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Exposome and Metabolome Analysis of Sugarcane Workers Reveals Predictors of Kidney Injury

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Introduction: Sugarcane workers are exposed to potentially hazardous agrochemicals, including pesticides, heavy metals, and silica. Such occupational exposures present health risks and have been implicated in a high rate of kidney disease seen in these workers.

Methods: To investigate potential biomarkers and mechanisms that could explain chronic kidney disease (CKD) among this worker population, paired urine samples were collected from sugarcane cutters at the beginning and end of a harvest season in Guatemala. Workers were then separated into 2 groups, namely those with or without kidney function decline (KFD) across the harvest season. Urine samples from these 2 groups underwent elemental analysis and untargeted metabolomics.

Results: Urine profiles demonstrated increases in silicon, certain pesticides, and phosphorus levels in all workers, whereas heavy metals remained low. The KFD group had a reduction in estimated glomerular filtration rate (eGFR) across the harvest season; however, kidney injury marker 1 did not significantly change. Cross-harvest metabolomic analysis found trends of fatty acid accumulation, perturbed amino acid metabolism, presence of pesticides, and other known signs of impaired kidney function.

Conclusion: Silica and certain pesticides were significantly elevated in the urine of sugarcane workers with or without KFD. Future work should determine whether long-term occupational exposure to silica and pesticides across multiple seasons contributes to CKD in these workers. Overall, these results confirmed that multiple exposures are occurring in sugarcane workers and may provide insight into early warning signs of kidney injury and may help explain the increased incidence of CKD among agricultural workers.

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See Commentary on Page 1163

Sugarcane is the most cultivated crop in the world, with nearly 2 billion tons produced every year. It has a variety of extremely valuable applications, ranging from its ubiquitous presence in food and increasing role in ethanol production to the use of sugarcane stalk pulp (bagasse) in energy production. Therefore, it comes as no surprise that production has continued to trend upward over recent decades.[1] Sugarcane field work involves a number of distinct job tasks, all of which demonstrate some degree of occupational risk and increased prevalence of renal injury, especially in cane cutters when preventive strategies are uncertain or not fully implemented. Cane cutters work an extremely physically demanding job for long hours in hot weather with limited rest breaks, shade, and opportunities for rehydration; they often work directly with recently burned sugarcane without respiratory protection and are exposed to high volumes of respirable ash.[2-4] Heat stress, exertional injury, dehydration, and exposure to agricultural toxicants are major occupational concerns that are posited as being responsible for the growing incidence of CKD among these communities.[5]

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In addition to increasing rates of CKD globally and in agricultural communities, an epidemic of CKD of unknown etiology (CKDu), also referred to as Mesoamerican nephropathy, has been posited to be the consequence of performing high exertion work in the face of increasing heat plus exposure to nephrotoxicants.[6–9] Agricultural communities in particular experience disproportionate CKDu rates, with hot spots in Latin America, India, and Sri Lanka, among other mostly subtropical countries.[10,11] Clinically, CKDu is defined as CKD that is not a comorbidity of diabetes, hypertension, kidney stones, or other known causes of kidney disease. It has a gradual onset characterized by a decline in kidney function as measured by serum creatinine or serum cystatin C with low or no proteinuria. Histopathologically, CKDu is characterized by tubulointerstitial nephritis and fibrosis, with glomerulosclerosis appearing in later stages of illness.[12,13] Unfortunately, the mechanistic understanding of CKDu remains extremely limited, impeding the development of effective treatments or diagnostic techniques. Given that CKDu and the rise in kidney injury are likely linked, investigating common exposures in these areas will help elucidate the primary risk factors responsible for the rapid degradation of renal health. Public health interventions addressing strenuous working conditions, heat stress, and dehydration have found some success in acute kidney injury mitigation, but such factors are unable to fully explain CKDu distribution and prevalence.[14–17] Thus, there remains a strong possibility that one or more environmental toxicants is contributing to the development of this disease via other mechanisms of kidney injury.

Sugarcane agricultural work involves the use of many pesticides, fertilizers, and heavy metals, which could result in exposure to these nephrotoxic chemicals.[18] Although pesticides and heavy metals have a well-documented risk of kidney damage, investigations into their role in the development of CKDu are ongoing. Current literature has yet to demonstrate a conclusive link, with many studies providing contradictory results and meta-analyses not finding any statistically significant risks.[19–24] A more recent hypothesis centers on a putative role for silica nanoparticles. Sugarcane stalks are comprised primarily of naturally occurring amorphous silica, which can be released during routine crop burning.[4] Evidence of such exposures have been found in the biopsies of some CKDu patients.[25] In addition, these particles have been found to be cytotoxic and disruptive of energy metabolism in human kidney cells.[26]

Understanding the mechanism of energy metabolism perturbation that occurs following exposure to nephrotoxicants could elucidate the exposures and stressors that contribute to pathogenesis. Kidney disease is known to be associated with inflammatory renal tubular injury, altered redox state, fibrosis, and mitochondrial dysfunction.[27,28] Altered redox state, glycolytic shift, and accumulation of fatty acids are hallmarks of CKD that have also been demonstrated to occur *in vitro* following exposure to amorphous silica nanoparticles.[26,29,30] Such processes and pathways have many associated metabolites that can be quantified via metabolomics to help understand exposures and risk factors correlated with higher rates of kidney injury. In addition, elemental and pesticide analysis provides a snapshot of the exposome of high-risk groups during key periods, clarifying associations between occupational and environmental exposures and biological indicators of kidney injury.

The hypothesis of this study was that the exposome and metabolome changes that occur concurrently in sugarcane cutters over the harvest season can provide a multifactorial snapshot of early kidney injury, which can be used to better understand potential mechanisms and biomarkers of CKDu. To this end, paired urine samples were collected from cutters at 2 timepoints across the 6-month harvest season. Cross-harvest changes to elemental abundance, presence of pesticides, and metabolic profiles were investigated to determine trends which may correlate with markers of kidney function.

METHODS

Study Design

The data for this analysis were derived from stored urine samples from a previous study of male agricultural workers (≥18 years) employed by a sugarcane agribusiness in Guatemala. The study was conducted during the 2017 to 2018 harvest among 202 sugarcane cutters. The harvest season lasted 6 months from November through May. For the original study, participants were recruited within 2 randomly selected work groups of male sugarcane cutters in November 2017. Clinical data and urine and blood samples were collected during November 2017 and April 2018 before the start of the work shift. These workers live in the Guatemala highlands during the rainy season and migrate to the lowlands for seasonal work where they are housed in dormitories on company property where they predominately consume the same water and prepared meals. During the harvest season, agrochemicals were applied, and the sugarcane fields were burned to facilitate harvest as a standard practice in this region. Additional work setting, worker population, work practice, and study method details have been previously described.[31,32] Participants provided written

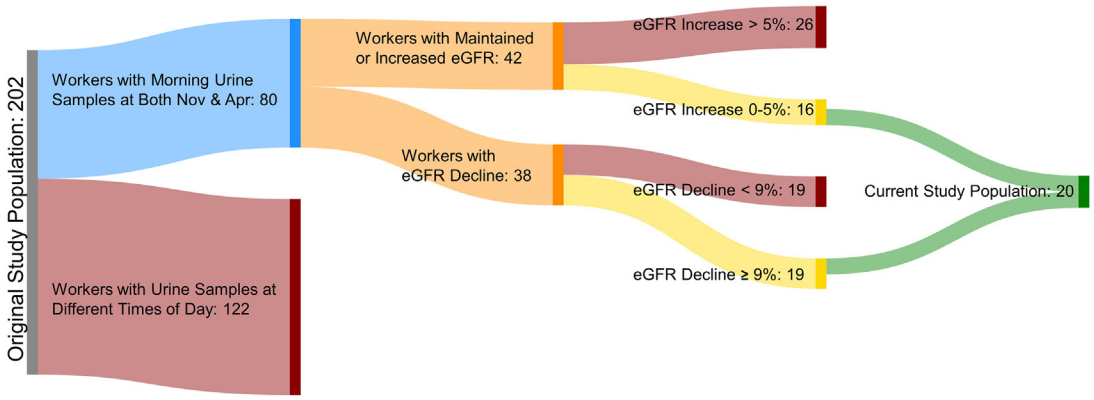

Figure 1. Study population selection.

informed consent at the time of recruitment, and institutional review board approval for this study was obtained from the Colorado Multiple Institutional Review Board of the University of Colorado and Comité de Ética Independiente ZUGUEME in Guatemala.

For this current analysis, untargeted metabolomics and elemental analysis were performed on a paired group of urine samples from 20 workers collected in November and April to determine cross-harvest changes, totaling 40 urine samples. To minimize diurnal variation, worker urine samples were excluded from selection if collection time during the day differed between November and April (Figure 1). Out of the original population of 202, 80 workers had morning urine samples collected in both November and April. Among these 80 workers, 38 had a decline in eGFR from November to April, 19 of whom experienced a decline greater than or equal to 9% and were randomly selected from to comprise the KFD group. For 42 of the 80 workers, their eGFR maintained or increased and had a value greater than 90 at both timepoints, 16 of whom maintained kidney function (0%–5% increased eGFR) and were randomly selected from to comprise the group with no decline in kidney function (non-KFD). The eGFR was calculated from serum creatinine values using established methods.[33] Urine creatinine, kidney injury marker 1, and electrolyte values were determined via previously discussed methods.[32]

Sample Collection and Storage

Urine samples were morning spot samples collected at approximately the same time in November and April before workers began their shift. Specific gravity was determined at the time of collection. Samples were placed on ice and transported to the on-site clinic within 1 hour after collection, where the urine was aliquoted into Fisherbrand sterile polypropylene tubes without preservatives before local storage at −20 °C and laboratory analysis at Guatemala City. Within 1 week, frozen urine aliquots were shipped on dry ice to the University of Colorado Anschutz Medical Campus. Upon arrival, they were immediately stored at −80 °C before metabolomic and elemental analysis.

Elemental Analysis

Urine samples were diluted 1:1 in 70% HNO3 and left to sit at least overnight for wet digestion. Samples were then diluted 1:100 in 1.5% HNO3 for instrumental analyses. Inductively coupled plasma-mass spectrometry analyses were performed on a NexION 2000B single quadrupole ICP-MS (Perkin-Elmer, Waltham, MA) equipped with a Meinhard nebulizer and a cyclonic spray chamber. Each day, prior to analyses, the instrument was tuned with a solution of 1 part per billion Li, Ce, In, Pb, and U to optimize sensitivity and robustness. Samples were analyzed via total quantitative analysis, scanning from m/z ratios of 7 to 238 to determine concentration of each individual element in the sample. Samples were calibrated to a 1-point external standard point of a 10-parts per billion cocktail of 40 metals. Data are presented in total parts per billion (μg/l) in urine normalized to creatinine (g/l).

Metabolomics

All solvents used for extraction and liquid chromatography-mass spectrometry analysis were of high-performance liquid chromatography or liquid chromatography-mass spectrometry grade, including water from Burdick and Jackson (Muskegon, MI) and acetonitrile, methanol, and formic acid from Fisher Scientific (Fair Lawn, NJ). Authentic standards were from Cayman Chemical (Ann Arbor, MI), Cambridge Isotope Laboratories (Tewksbury, MA), CDN Isotopes (Pointe-Claire, Quebec, Canada), and Millipore-Sigma (St. Louis, MO).

Sample Preparation

Urine samples were analyzed neat as previously described with some modifications as previously

described.[34] Briefly, urine samples and a pooled urine quality control (QC) sample were thawed at 4 °C, and 100 µl aliquots were stored in 1.5 ml microcentrifuge tubes overnight at 4°C until sample preparation. Samples and the pooled QC samples were centrifuged for 10 minutes at 3000g and 4°C (Beckman-Coulter). Then 35 µl of sample supernatants were transferred to autosampler vials (Cornerstone Scientific) in a 4 °C cold room. A 160 µl aliquot of the pooled QC sample, made from 4 µl aliquots of each urine sample, was transferred to a fresh 1.5 ml microcentrifuge tube (Fisher Scientific) and was spiked with 20 µl each of an in-house hydrophilic spike mix and 8-iso PGF2α (Cayman Chemical).[35] The spiked pooled QC sample was vortexed well to mix and 40 µl aliquots were transferred to autosampler vials. All samples and pooled QC aliquots were stored at −80 °C until instrumental analysis.

Liquid Chromatography-Mass Spectrometry

Urine samples were analyzed by reverse-phase chromatography as previously described with the following modifications: the SB-AQ analytical column was fitted with an in-line filter frit (Agilent Technologies, Santa Clara, CA); mobile phase A was composed of water with 0.1% formic acid and 0.1% InfinityLab deactivator additive (Agilent Technologies); mobile phase B was composed of acetonitrile with 0.1% formic acid and 0.1% InfinityLab deactivator additive; the gradient was as follows: 0 to 3 minutes, 1.8% B; 3 to 10 minutes, 1.8% to 54% B; 10 to 15 minutes, 54% to 90% B; 15 to 20 minutes, hold at 90% B; 20 to 20.1 minutes, 90% to 1.8% B; hold at 1.8% B until 25 minutes.[34] The Agilent Technologies 6545 Time-of-Flight Mass Spectrometer conditions were as previously described.[35]

QC

Pooled QC samples were injected after every 10 samples to monitor instrument variability across the run. Detected spiked-in standards had coefficient of variations of 6.5% or lower across 7 pooled QC injections.

Data Processing and Annotation

Raw liquid chromatography-mass spectrometry data were extracted using a recursive workflow in MassHunter Profinder Version B.10, SP1 (Agilent Technologies), similar to that previously described with the following modifications: retention time extraction range of 0.5 to 13 minutes with a noise peak height filter ≥7500 counts; ion species: +H, +Na, +K, +NH4; and charge state maximum of 1. Alignment tolerance for RT was 0% + 0.20 minutes with a mass tolerance of 10 ppm + 2 mDa (millidalton).[34] Data were imported into Mass Profiler Professional Version 14.1 (Agilent Technologies) for quality filtering. Compounds remaining after filtering were used for targeted feature extraction in Profinder. Compounds resulting from targeted data extraction were imported into Mass Profiler Professional Version for differential analysis. Differential analysis was performed using Mass Profiler Professional Version 14.1 (Agilent Technologies), using a workflow similar to that previously described.[34] Analysis of variance was performed using a *P*-value of <0.05. A fold change filter of 1.5 was applied to analysis of variance results.

Compound Annotation

Compounds were annotated using MassHunter ID Browser B.08 (Agilent technologies) to search in-house and publicly available or commercial databases. The in-house database is composed of a set of 683 authentic standards; database matches were based on isotope ratios, neutral mass (using a 10 ppm window), and retention time. Public and commercial databases included HMDB 4.0, Lipid Maps, Metlin and KEGG.[36–39] Annotations from public or commercial databases were based on accurate mass, with a mass error cutoff of 10 ppm, isotope ratios. A match score cutoff of 60 was applied.

Statistical Analysis

Demographic and biomarker data were compared between time points (November and April) and between groups (non-KFD and KFD) using the Wilcoxon-Mann Whitney test. Principal component analysis graphs and pathway enrichment or topology analysis tables were created using MetaboAnalyst 5.0 and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database or Small Molecule Pathway Database with a false discovery rate corrected *P*-value (q-value) threshold of 0.05 used to determine statistical significance. All other statistical analyses and graphics were performed or generated in GraphPad Prism (Version 8.4.3; La Jolla, CA) using a paired *t*-test with Holm-Šídák multiple comparisons correction and a *P*-value threshold of 0.05 to determine statistical significance.

RESULTS

Demographics and Laboratory Features

The demographic features of both groups (non-KFD vs. KFD) reveal that the workers with evidence of KFD were, on average, older and weighed more at the start of the season (Table 1). Urine creatinine and specific gravity indicate more dilute urine at the end of the harvest season; however, kidney injury marker 1 levels were still within normal ranges at both timepoints. In April, workers with KFD had significantly higher levels of urine potassium and chloride compared to non-KFD workers.

Table 1. Demographic and biomarkers descriptives among sugarcane cutters at the start of the harvest, November, and the end of the harvest, April, stratified by workers with normal kidney function (non-KFD) and workers with evidence of kidney function decline (KFD)

	Normal Kidney Function (non-KFD) (n = 10)		Kidney Function Decline (KFD) (n = 10)	
Characteristics	**November**	**April**	**November**	**April**
Demographics				
Age, yr, mean (SD)	24 (7)	-	29 (3)	-
HbA1c, %, mean (SD)	5.3 (0.1)	-	5.4 (0.3)	
Systolic blood pressure, mm Hg, mean (SD)	108 (14.8)	109.6 (7.7)	106 (8.4)	109.6 (8)
Diastolic blood pressure, mm Hg, mean (SD)	75 (7.1)	62.9 (4.2)[a]	70 (9.4)	59.5 (9.4)
Height, m, mean (SD)	1.6 (0.1)	-	1.6 (0.1)	-
Weight, kg, mean (SD)	56.5 (6.0)	53.9 (5.5)	62.3 (6.6)	59.2 (8.4)
Markers of kidney function				
Serum creatinine, mg/dl, mean (SD)	0.79 (0.10)	0.77 (0.10)	0.79 (0.14)	1.02 (0.18)[a]
eGFR, ml/min per 1.73 m^2, mean (SD)	125.9 (8.1)	127.3 (7.7)	122.9 (11.6)	103.2 (17.8)[a]
Percent change in eGFR, mean (SD), min–max	-	1.1 (0.99), 0–3.00		−21.00 (13.48), −53.00 to −9.00
Albumin-to-creatinine ratio, mg/g, mean (SD)	9.3 (6.0)	8.4 (4.5)	6.4 (5.2)	7.6 (2.3)
Urine KIM-1, pg/ml (SD)	1014 (817.2)	352.5 (598.3)	649.3 (691.7)	239.7 (196.9)
Urine KIM-1, pg/g creatinine	869 (677)	690 (1432)	464 (514)	299 (204)
Urine biomarkers				
Urine creatinine, mg/dl, mean (SD)	135.8 (67.7)	69.2 (35.3)[a]	135.4 (67.6)	80.5 (40.6)[a]
Urine albumin, mg/l, mean (SD)	14.59 (16.31)	4.67 (1.48)[a]	8.19 (7.13)	5.51 (1.79)
Urine lactate, mmol/l, mean (SD)	3.09 (1.41)	1.84 (1.06)[a]	5.47 (3.24)	2.02 (0.72)[a]
Urine phosphate, mg/dl, mean (SD)	38.35 (27.75)	22.48 (11.92)	19.44 (12.66)	29.44 (24.43)
Urine magnesium, md/dl, mean (SD)	9.54 (5.59)	7.93 (3.21)	7.19 (3.06)	8.17 (4.24)
Urine sodium, mmol/l, mean (SD)	60.90 (33.39)	44.6 (28.91)	73.56(31.34)	63.2 (41.56)
Urine potassium, mmol/l, mean (SD)	60.96 (26.37)	19.43 (12.82)[a]	47.68 (16.4)	33.38 (10.33)[a]
Urine chloride, mmol/l, mean (SD)	139.0 (52.51)	56.1 (27.79)[a]	130.11 (39.38)	91.2 (38.7)
Urine specific gravity, mean (SD)	1.019 (0.006)	1.010 (0.005)[a]	1.018 (0.006)	1.013 (0.004)
Urine pH, mean (SD)	5.85 (0.63)	6.45 (0.83)	6.00 (1.00)	6.05 (0.92)

eGFR, estimated glomerular filtration rate; KFD, kidney function decline; KIM-1, Kidney Injury Molecule-1.
[a]indicates a P-value <0.05 between November and April within groups using the Wilcoxon-Mann Whitney test.

Elemental Analysis

Inductively coupled plasma mass spectrometry analysis revealed consistent trends in elemental levels in urine over the harvest season. Silicon and phosphorus were found to increase significantly from November to April, with silicon levels being higher in every sample (Figure 2a) and phosphorus in 85% of samples (Figure 2b). Interestingly, the concentration of metals hypothesized to be associated with CKDu (i.e., nickel, cadmium, lead, and arsenic) remained stable over the harvest season (Figure 2c). Individual values for all samples can be found in the supplementary material (Supplementary Table S1).

Metabolomic Analysis

Untargeted metabolomics detected 4799 compounds, 3150 of which were present in ≥50% of samples, 3419 of which were present in ≥50% of November samples, 2963 of which were present in ≥50% of April samples, and 1154 of which were detectable in all samples (Supplementary Table S2). Metabolite data were normalized to creatinine, with the metabolites demonstrating the greatest change over the harvest season expressed in the form of an increased or decreased foldchange heat map (Figure 3a). These metabolites consisted primarily of amino acids, botanical compounds, and fatty acids. Metabolic changes were consistent among subgroups, with minimal variation between KFD and non-KFD workers. When visualized in the context of a principal component analysis plot (Figure 3b), this becomes even more evident. Despite having reduced eGFR, metabolic profiles of KFD workers demonstrated high intergroup clustering with non-KFD workers. However, there was evidence of cross-harvest changes, with samples from the start of the harvest season (November) clearly separating from samples at the end of the harvest season (April). To further explore these changes, data were uploaded to MetaboAnalyst and underwent pathway enrichment and topology analysis within the context of the Small Molecule Pathway Database (Table 2) and the KEGG database (Supplementary Table S3). There were several significantly impacted pathways, including altered amino acid metabolism, fatty acid oxidation, glutathione homeostasis, and various energetic pathways (i.e., pyruvate and carnitine metabolism). In addition, there were signs of perturbation to nicotinamide adenine dinucleotide (NAD+) biosynthesis in the form

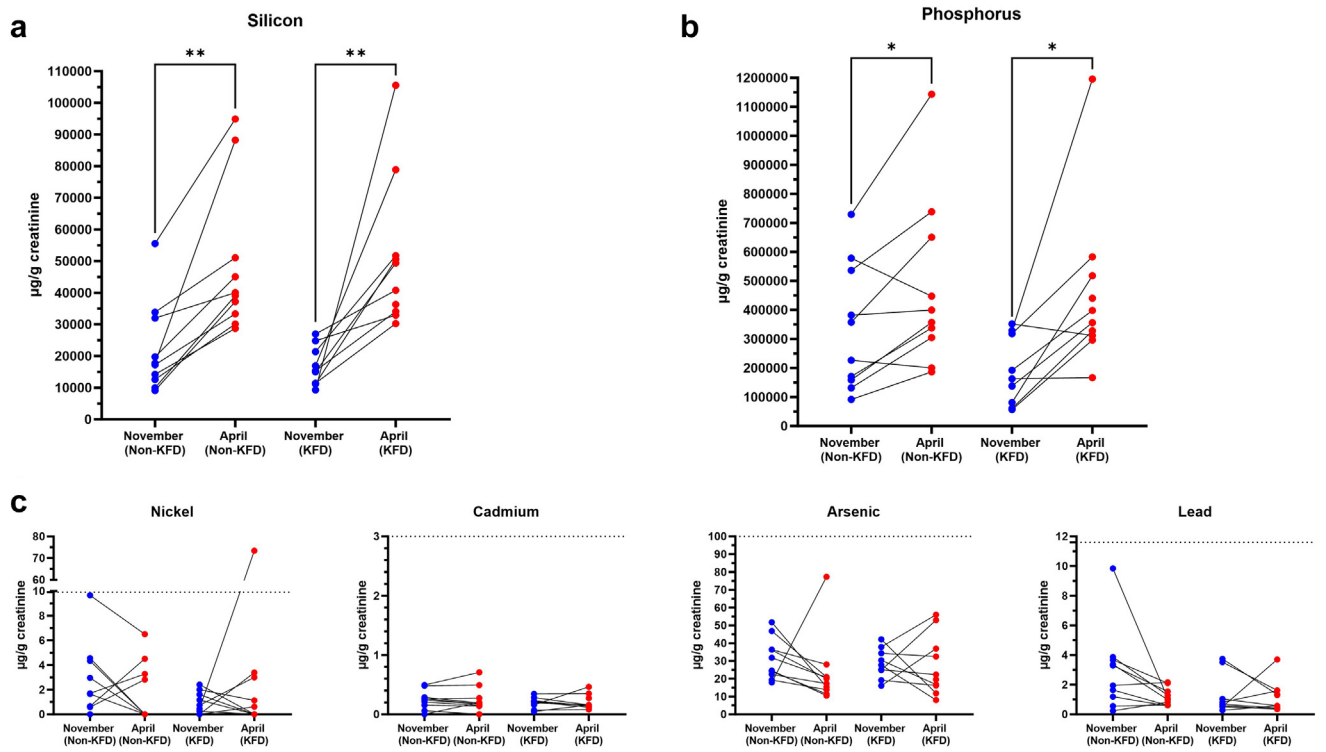

Figure 2. Inductively coupled plasma mass spectrometry (ICPMS) analysis found urinary concentrations of silicon (a) and phosphorus (b) significantly increased over the harvest season, whereas nephrotoxic metals, nickel, cadmium, lead, and arsenic (c) did not change significantly. Each dot represents an individual worker sample ($n = 20$). Dashed lines represent upper reference values (in µg/l) from the literature for nickel, from the Occupational Safety and Health Administration for cadmium, and from the Agency for Toxic Substances and Disease Registry (ATSDR) for arsenic.[40-42] For lead, the dashed line is based on the geometric mean of nonsmokers from the ATSDR's 2020 Toxicological Profile for lead.[43] Data were analyzed with a paired t-test with Holm-Šídák multiple comparisons correction (*indicates a *P*-value <0.05, ** indicates a *P*-value <0.01). KFD, kidney function decline.

of altered tryptophan, nicotinate, and nicotinamide metabolism. Specific compound hits from pathway analysis (Table 3) followed similar trends across the harvest season, with comparable fold change values between KFD and non-KFD workers (Supplementary Figure S1).

Metabolite Abundance Trends

Fatty acid accumulation is a hallmark of CKD and is indicative of impaired beta oxidation, reduced mitochondrial function, and perturbed energy metabolism. Metabolites associated with such pathways were quantified and analyzed via paired *t*-tests between November and April timepoints (Figure 4a). N6,N6,N6-trimethyl-L-lysine, valerylcarnitine, deoxycarnitine, dodecanedioylcarnitine, L-hexanoylcarnitine, and octanoyl-L-carnitine were found to increase significantly in abundance. Propionyl-L-carnitine abundance was found to decrease. Next, we similarly investigated amino acid metabolites known to correlate with compromised renal function, kidney injury, and disease progression (Figure 4b). Histidine, L-isoleucine, methionine, proline, valine, sarcosine, pyrimidine, and homocysteine were all found to be higher in abundance at the end of the harvest season.

Pesticide Metabolomics

Metabolite compounds demonstrating ≥1.5-fold change over the harvest season (Supplementary Table S4) were searched against a pesticide database and expressed as a heat map (Figure 5a). Although the majority of detected pesticide metabolites were not found to increase from November to April, carbofuran-3-keto, metolachlor, diquat, and paraquat were found to increase significantly over the harvest season (Figure 5b).

DISCUSSION

This study demonstrates the value of a combined exposome and metabolome analysis, providing insights into agricultural workers' key occupational risk factors and potential mechanisms of CKDu pathogenesis in agricultural field workers exposed to complex climatologic and environmental exposure conditions.

Exposomic analysis of urine samples revealed elevated levels of silicon over the harvest season, indicating a consistent occupational exposure among sugarcane cutters, with potential renal health risks. Nephrotoxic metals remained low, suggesting minimal exposure. Several pesticides, including some which

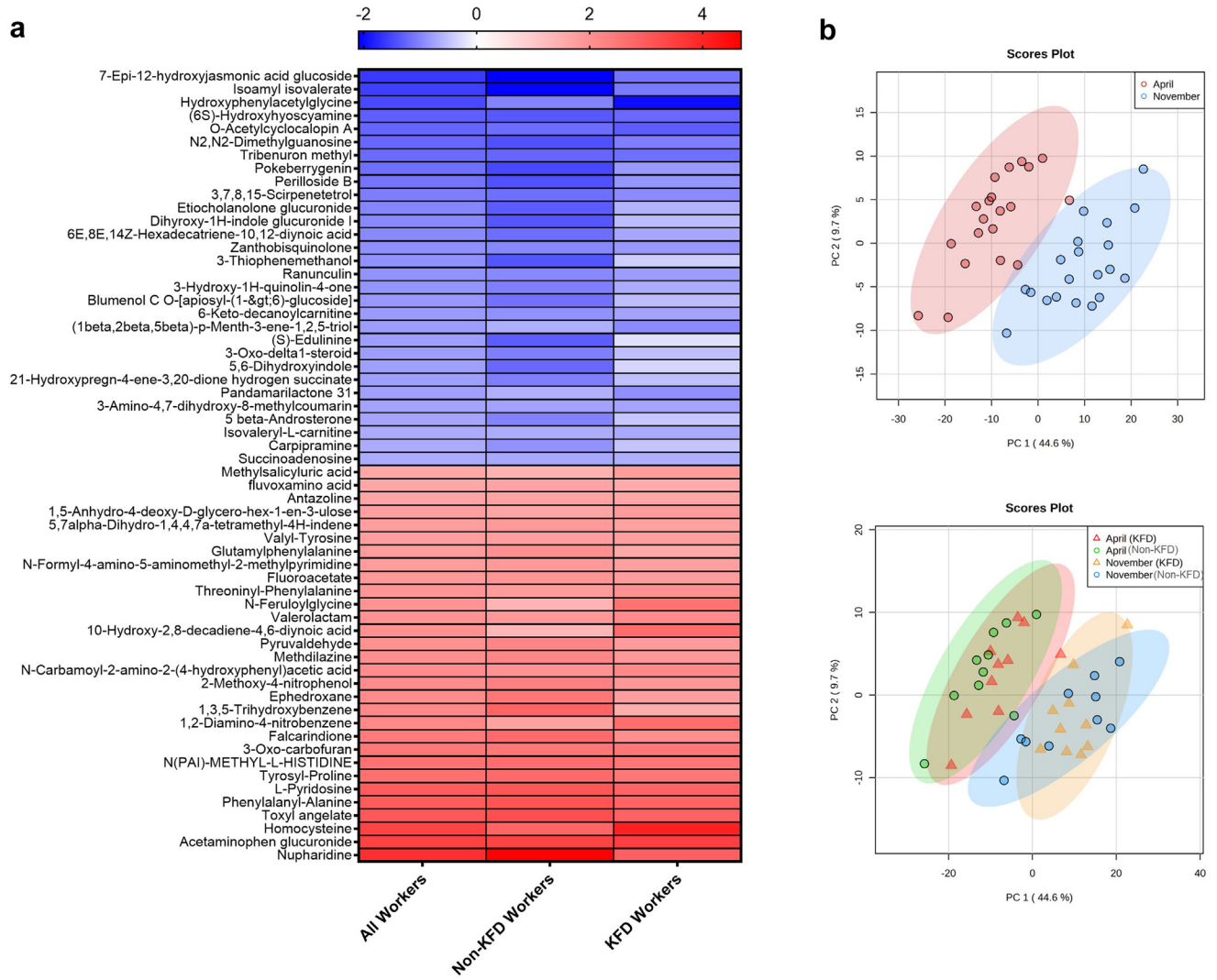

Figure 3. Heat map of metabolite fold change from November to April (a) reveals highest up and down regulated metabolites are primarily those associated with fatty acid and amino acid metabolism. Principle component analysis (PCA) plots (b) demonstrate high clustering based on time point among all workers (top figure) and among both kidney function decline (KFD) and non-KFD agricultural workers (bottom figure). Original compound values were log2 transformed, normalized to creatinine, and analyzed via metaboanalyst ($n = 20$). KFD, kidney function decline.

have been implicated as potentially nephrotoxic, were found to increase in abundance from November to April. This demonstrates evidence of pesticide uptake and may help explain the metabolome changes which were found to occur over the harvest season, including altered energy metabolism and trends in metabolite abundance consistent with CKD biomarkers. Understanding these exposures and the key risk factors at play is critical as new epidemics of kidney disease, including CKDu, continue to increase around the world.[44]

Exposure to heavy metals has long been known to cause renal damage and is suspected of playing a key role in recent epidemics of kidney injury, due to use of agrochemicals known to contain nephrotoxic metals and some evidence of elevated heavy metal levels in agricultural communities' water sources.[21,45-49] Indeed, arsenic, cadmium, and lead are pervasive toxicants with established mechanisms for inducing both acute kidney injury and CKD, resulting in ongoing investigations into potential contributions to the epidemics of CKDu occurring worldwide.[50,51] Although some association of heavy metals have been made with increased risk of CKDu, such findings have been inconsistent.[23,24] Thus, it is unsurprising that elemental analysis found that urinary levels of heavy metals were relatively low and did not increase significantly over the harvest.[40-43] It is important to emphasize that the agricultural company where these workers were employed had implemented a program to reduce worker exposure to heavy metal contaminated water through routine QC testing for metals in their supplied drinking water. We would caution against generalizing from the observation of low heavy metal exposure

Table 2. Significantly impacted pathways based on the Small Molecule Pathway Database with *P*-values from pathway enrichment analysis and pathway impact values from pathway topology analysis

Pathway	Total compounds	Hits	November vs. April (All)	November (Non-KFD) vs. April (Non-KFD)	November (KFD) vs. April (KFD)	April (Non-KFD) vs. April (KFD)	Impact
Betaine Metabolism	18	3 (Betaine aldehyde; Betaine;Homocysteine)	5.0252E-07	0.008983	0.000020551	0.11756	0.34862
Carnitine Synthesis	16	1 (3-Hydroxy-N6,N6,N6-trimethyl-L-lysine)	0.00084994	0.038378	0.0047409	0.88928	0.32941
Histidine Metabolism	35	1 (L-Histidine)	0.014709	0.010182	0.18375	0.75647	0.23416
Methionine Metabolism	39	3 (Homocysteine; Sarcosine; Betaine)	3.8911E-07	0.0085505	0.000020551	0.75074	0.14308
Tryptophan Metabolism	55	2 (3-Hydroxyanthranilic acid; Tetrahydrobiopterin)	0.000058313	0.01131	0.004632	0.32579	0.071296
Glycine and Serine Metabolism	50	4 (Pyruvaldehyde; Homocysteine; Betaine; Sarcosine)	1.0558E-08	0.00080861	0.000020551	0.011282	0.067324
Beta-Alanine Metabolism	26	2 (L-Histidine; Ureidopropionic acid)	0.0027437	0.010182	0.078916	0.006329	0.066116
Androstenedione Metabolism	23	2 (Etiocholanolone glucuronide; Etiocholanolone)	0.00036168	0.0085505	0.072899	0.3314	0.053333
Pyrimidine Metabolism	54	2 (Ureidopropionic acid; Ureidoisobutyric acid)	0.00015386	0.010182	0.013997	0.006329	0.029225
Pterine Biosynthesis	18	1 (Tetrahydrobiopterin)	0.0031783	0.029289	0.073112	0.3751	0.012869
Nicotinate and Nicotinamide Metabolism	32	1 (Nicotinamide riboside)	0.0010067	0.018867	0.039495	0.34175	0.010436
Tyrosine Metabolism	55	3 (Ascorbic acid; Tetrahydrobiopterin; 5,6-Dihydroxyindole)	0.000058119	0.000066355	0.06462	0.32579	0.0014347
Ammonia Recycling	25	1 (L-Histidine)	0.014709	0.010182	0.18375	0.32658	0
Catecholamine Biosynthesis	14	1 (Ascorbic acid)	0.00056843	0.0085505	0.058653	0.13075	0
Homocysteine Degradation	7	1 (Homocysteine)	1.4172E-06	0.010182	0.000020551	0.74332	0
Pyruvaldehyde Degradation	7	1 (Pyruvaldehyde)	3.2267E-09	0.000054311	0.00012806	0.8889	0
Pyruvate Metabolism	37	1 (Pyruvaldehyde)	3.2267E-09	0.000054311	0.00012806	0.89075	0
Vitamin B6 Metabolism	15	1 (4-Pyridoxic acid)	0.00064053	0.010587	0.031123	0.077747	0

KFD, kidney function decline.
Data is ordered by descending impact factor (q value <0.05 is indicated by a bolded value). Original compound values were log2 transformed and analyzed via metaboanalyst ($N = 20$).

because the exposure control efforts of this company may not be representative of the industry at large and urine samples are less accurate than blood tests for determining chronic exposure to certain metals.

Unlike heavy metals, urinary abundance of silicon increased over the harvest season. This confirms that silicon is a concerning occupational exposure and lends additional support to evidence of cane worker exposure

Table 3. Compound hits from pathway analysis with fold change from November to April and across groups with or without KFD

Compounds	November vs. April (All)	November (Non-KFD) vs. April (Non-KFD)	November (KFD) vs. April (KFD)	April (Non-KFD) vs. April (KFD)
Betaine aldehyde	1.0394004	1.1351618	0.943639	−0.7584812
3-Hydroxy-N6, N6,N6-trimethyl-L-lysine	1.1236717	1.1721541	1.0751893	−0.4505893
L-Histidine	0.7307202	0.9475248	0.5139156	−1.18501835
Homocysteine	3.42772305	2.8106313	4.0448148	−0.0611859
Sarcosine	1.359087625	1.44399085	1.2741844	−1.0571272
Betaine	0.6151725	0.55932625	0.67101875	−0.07658775
3-Hydroxyanthranilic acid	1.45654495	0.5908845	2.3222054	−0.1527283
Tetrahydrobiopterin	0.956942125	1.01491395	0.8989703	−0.1508771
Pyruvaldehyde	2.0163203	2.2148971	1.8177435	−0.3794771
Ureidopropionic acid	0.670869	0.73045375	0.61128425	−0.19337995
Etiocholanolone glucuronide	−0.19546855	−0.32636545	−0.06457165	−0.0285683
Etiocholanolone	−0.993823325	−1.3519187	−0.63572795	0.26880215
Ureidoisobutyric acid	1.03568835	1.16121055	0.91016615	−0.0829997
Nicotinamide riboside	1.030189025	1.1395568	0.92082125	−0.40516915
Ascorbic acid	1.3980836	1.5413648	1.2548024	0.0890468
5,6−Dihydroxyindole	−0.79335603	−1.23406921	−0.35264285	0.93608066
4-Pyridoxic acid	2.276566175	3.1774513	1.37568105	−0.4154801

KFD, kidney function decline.
Original compound values were log2 transformed, normalized to creatinine, and analyzed via metaboanalyst ($n = 20$).

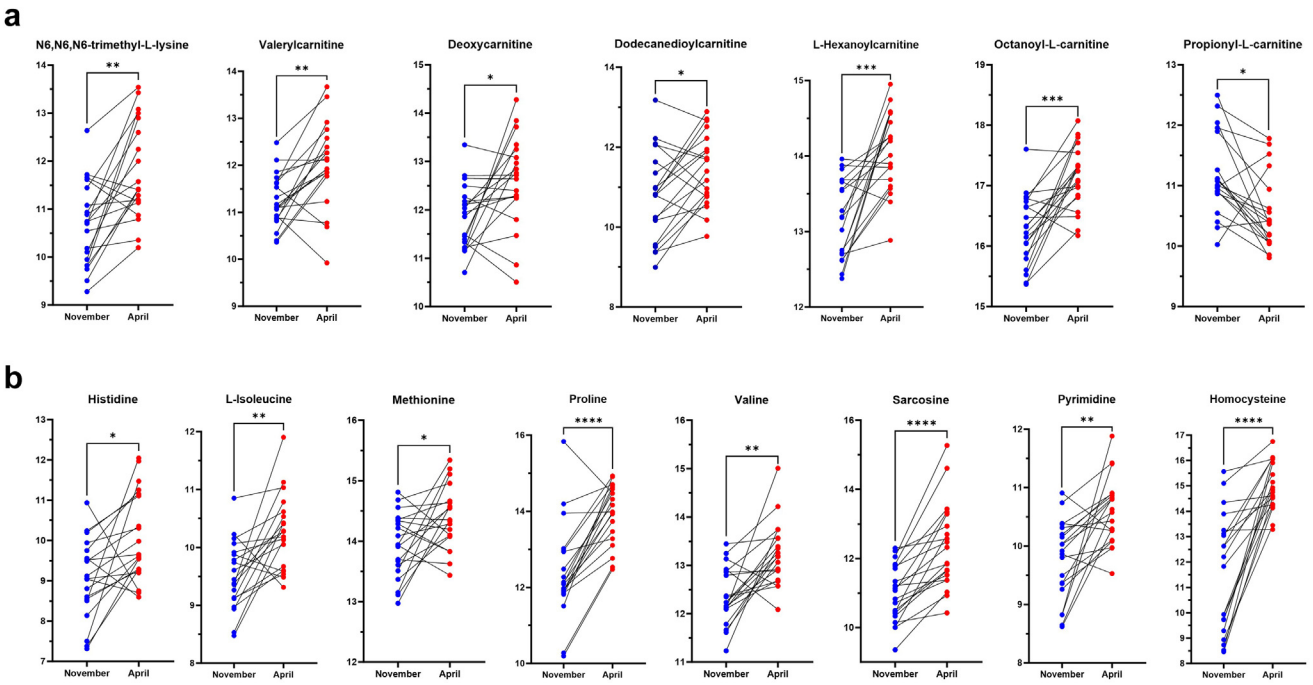

Figure 4. Trends in fatty acid (a) and amino acid (b) metabolites over the harvest season among all workers were consistent with known signs of impaired renal function and kidney disease. Data is presented as log2 signal intensity normalized to creatinine and each dot represents an individual worker sample ($n = 20$). Data were analyzed with a paired t-test with Holm-Šídák multiple comparisons correction (* indicates a *P*-value <0.05, ** indicates a *P*-value <0.01, *** and **** indicate a *P*-value <0.001 and <0.0001, respectively).

and absorption of silica nanoparticles via ash inhalation from sugarcane burning and/or groundwater consumption.[52] Such amorphous silica carries known risks and can be resistant to clearance, potentially leading to accumulation after each harvest.[53-58] This accumulation in the kidney tissue, as has been demonstrated in worker biopsies, may limit urinary excretion and could explain why abundance did not differ significantly between KFD and non-KFD groups.[59] Elevated phosphorus is also notable as a known indicator of renal damage resulting in altered kidney function. Renal proximal tubular cells are responsible for maintaining phosphate homeostasis through reabsorption; exposure to nephrotoxic metals, pesticides, and/or silica could damage these cells and result in increased urinary phosphate excretion.[60,61] However, such damage would generally be expected to be accompanied by increased kidney injury marker 1 levels, rather than the slight cross-harvest decrease observed. High phosphate has been found to be associated with reactive oxygen species generation, mitochondrial dysfunction, cell death, and progression of CKD.[62-66] Interestingly, similar hallmarks of kidney disease have been found *in vitro* following silica nanoparticle exposure and in animal models following sugarcane ash exposure.[26,57,58] As with silicon, phosphorus abundance was comparable between KFD and non-KFD workers despite increasing over the harvest season. One reason for this could be that reduced glomerular filtration rate can limit renal clearance, causing levels to appear lower than expected, particularly when normalizing elemental expression to creatinine.

Pesticides are another occupational and community exposure of concern, due in part to their abundant use in agricultural communities and tendency to contain nephrotoxic components. Previous epidemiologic investigations into the link between pesticide exposure and CKDu have produced varying results, with several finding positive associations.[20,21,67-69] Carbofuran, metolachlor, paraquat, and diquat are 4 of such compounds which are known to cause acute kidney injury and have been previously implicated in CKD.[67,69-74] In particular, a recent study found evidence that paraquat might have a participatory role in CKDu from Central America.[75] These pesticides and associated metabolites were found to increase in abundance over the harvest season, across both KFD and non-KFD workers. Even if such exposures are not solely responsible for elevated rates of kidney disease seen among this population, they are yet another nephrological stressor that could be contributing to pathogenesis. CKDu etiology is likely multifactorial. We speculate that even low levels of pesticide exposure could be hazardous if occurring alongside chronic exposure to silica, heavy metals, heat stress, and dehydration. Characterizing the metabolomic state of individuals who experience multiple exposures is essential to determine specific mechanisms and pathways involved in pathogenesis.

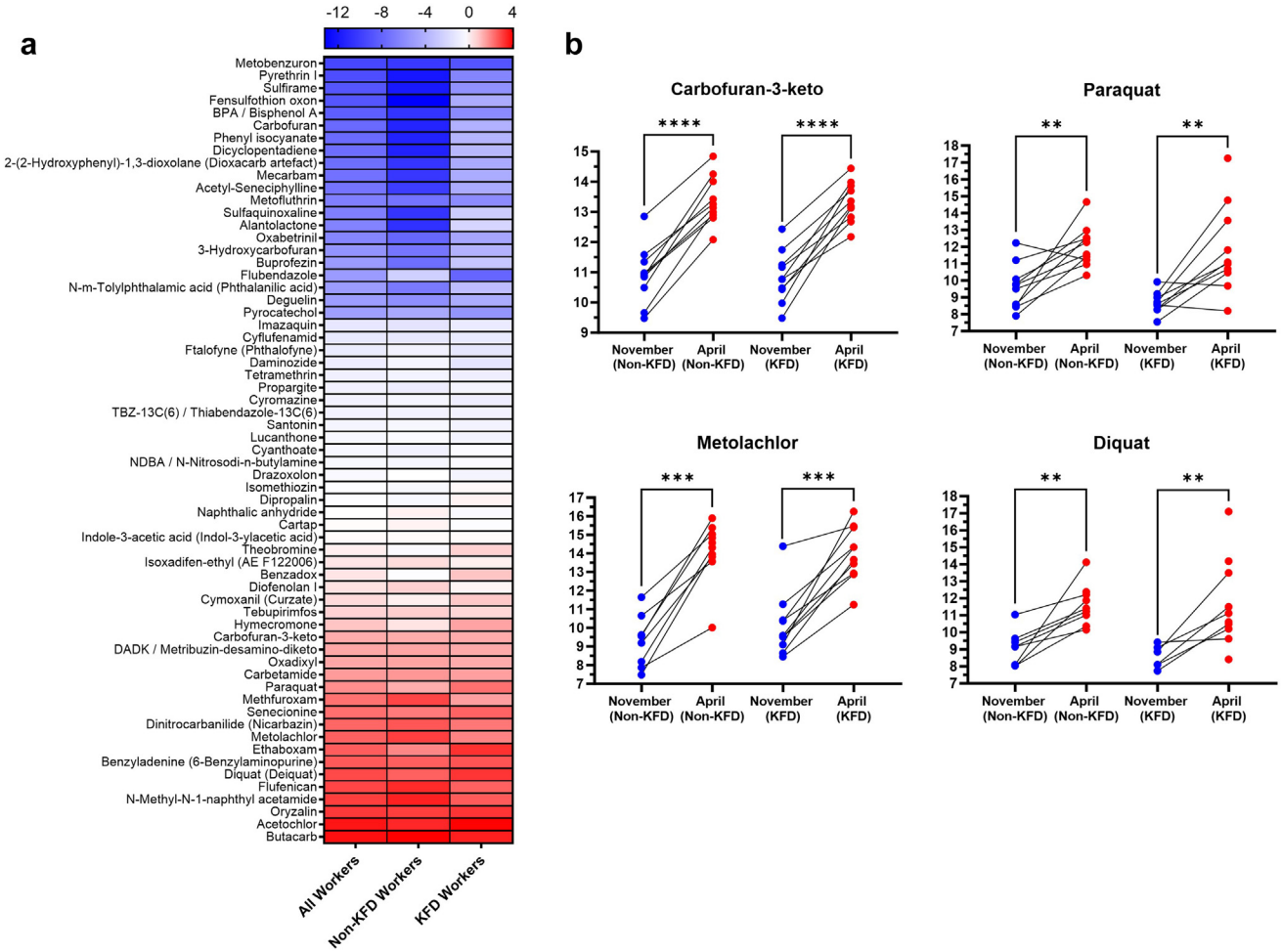

Figure 5. Heat map of pesticide metabolite trends from November to April. Overall pesticide metabolite trends remained consistent across groups (a). Workers with normal kidney function (non-KFD) demonstrated greater apparent decreases in some metabolites over the harvest season; however, metabolite abundance of certain potentially nephrotoxic pesticides increased across the season in both groups (b). Original compounds were referenced to a pesticide database, values were log2 transformed, and normalized to creatinine. Data is presented as fold change over the harvest season ($n = 20$). Potentially nephrotoxic pesticide metabolites were analyzed with a paired t-test with Holm-Šídák multiple comparisons correction (* indicates a P-value <0.05, ** indicates a P-value <0.01). KFD, kidney function decline.

Alteration in energy metabolism is a hallmark of CKD, which has been found to occur in populations at high risk for CKDu.[28] The kidney is an extremely energy-intensive organ, requiring large amounts of functional mitochondria to remain healthy.[76] It is therefore highly sensitive to any changes in energy production, with disease states demonstrating pronounced shifts in energy metabolism. Nicotinamide metabolism was altered significantly over the harvest season, along with carnitine synthesis, pyruvate metabolism, and expression of several fatty acid metabolites. NAD+ is a crucial coenzyme for a variety of cellular processes, including DNA repair, cellular senescence, and energy metabolism.[77] Perturbed energy metabolism associated with NAD+ is known to occur in states of kidney stress, with depletion leading to ischemia and renal tubular injury.[78–80] Increased abundance of free fatty acids is indicative of inhibited beta oxidation of fatty acids, a key mechanism of ATP generation in the kidney.[29,81–85] This trend is not only linked with CKD, but specific fatty acid metabolites were found to follow the same pattern as occurred *in vitro* following exposure to silica nanoparticles derived from sugarcane ash.[26] Furthermore, the only fatty acid associated metabolite that was found to undergo a significant decrease both over the harvest season and *in vitro* was propionyl-l-carnitine, a metabolite which has known nephroprotective effects.[86,87] Beyond nicotinamide and lipid metabolism perturbation, a variety of amino acid metabolites (i.e., histidine, methionine, or valine) were found to demonstrate signs consistent with impaired renal function and kidney disease.[88–95] Phenylacetic acid and acetaminophen glucuronide were other metabolites of concern found to increase in abundance over

the harvest season, the former of which has been found to be elevated in CKD patient and both of which are capable of inducing inflammation and oxidative stress.[96–100] These changes are consistent with inhibition of mitochondrial respiration, perturbed lipid metabolism, and glycolytic shifts that occur during ischemic acute kidney injury. If injury is so severe that cells are unable to recover from the stress and energy production remains insufficient, this can be followed by proximal tubule atrophy, fibrosis, and a transition to CKD.[101] It is this pathology that is seen both in CKDu patient biopsies as well as *in vivo* models of silica exposure.

There are several limitations which must be acknowledged and considered to contextualize these findings. The sample size and timepoints of this study are key considerations. Although many elemental and metabolic trends were consistent throughout groups and over the harvest season, it is unclear whether such findings would remain consistent over multiple harvests or would be observed in other CKD-prone populations (i.e., Sri Lankan/Indian sugarcane harvesters). In addition, it is unknown if any of the workers included in this study later developed signs of renal dysfunction, it is possible that observed trends might be more closely associated with long-term kidney health issues rather than the "snapshot" eGFR values used to assign groups in the current study. The use of creatinine to normalize for sample concentration also may obscure some group variation. Such factors could explain why elemental abundance and metabolic trends were consistent over the harvest but varied minimally across KFD/non-KFD groups. Another limitation is the use of untargeted liquid chromatography-mass spectrometry, which is valuable for initial exploration and trend identification, and should ideally be followed-up with more targeted approaches to confirm suspected metabolic and pathway changes. The use of urine as a biomatrix also limits heavy metal analysis, particularly given the discussed company water interventions. To properly evaluate chronic metal exposure, a blood test in addition to urine analysis would be ideal. Lastly, it is important to recognize that the experimental approach of this study is unable to determine directionality of associations. Changes to renal function, such as altered glomerular filtration rate or compromised proximal convoluted tubule uptake, could greatly impact elemental and metabolic expression. Therefore, though the findings of this study provide a promising start, future research is needed to further elucidate the role of perturbed lipid or amino acid msilica,ism, silica, and pesticides in CKD pathogenesis.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

ADS and KLR contributed to project conception, experimental design, data acquisition, and data analysis and interpretation. SB, JB-D, LK, and LSN contributed to sample collection, experimental design, data acquisition, and data analysis. AS contributed to data analysis. CAR-J and RJJ contributed to project conception and data analysis. JMB contributed to project conception, experimental design, and data analysis and interpretation. All authors were involved in drafting, revision, and final approval of the publishable version.

SUPPLEMENTARY MATERIAL

Supplementary file (PDF)

Figure S1. Specific compound hits from pathway analysis expressed as a fold change heat map across groups (PNG).
Table S1. Complete raw inductively coupled plasma mass spectrometry elemental analysis table (Excel).
Table S2. Complete raw metabolomic results table (Excel).
Table S3. Significantly impacted pathways based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) with *P*-values from pathway enrichment analysis and pathway impact values from pathway topology analysis (Excel).
Table S4. Complete raw pesticide metabolomic results table (Excel).

REFERENCES

1. Food and Administration Organization of the United Nations. FAOSTAT. Accessed February 21, 2024. http://www.fao.org/faostat/en/#data/QC

2. Leite MR, Zanetta DMT, Trevisan IB, Burdmann Ede A, Santos Ude P. Sugarcane cutting work, risks, and health effects: a literature review. *Rev Saúde Publ*. 2018;52:80.
3. Laws RL, Brooks DR, Amador JJ, et al. Biomarkers of kidney injury among Nicaraguan sugarcane workers. *Am J Kidney Dis Off J Natl Kidney Found*. 2016;67:209–217. https://doi.org/10.1053/j.ajkd.2015.08.022
4. Le Blond JS, Woskie S, Horwell CJ, Williamson BJ. Particulate matter produced during commercial sugarcane harvesting and processing: a respiratory health hazard? *Atmos Environ*. 2017;149:34–46. https://doi.org/10.1016/j.atmosenv.2016.11.012
5. Johnson RJ, Wesseling C, Newman LS. Chronic kidney disease of unknown cause in agricultural communities. *N Engl J Med*. 2019;380:1843–1852. https://doi.org/10.1056/NEJMra1813869
6. Sasai F, Roncal-Jimenez C, Rogers K, et al. Climate change and nephrology. *Nephrol Dial Transplant*. 2023;38:41–48. https://doi.org/10.1093/ndt/gfab258
7. Chang C-J, Yang H-Y. Chronic kidney disease among agricultural workers in Taiwan: a nationwide population-based study. *Kidney Int Rep*. 2023;8:2677–2689. https://doi.org/10.1016/j.ekir.2023.09.004
8. Broe MED, Vervaet BA. Is an environmental nephrotoxin the primary cause of CKDu (Mesoamerican nephropathy)? PRO. *Kidney360*. 2020:591–595.
9. Pearce N. Is CKDu an occupational or an environmental disease? *Environ Epidemiol*. 2019;3:306.
10. Floris M, Lepori N, Angioi A, et al. Chronic kidney disease of undetermined etiology around the world. *Kidney Blood Press Res*. 2021;46:142–151. https://doi.org/10.1159/000513014
11. Ranasinghe AV, Kumara GWGP, Karunarathna RH, et al. The incidence, prevalence and trends of Chronic Kidney Disease and Chronic Kidney Disease of uncertain aetiology (CKDu) in the North Central Province of Sri Lanka: an analysis of 30, 566 patients. *BMC Nephrol*. 2019;20:338. https://doi.org/10.1186/s12882-019-1501-0
12. Fischer RSB, Mandayam S, Chavarria D, et al. Clinical evidence of acute Mesoamerican nephropathy. *Am J Trop Med Hyg*. 2017;97:1247–1256. https://doi.org/10.4269/ajtmh.17-0260
13. Selvarajah M, Weeratunga P, Sivayoganthan S, Rathnatunga N, Rajapakse S. Clinicopathological correlates of chronic kidney disease of unknown etiology in Sri Lanka. *Indian J Nephrol*. 2016;26:357–363. https://doi.org/10.4103/0971-4065.167280
14. Wimalawansa SJ. Public health interventions for chronic diseases: cost-benefit modelizations for eradicating chronic kidney disease of multifactorial origin (CKDmfo/ CKDu) from tropical countries. *Heliyon*. 2019;5:e02309. https://doi.org/10.1016/j.heliyon.2019.e02309
15. Butler-Dawson J, Krisher L, Asensio C, et al. Risk factors for declines in kidney function in sugarcane workers in Guatemala. *J Occup Environ Med*. 2018;60:548–558. https://doi.org/10.1097/JOM.0000000000001284
16. Sorensen CJ, Butler-Dawson J, Dally M, et al. Risk factors and mechanisms underlying cross-shift decline in kidney function in Guatemalan sugarcane workers. *J Occup Environ Med*. 2019;61:239–250. https://doi.org/10.1097/JOM.0000000000001529
17. Glaser J, Hansson E, Weiss I, et al. Preventing kidney injury among sugarcane workers: promising evidence from enhanced workplace interventions. *Occup Environ Med*. 2020;77:527–534. https://doi.org/10.1136/oemed-2020-106406
18. Zúñiga-Venegas LA, Hyland C, Muñoz-Quezada MT, , et alZ úñiga-V L. Health Effects of Pesticide Exposure in Latin American and the Caribbean Populations: a Scoping Review. *Environ Health Perspect*. 2022;130:096002. https://doi.org/10.1289/EHP9934
19. Rajapaksha H, Pandithavidana DR, Dahanayake JN. Demystifying chronic kidney disease of unknown etiology (CKDu): computational interaction analysis of pesticides and metabolites with vital renal enzymes. *Biomolecules*. 2021;11:261. https://doi.org/10.3390/biom11020261
20. Alvand S, Alatab S, Dalvand S, et al. Association of indoor use of pesticides with CKD of unknown origin. *PLoS One*. 2023;18:e0277151. https://doi.org/10.1371/journal.pone.0277151
21. Valcke M, Levasseur M-E, Soares da Silva A, Wesseling C. Pesticide exposures and chronic kidney disease of unknown etiology: an epidemiologic review. *Environ Health Glob Access Sci Source*. 2017;16:49. https://doi.org/10.1186/s12940-017-0254-0
22. Fischer RSB, Unrine JM, Vangala C, Sanderson WT, Mandayam S, Murray KO. Evidence of nickel and other trace elements and their relationship to clinical findings in acute Mesoamerican Nephropathy: a case-control analysis. *PLoS One*. 2020;15:e0240988. https://doi.org/10.1371/journal.pone.0240988
23. Anupama YJ, Kiran SK, Hegde SN. Heavy metals and pesticides in chronic kidney disease - results from a matched case-control study from a rural population in Shivamogga District in South India. *Indian J Nephrol*. 2019;29:402–409. https://doi.org/10.4103/ijn.IJN_325_18
24. Kulathunga MRDL, Ayanka Wijayawardena MA, Naidu R, Wijeratne AW. Chronic kidney disease of unknown aetiology in Sri Lanka and the exposure to environmental chemicals: a review of literature. *Environ Geochem Health*. 2019;41:2329–2338. https://doi.org/10.1007/s10653-019-00264-z
25. Rogers KL, Roncal-Jimenez CA, Leiva R, et al. Silica Nanoparticles and Mesoamerican Nephropathy: a case series. Am J Kidney Dis. https://doi.org/10.1053/j.ajkd.2023.06.010
26. Stem AD, Rogers KL, Roede JR, Roncal-Jimenez CA, Johnson RJ, Brown JM. Sugarcane ash and sugarcane ash-derived silica nanoparticles alter cellular metabolism in human proximal tubular kidney cells. *Environ Pollut*. 2023;332:121951. https://doi.org/10.1016/j.envpol.2023.121951
27. Paidi G, Iroshani Jayarathna AI, Salibindla DBAMR, et al. Chronic kidney disease of unknown origin: a mysterious epidemic. *Cureus*. 2022;13:e17132. https://doi.org/10.7759/cureus.17132
28. Raines NH, Leone DA, O'Callaghan-Gordo C, et al. Metabolic features of increased gut permeability, inflammation, and altered energy metabolism distinguish agricultural workers at risk for Mesoamerican nephropathy. *Metabolites*. 2023;13:325. https://doi.org/10.3390/metabo13030325
29. Jang H-S, Noh MR, Kim J, Padanilam BJ. Defective mitochondrial fatty acid oxidation and lipotoxicity in kidney diseases. *Front Med (Lausanne)*. 2020;7:65. https://doi.org/10.3389/fmed.2020.00065

30. Liu X, Du H, Sun Y, Shao L. Role of abnormal energy metabolism in the progression of chronic kidney disease and drug intervention. *Ren Fail*. 2022;44:790–805. https://doi.org/10.1080/0886022X.2022.2072743
31. Butler-Dawson J, Barnoya J, Brindley S, et al. Cross-sectional study examining the accuracy of self-reported smoking status as compared to urinary cotinine levels among workers at risk for chronic kidney disease of unknown origin in Guatemala. *BMJ Open*. 2021;11:e050374. https://doi.org/10.1136/bmjopen-2021-050374
32. Butler-Dawson J, Krisher L, Yoder H, et al. Evaluation of heat stress and cumulative incidence of acute kidney injury in sugarcane workers in Guatemala. *Int Arch Occup Environ Health*. 2019;92:977–990. https://doi.org/10.1007/s00420-019-01426-3
33. Inker LA, Eneanya ND, Coresh J, et al. New creatinine- and cystatin C-based equations to estimate GFR without race. *N Engl J Med*. 2021;385:1737–1749. https://doi.org/10.1056/NEJMoa2102953
34. Reisdorph NA, Hendricks AE, Tang M, et al. Nutrimetabolomics reveals food-specific compounds in urine of adults consuming a DASH-style diet. *Sci Rep*. 2020;10:1157. https://doi.org/10.1038/s41598-020-57979-8
35. Uffelman CN, Doenges KA, Armstrong ML, et al. Metabolomics profiling of white button, crimini, portabella, Lion's mane, maitake, oyster, and shiitake mushrooms using untargeted metabolomics and targeted amino acid analysis. *Foods Basel Switz*. 2023;12:2985. https://doi.org/10.3390/foods12162985
36. Wishart DS, Feunang YD, Marcu A, et al. HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Res*. 2018;46:D608–D617. https://doi.org/10.1093/nar/gkx1089
37. O'Donnell VB, Dennis EA, Wakelam MJO, Subramaniam S. LIPID MAPS: serving the next generation of lipid researchers with tools, resources, data, and training. *Sci Signal*. 2019;12:eaaw2964. https://doi.org/10.1126/scisignal.aaw2964
38. Smith CA, O'Maille G, Want EJ, et al. Metlin: a metabolite mass spectral database. *Ther Drug Monit*. 2005;27:747–751. https://doi.org/10.1097/01.ftd.0000179845.53213.39
39. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;28:27–30. https://doi.org/10.1093/nar/28.1.27
40. Sunderman FW. Biological monitoring of nickel in humans. *Scand J Work. Environ Health*. 1993;19(suppl 1):34–38.
41. U.S. Department of Labor. Occupational safety and health administration. Accessed September 27, 2023. https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1027
42. Toxicological profile for arsenic. Centers for Disease Control and Prevention. Accessed September 27, 2023. https://stacks.cdc.gov/view/cdc/11481
43. Toxicological profile for lead. Centers for Disease Control and Prevention. Accessed September 27, 2023. https://stacks.cdc.gov/view/cdc/95222
44. Jayasumana C, Orantes C, Herrera R, et al. Chronic interstitial nephritis in agricultural communities: a worldwide epidemic with social, occupational and environmental determinants. *Nephrol Dial Transplant*. 2017;32:234–241. https://doi.org/10.1093/ndt/gfw346
45. Nanayakkara S, Senevirathna ST, Abeysekera T, et al. An integrative study of the genetic, social and environmental determinants of chronic kidney disease characterized by tubulointerstitial damages in the North Central Region of Sri Lanka. *J Occup Health*. 2014;56:28–38. https://doi.org/10.1539/joh.13-0172-oa
46. Nayak S, Rehman T, Patel K, et al. Factors associated with chronic kidney disease of unknown etiology (CKDu): a systematic review. *Healthcare*. 2023;11:551. https://doi.org/10.3390/healthcare11040551
47. Zheng L, Kuo CC, Fadrowski J, Agnew J, Weaver VM, Navas-Acien A. Arsenic and chronic kidney disease: a systematic review. *Curr Environ Health Rep*. 2014;1:192–207. https://doi.org/10.1007/s40572-014-0024-x
48. Nyachoti S, Godebo TR, Okwori OF, Jeuland MA, Manthrithilake H. Dietary exposures to metals in relation to chronic kidney disease of unknown cause (CKDu) in Sri Lanka. *Expo Health*. 2022;14:63–73. https://doi.org/10.1007/s12403-021-00418-4
49. Rango T, Jeuland M, Manthrithilake H, McCornick P. Nephrotoxic contaminants in drinking water and urine, and chronic kidney disease in rural Sri Lanka. *Sci Total Environ*. 2015;518-519:574–585. https://doi.org/10.1016/j.scitotenv.2015.02.097
50. Moody EC, Coca SG, Sanders AP. Toxic metals and chronic kidney disease: a systematic review of recent literature. *Curr Environ Health Rep*. 2018;5:453–463. https://doi.org/10.1007/s40572-018-0212-1
51. Kwon JY, Lee S, Surenbaatar U, et al. Association between levels of exposure to heavy metals and renal function indicators of residents in environmentally vulnerable areas. *Sci Rep*. 2023;13:2856. https://doi.org/10.1038/s41598-022-27292-7
52. Mascarenhas S, Mutnuri S, Ganguly A. Deleterious role of trace elements - Silica and lead in the development of chronic kidney disease. *Chemosphere*. 2017;177:239–249. https://doi.org/10.1016/j.chemosphere.2017.02.155
53. Chen L, Liu J, Zhang Y, et al. The toxicity of silica nanoparticles to the immune system. *Nanomedicine (Lond)*. 2018;13:1939–1962. https://doi.org/10.2217/nnm-2018-0076
54. Chou CC, Chen W, Hung Y, Mou CY. Molecular elucidation of biological response to mesoporous silica nanoparticles in vitro and in vivo. *ACS Appl Mater Interfaces*. 2017;9:22235–22251. https://doi.org/10.1021/acsami.7b05359
55. Schütz I, Lopez-Hernandez T, Gao Q, et al. Lysosomal dysfunction caused by cellular accumulation of silica nanoparticles. *J Biol Chem*. 2016;291:14170–14184. https://doi.org/10.1074/jbc.M115.710947
56. Hornung V, Bauernfeind F, Halle A, et al. Silica crystals and aluminum salts mediate NALP-3 inflammasome activation via phagosomal destabilization. *Nat Immunol*. 2008;9:847–856. https://doi.org/10.1038/ni.1631
57. Sasai F, Rogers KL, Orlicky DJ, et al. Inhaled silica nanoparticles cause chronic kidney disease in rats. *Am J Physiol Ren Physiol*. 2022;323:F48–F58. https://doi.org/10.1152/ajprenal.00021.2022
58. Boudard D, Aureli F, Laurent B, et al. Chronic oral exposure to synthetic amorphous silica (NM-200) results in renal and liver lesions in mice. *Kidney Int Rep*. 2019;4:1463–1471. https://doi.org/10.1016/j.ekir.2019.06.007

59. Rogers KL, Roncal-Jimenez CA, Leiva R, et al. Silica nanoparticles and Mesoamerican nephropathy: a case series. *Am J Kidney Dis*. 2023. https://doi.org/10.1053/j.ajkd.2023.06.010
60. Prasad N, Bhadauria D. Renal phosphate handling: physiology. *Indian J Endocrinol Metab*. 2013;17:620–627. https://doi.org/10.4103/2230-8210.113752
61. Murer H, Hernando N, Forster I, Biber J. Proximal tubular phosphate reabsorption: molecular mechanisms. *Physiol Rev*. 2000;80:1373–1409. https://doi.org/10.1152/physrev.2000.80.4.1373
62. Cupisti A, Gallieni M. Urinary phosphorus excretion: not what we have believed it to be? *Clin J Am Soc Nephrol CJASN*. 2018;13:973–974. https://doi.org/10.2215/CJN.06260518
63. Vervloet MG, Sezer S, Massy ZA, et al. The role of phosphate in kidney disease. *Nat Rev Nephrol*. 2017;13:27–38. https://doi.org/10.1038/nrneph.2016.164
64. Suki WN, Moore LW. Phosphorus regulation in chronic kidney disease. *Methodist DeBakey Cardiovasc J*. 2016;12(suppl):6–9. https://doi.org/10.14797/mdcj-12-4s1-6
65. González-Parra E, Gracia-Iguacel C, Egido J, Ortiz A. Phosphorus and nutrition in chronic kidney disease. *Int J Nephrol*. 2012;2012:597605. https://doi.org/10.1155/2012/597605
66. Kawasaki T, Maeda Y, Matsuki H, Matsumoto Y, Akazawa M, Kuyama T. Urinary phosphorus excretion per creatinine clearance as a prognostic marker for progression of chronic kidney disease: a retrospective cohort study. *BMC Nephrol*. 2015;16:116. https://doi.org/10.1186/s12882-015-0118-1
67. Lebov JF, Engel LS, Richardson D, Hogan SL, Hoppin JA, Sandler DP. Pesticide use and risk of end-stage renal disease among licensed pesticide applicators in the Agricultural Health Study. *Occup Environ Med*. 2016;73:3–12. https://doi.org/10.1136/oemed-2014-102615
68. Siddarth M, Datta SK, Mustafa M, et al. Increased level of organochlorine pesticides in chronic kidney disease patients of unknown etiology: role of GSTM1/GSTT1 polymorphism. *Chemosphere*. 2014;96:174–179. https://doi.org/10.1016/j.chemosphere.2013.10.029
69. Stalin P, Purty AJ, Abraham G. Distribution and determinants of chronic kidney disease of unknown etiology: A brief overview. *Indian J Nephrol*. 2020;30:241–244. https://doi.org/10.4103/ijn.IJN_313_18
70. Kaur B, Khera A, Sandhir R. Attenuation of cellular antioxidant defense mechanisms in kidney of rats intoxicated with carbofuran. *J Biochem Mol Toxicol*. 2012;26:393–398. https://doi.org/10.1002/jbt.21433
71. Kim S, Gil HW, Yang JO, Lee EY, Hong SY. The clinical features of acute kidney injury in patients with acute paraquat intoxication. *Nephrol Dial Transplant*. 2009;24:1226–1232. https://doi.org/10.1093/ndt/gfn615
72. Soloukides A, Moutzouris DA, Kassimatis T, Metaxatos G, Hadjiconstantinou V. A fatal case of paraquat poisoning following minimal dermal exposure. *Ren Fail*. 2007;29:375–377. https://doi.org/10.1080/08860220601184134
73. Wesseling C, Hogstedt C, Picado A, Johansson L. Unintentional fatal paraquat poisonings among agricultural workers in Costa Rica: report of 15 cases. *Am J Ind Med*. 1997;32:433–441. https://doi.org/10.1002/(sici)1097-0274(199711)32:5<433::aid-ajim1>3.0.co;2-t
74. Yu G, Wang J, Jian T, et al. Case series: Diquat poisoning with acute kidney failure, myocardial damage, and rhabdomyolysis. Case series. *Front Public Health*. 2022;10:991587. https://doi.org/10.3389/fpubh.2022.991587
75. Holliday MW, Majeti RN, Sheikh-Hamad D. Chronic interstitial nephritis in agricultural communities: observational and mechanistic evidence supporting the role of nephrotoxic agrochemicals. *Clin J Am Soc Nephrol*. 2024;19:538–545. https://doi.org/10.2215/CJN.0000000000000312
76. Bhargava P, Schnellmann RG. Mitochondrial energetics in the kidney. *Nat Rev Nephrol*. 2017;13:629–646. https://doi.org/10.1038/nrneph.2017.107
77. Covarrubias AJ, Perrone R, Grozio A, Verdin E. NAD^+ metabolism and its roles in cellular processes during ageing. *Nat Rev Mol Cell Biol*. 2021;22:119–141. https://doi.org/10.1038/s41580-020-00313-x
78. Bignon Y, Rinaldi A, Nadour Z, et al. Cell stress response impairs de novo NAD^+ biosynthesis in the kidney. *JCI Insight*. 2022;7. https://doi.org/10.1172/jci.insight.153019
79. Katsyuba E, Mottis A, Zietak M, et al. De novo NAD^+ synthesis enhances mitochondrial function and improves health. *Nature*. 2018;563:354–359. https://doi.org/10.1038/s41586-018-0645-6
80. Poyan Mehr A, Tran MT, Ralto KM, et al. De novo NAD^+ biosynthetic impairment in acute kidney injury in humans. *Nat Med*. 2018;24:1351–1359. https://doi.org/10.1038/s41591-018-0138-z
81. Kang HM, Ahn SH, Choi P, et al. Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. *Nat Med*. 2015;21:37–46. https://doi.org/10.1038/nm.3762
82. Gao Z, Chen X. Fatty acid β-oxidation in kidney diseases: perspectives on pathophysiological mechanisms and therapeutic opportunities. *Front Pharmacol*. 2022;13:805281. https://doi.org/10.3389/fphar.2022.805281
83. Chen H, Chen L, Liu D, et al. Combined clinical phenotype and lipidomic analysis reveals the impact of chronic kidney disease on lipid metabolism. *J Proteome Res*. 2017;16:1566–1578. https://doi.org/10.1021/acs.jproteome.6b00956
84. Gai Z, Wang T, Visentin M, Kullak-Ublick GA, Fu X, Wang Z. Lipid accumulation and chronic kidney disease. *Nutrients*. 2019;11:722. https://doi.org/10.3390/nu11040722
85. Szeto HH, Liu S, Soong Y, Alam N, Prusky GT, Seshan SV. Protection of mitochondria prevents high-fat diet-induced glomerulopathy and proximal tubular injury. *Kidney Int*. 2016;90:997–1011. https://doi.org/10.1016/j.kint.2016.06.013
86. Mister M, Noris M, Szymczuk J, et al. Propionyl-l-carnitine prevents renal function deterioration due to ischemia/reperfusion. *Kidney Int*. 2002;61:1064–1078. https://doi.org/10.1046/j.1523-1755.2002.00212.x
87. Origlia N, Migliori M, Panichi V, et al. Protective effect of l-propionylcarnitine in chronic cyclosporine-a induced nephrotoxicity. *Biomed Pharmacother*. 2006;60:77–81. https://doi.org/10.1016/j.biopha.2005.06.014
88. Lee H, Jang HB, Yoo M-G, Park SI, Lee H-J. Amino acid metabolites associated with chronic kidney disease: an eight-year follow-up Korean epidemiology study. *Biomedicines*. 2020;8:222. https://doi.org/10.3390/biomedicines8070222

89. Davies R. The metabolomic quest for a biomarker in chronic kidney disease. *Clin Kidney J*. 2018;11:694–703. https://doi.org/10.1093/ckj/sfy037

90. Zhang Z-H, Wei F, Vaziri ND, et al. Metabolomics insights into chronic kidney disease and modulatory effect of rhubarb against tubulointerstitial fibrosis. *Sci Rep*. 2015;5:14472. https://doi.org/10.1038/srep14472

91. Silva RE, Baldim JL, Chagas-Paula DA, et al. Predictive metabolomic signatures of end-stage renal disease: a multivariate analysis of population-based data. *Biochimie*. 2018;152:14–30. https://doi.org/10.1016/j.biochi.2018.06.009

92. Chasapi SA, Karagkouni E, Kalavrizioti D, et al. NMR-based metabolomics in differential diagnosis of chronic kidney disease (CKD) subtypes. *Metabolites*. 2022;12:490. https://doi.org/10.3390/metabo12060490

93. Rhee EP, Clish CB, Wenger J, et al. Metabolomics of CKD progression: a case-control analysis in the chronic renal insufficiency cohort study. *Am J Nephrol*. 2016;43:366–374. https://doi.org/10.1159/000446484

94. Mutter S, Valo E, Aittomäki V, et al. Urinary metabolite profiling and risk of progression of diabetic nephropathy in 2670 individuals with type 1 diabetes. *Diabetologia*. 2022;65:140–149. https://doi.org/10.1007/s00125-021-05584-3

95. Shih Y-L, Shih C-C, Chen J-Y. Elevated homocysteine level as an indicator for chronic kidney disease in community-dwelling middle-aged and elderly populations in Taiwan: a community-based cross-sectional study. *Front Med (Lausanne)*. 2022;9:964101. https://doi.org/10.3389/fmed.2022.964101

96. Jankowski J, van der Giet M, Jankowski V, et al. Increased plasma phenylacetic acid in patients with end-stage renal failure inhibits iNOS expression. *J Clin Invest*. 2003;112:256–264. https://doi.org/10.1172/JCI15524

97. Morita M, Yano S, Yamaguchi T, Yamauchi M, Sugimoto T. Phenylacetic acid stimulates reactive oxygen species generation and tumor necrosis factor-α secretion in vascular endothelial cells. *Ther Apher Dial*. 2011;15:147–150. https://doi.org/10.1111/j.1744-9987.2010.00887.x

98. Schulz AM, Terne C, Jankowski V, et al. Modulation of NADPH oxidase activity by known uraemic retention solutes. *Eur J Clin Investig*. 2014;44:802–811. https://doi.org/10.1111/eci.12297

99. Kanchanasurakit S, Arsu A, Siriplabpla W, Duangjai A, Saokaew S. Acetaminophen use and risk of renal impairment: a systematic review and meta-analysis. *Kidney Res Clin Pract*. 2020;39:81–92. https://doi.org/10.23876/j.krcp.19.106

100. Miettinen TP, Björklund M. NQO2 is a reactive oxygen species generating off-target for acetaminophen. *Mol Pharm*. 2014;11:4395–4404. https://doi.org/10.1021/mp5004866

101. Schaub JA, Venkatachalam MA, Weinberg JM. Proximal tubular oxidative metabolism in acute kidney injury and the transition to CKD. *Kidney360*. 2021;360:355–364. https://doi.org/10.34067/KID.0004772020