Beta2 microglobulin PM. For Non-Agriculture, there was 1 missing PM and PM-AM value for each characteristic.

[3] Wilcoxon rank sum test.

Table 3

Number of significant features for each set of Metabolomics-Wide Association Study (MWAS) Models by the HILIC and C18 Columns.

Predictor	Outcome Model	HILIC			C18		
		FDR q < 0.2	RAW p < 0.05	RAW p < 0.005	FDR q < 0.2	RAW p < 0.05	RAW p < 0.005
AGRI_TERM[a]	Pre-shift	0	394	41	0	284	27
	Post-shift	0	336	29	0	197	20
Heat exposure[b]	Previous day	0	636	62	0	526	67
	Current day	1	666	88	1	468	79

[a]Whether the participant worked for agriculture and non-agriculture.

[b]Wet-Bulb Globe temperature.

Table 4

Chemical identity of metabolites that are significantly different between agricultural and non-agricultural workers (raw $p < 0.05$) with separate models for pre-workday and post-workday[1].

m / z	RT (s)	Identified Metabolite	Adduct Form	β_{pre}	p_{pre}	β_{post}	p_{post}	Pathways
144.0661	21.7	4-ACETAMIDOBUTANOATE	M−H	0.036	0.05	0.237	0.01	Arginine and proline metabolism
174.0879	21.6	CITRULLINE [a]	M−H	0.318	0.03	0.056	0.05	Arginine biosynthesis
214.0489	26.3	GLUCOSAMINE	M + Cl	−0.131	0.03	−0.317	0.05	Amino sugar and nucleotide sugar metabolism
157.0273	22	ERYTHRITOL/THREITOL	M + CI	−0.057	0.04	−1.161	0.02	Renal disorder
167.035	25	HOMOGENTISATE	M−H	1.046	0.01	0.593	0.03	Tyrosine metabolism
377.0856	20.4	LACTOSE/MALTOSE/ MELIBIOSE [a]	M + Cl	− 0.551	0.03	−1.523	0.01	Starch and sucrose metabolism; Galactose metabolism
173.045	18.1	SHIKIMATE	M−H	−0.102	0.42	0.344	0.05	Phenylalanine, tyrosine and tryptophan biosynthesis
157.0273	22	THREITOL	M + CI	−0.057	<0.001	−1.161	0.02	Renal disorder
168.1024	30.4	3-METHOXYTYRAMINE/4-METHOXYTYRAMINE	M + H	0.468	0.02	−0.378	0.85	Tyrosine metabolism/ Central nervous system disorder
176.1035	91.2	CITRULLINE [a]	M + H	0.266	0.04	0.096	0.01	Arginine biosynthesis
522.356	40	LYSOPC(18:1)	M + H	−0.266	0.01	−0.174	<0.001	Fatty Acid Metabolism
343.124	94.4	MELIBIOSE [a]	M + H	−1.794	0.02	−2.300	0.04	Galactose metabolism
170.0924	97	METHYLHISTIDINE	M + H	−0.120	0.05	−0.644	0.03	Tyrosine metabolism/ Histidine metabolism
149.0233	30	PHTHALIC ANHYDRIDE	M + H	0.460	0.03	1.092	0.05	NA—exogenous chemical, precursor to phthalate esters
667.2297	250.2	STACHYOSE	M + H	−2.743	0.01	−1.176	0.04	Galactose metabolism
343.124	94.4	SUCROSE [a]	M + H, M−H2O + H	−1.794	0.02	−2.300	0.03	Galactose metabolism
113.0346	38	URACIL	M + H	−0.070	0.05	0.009	0.51	Pyrimidine metabolism
139.0507	38	UROCANATE	M + H	−0.147	0.05	−0.888	0.03	Histidine metabolism

Note: Chemical identity of metabolic features was confirmed by matching peaks via accurate mass to charge ratio and retention time to authentic reference standards under the same conditions using tandem mass spectrometry.

Abbreviations: *m/z*: mass to charge ratio RT: retention time.

[a] Metabolites were identified in both $HILIC^{+}$ with positive electrospray ionization mode and $C18^{-}$ with negative electrospray ionization mode.

[1] Betas represent the comparison of agricultural workers to the referent group non-agricultural workers; for example, positive values indicate comparatively higher level of intensities of the metabolites among the agricultural samples compared to non-agricultural samples.

Table 5

Chemical identity of metabolites associated with heat exposure for previous day (raw $p < 0.05$).

m / z	RT (s)	Identified Metabolite	Adduct Form	β^b	Pathways
94.06565	36	ANILINE	M + H	−0.863	Aminobenzoate degradation
108.0813	30.7	BENZYLAMINE	M + H	0.231	High concentration within foods
126.1026	73	METHYLHISTAMINE	M + H	0.095	Histidine metabolism
133.0977	109.5	ORNITHINE	M + H	−0.108	Arginine biosynthesis
190.0868	29.2	METHYL INDOLE-3-ACETATE	M + H	0.372	Tryptophan metabolism
239.1493	24	PIRIMICARB	M + H	−0.468	NA/ Exogenous chemical- carbamate insecticide
241.0317	211	CYSTINE[a]	M + H	0.089	Cysteine and methionine metabolism
261.0375	263	D-MANNOSE 6-PHOSPHATE	M + H	−0.157	Fructose and mannose metabolism
400.3421	36	PALMITOYLCARNITINE	M + H	−0.068	Fatty acid metabolism
89.0239	22.9	GLYCERALDEHYDE	M−H	−0.126	Genetic disorder
115.0032	22.2	MALEIC ACID	M−H	−0.262	Tyrosine metabolism
133.0137	21.2	MALATE	M−H	−0.271	Butanoate metabolism
136.0399	26.4	ANTHRANILATE	M−H	0.043	Tryptophan metabolism
136.0399	25.8	4-AMINOBENZOATE	M−H	0.043	Biosynthesis of phenylpropanoids
149.0455	22	XYLOSE/RIBOSE	M−H	−0.142	Renal disorder
191.0561	22	QUINIC ACID	M−H	−0.516	Phenylalanine, tyrosine and tryptophan biosynthesis
239.0161	27.9	CYSTINE[a]	M−H	0.129	Cysteine and methionine metabolism
241.0726	36	LUMICHROME	M−H	−0.294	Riboflavin metabolism
464.3012	33	GLYCOCHOLATE	M−H	−0.281	Cholesterol metabolism

Note: Chemical identity of metabolic features was confirmed by matching peaks via accurate mass to charge ratio and retention time to authentic reference standards under the same conditions using tandem mass spectrometry.

Abbreviations: *m/z*: mass to charge ratio; RT: retention time.

[a]Metabolites were identified in both HILIC + with positive electrospray ionization mode and C18− with negative electrospray ionization mode.

[b]The beta coefficient represents the change in log-transformed metabolite intensity per 1-unit increase in Wet-Bulb Globe temperature level.

Table 6

Chemical identity of metabolites associated with heat exposure for current day (raw $p < 0.05$).

m / z	RT (*s*)	Identified Metabolite	Adduct Form	β[b]	Pathways
110.0276	77.1798	HYPOTAURINE	M + H	−0.129	Taurine and hypotaurine metabolism
112.0875	73.8	HISTAMINE	M + H	0.105	Histidine metabolism
126.0225	62.5	TAURINE	M + H	−0.096	Taurine and hypotaurine metabolism
130.0868	60.6	PIPECOLATE	M + H	0.108	Biosynthesis of alkaloids derived from ornithine, lysine and nicotinic acid
136.0432	59	HOMOCYSTEINE	M + H	0.214	Cysteine and methionine metabolism
137.0463	44.2	HYPOXANTHINE	M + H	−0.135	Purine metabolism
148.0762	29.4	3-METHYL-2-OXINDOLE	M + H	0.186	Valine, leucine and isoleucine biosynthesis
154.0868	38	DOPAMINE	M + H	−0.094	Tyrosine metabolism
175.1195	118	L-ARGININE	M + H	0.086	Arginine and proline metabolism
176.0671	74	N-AMIDINO-L-ASPARTATE	M + H	0.079	Renal disorder
176.0666	74	GUANIDINOSUCCINATE	M + H	0.079	Renal disorder
190.0504	48.1	4-HYDROXY-2-QUINOLINECARBOXYLIC ACID	M + H	−0.263	Tryptophan metabolism
190.0868	29.2	METHYL INDOLE-3-ACETATE	M + H	0.286	Tryptophan metabolism
203.0529	60.1	D-GALACTOSE /ALPHA-D-GLUCOSE	M + Na	0.074	Amino sugar and nucleotide sugar metabolism
206.0673	29.7	LIPOAMIDE	M + H	−0.097	Genetic disorder
244.0796	59.2	N-ACETYL-D-GALACTOSAMINE [a]	M + Na	0.072	Amino sugar and nucleotide sugar metabolism & Galactose metabolism
282.1202	40.5	1-METHYLADENOSINE	M + H	−0.186	Renal disorder
301.2167	26.5	RETINOATE	M + H	−0.467	Retinol metabolism
306.0491	100.8	CYTIDINE 2′,3′-CYCLIC MONOPHOSPHATE	M + H	0.060	Pyrimidine metabolism
332.076	122	DEOXYADENOSINE MONOPHOSPHATE	M + H	−0.288	Purine metabolism
377.1461	44.7	RIBOFLAVIN	M + H	0.066	Riboflavin metabolism
480.3449	39	LYSOPC(O/P-16:1)	M + H	−0.111	Fatty Acid Metabolism
508.3398	42	LYSOPE(20:1)	M + H	−0.158	Fatty Acid Metabolism
522.356	40	LYSOPC(18:1)	M + H	−0.107	Fatty Acid Metabolism
88.98802	21	OXALIC ACID	M−H	0.127	Glyoxylate and dicarboxylate metabolism & Purine metabolism
101.0244	22	ACETOACETATE / 2-KETOBUTYRIC ACID	M−H	0.100	Glycine, serine and threonine metabolism, Tyrosine metabolism & Cysteine and methionine metabolism
109.029	33.6	CATECHOL	M−H	0.324	Tyrosine metabolism

m / z	RT (*s*)	Identified Metabolite	Adduct Form	β[b]	Pathways
125.0351	21.6	4-IMIDAZOLEACETIC ACID	M−H	0.103	Histidine metabolism
161.0456	23	HYDROXYMETHYLGLUTARATE	M−H	0.083	Upper GI Disorder
175.0243	20.5	D-GLUCURONOLACTONE	M−H	−0.212	Ascorbate and aldarate metabolism
239.0161	27.9	L-CYSTINE	M−H	0.131	Cysteine and methionine metabolism
256.0588	23	N-ACETYL-D-GALACTOSAMINE [a]	M + Cl	0.091	Amino sugar and nucleotide sugar metabolism & Galactose metabolism
427.0056	20.3	INOSINE 5′-DIPHOSPHATE	M−H	0.190	Purine metabolism

Note: Chemical identity of metabolic features was confirmed by matching peaks via accurate mass to charge ratio and retention time to authentic reference standards under the same conditions using tandem mass spectrometry.

Abbreviations: *m/z*: mass to charge ratio; RT: retention time.

[a]Metabolites were identified in both HILIC + with positive electrospray ionization mode and $C18^{-}$ with negative electrospray ionization mode.

[b]The beta coefficient represents the change in log-transformed metabolite intensity per 1-unit increase in Wet-Bulb Globe temperature level.