Environmental Science & Technology

pubs.acs.org/est

Article

Health Risks of Chronic Exposure to Small Doses of Microcystins: An Integrative Metabolomic and Biochemical Study of Human Serum

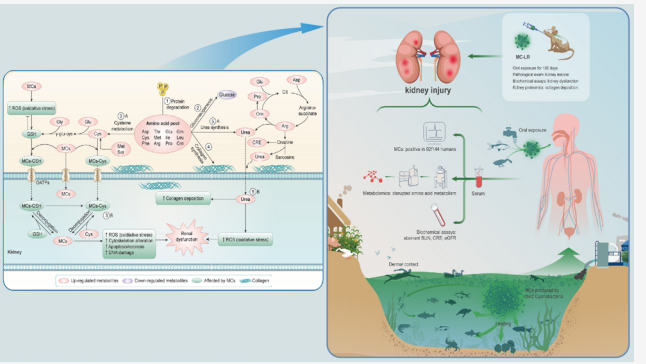
Jun He,[♦] Jun Chen,[♦] Feng Chen, Liang Chen, John P. Giesy, Yuming Guo, Gaodao Liang, Xuwei Deng, Wenjing Wang, and Ping Xie*

Cite This: https://doi.org/10.1021/acs.est.2c00973

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Health risks of chronic exposure to microcystins (MCs), a family of aquatic contaminants produced mainly by cyanobacteria, are critical yet unsolved problems. Despite a few epidemiological studies, the metabolic profiles of humans exposed to MCs remain unknown, hindering the deep understanding of the molecular toxicity mechanisms. Here, sensitive nuclear magnetic resonance (NMR)- and liquid chromatography−mass spectrometry (LC−MS)-based metabolomics were applied to investigate the serum metabolic profiles of humans living near Lake Chao, where toxic cyanobacterial blooms occur annually. MCs were positively detected in 92 of 144 sera by ultra-high-pressure liquid chromatography−tandem mass spectrometry (UPLC−MS/MS) with a median concentration of 0.016 μg/L. The estimated daily intake (0.15−0.27 μg MC-LReq/day) was less than the tolerable daily intake (TDI, 2.4 μg MC-LR for 60 kg adults) recommended by the World Health Organization (WHO). Obvious disruptions of the amino acid metabolism were confirmed and played important roles in renal impairments associated with serum MC burdens. Chronic oral exposure of mice to 30 μg MC-LR/kg body mass, which is less than the no observed adverse effect level, also led to obvious renal lesions and metabolic dysfunction. These observations provide the first evidence of metabolic disturbance of humans exposed to MCs and indicate that the WHO's TDI value determined traditionally should be lessened to protect human health effectively.



KEYWORDS: *metabolomics, microcystin, epidemiological study, health risk, renal injury*

1. INTRODUCTION

Due to increasing eutrophication and climate warming, occurrences of cyanobacteria blooms in freshwater have been increasing globally.[1−3] Cyanobacteria, including the genera *Microcystis*, *Anabaena*, *Planktothrix*, *Anabaenopsis*, *Nostoc*, and *Aphanizomenon*, can produce cyanotoxins. The most ubiquitous cyanotoxins are microcystins (MCs), which can result in public health emergencies.[4,5] MCs are a family of cyclic heptapeptides, and more than 200 specific congeners have been identified so far.[6] MCs contain two variable residues of L-amino acids. In variant microcystin-leucine arginine (MC-LR), these variable residues are leucine (L) and arginine (R), while in microcystin-arginine arginine (MC-RR), these are two arginine residues (RR). MC-LR and MC-RR are the most commonly encountered and studied congeners.[6−8] Humans are mostly exposed to MCs through ingestion of contaminated water and/or food but also through dermal contact during recreation, bathing, and agriculture, as well as inhalation and other occupational activities.[9−11] Cases of lethality of wildlife and domestic livestock have been associated with ingestion of MCs.[5,12−16] Acute exposure to MCs can cause severe poisoning death.[17−19]

There have been a few epidemiological studies of effects of chronic exposure to MCs on health. Positive correlations were identified between concentrations of MCs in drinking water supplies and greater incidences and/or mortalities from cancer of the liver,[20−23] colon, rectum,[24] and stomach.[25] The results of several cross-sectional studies showed associations of estimated daily intake (EDI) of MCs with some serum liver enzymes[26,27] and indicators of renal dysfunction,[28] implying risks of liver or renal diseases in populations exposed to MCs. However, in these studies, estimates of external exposure doses based solely on concentrations of MCs in various environmental samples were limited by uncertainties and therefore could not represent accurate exposure doses (determined by internal MC burden). In addition, most previous epidemiological studies for evaluating toxic effects used traditional

Received: February 10, 2022
Revised: April 19, 2022
Accepted: April 20, 2022

serum biochemical parameters that are not sufficiently sensitive to subtle injuries, while changes in the human metabolome in response to MCs and their relation to the toxicity mechanism remain largely unknown. Therefore, a more accurate and sensitive assessment is necessary, for which metabolomics is a useful technology.

Recent developments in metabolomics facilitate the elucidation of molecular signatures of chronic exposure to MCs *in vitro* and *in vivo*.[29−33] In general, profiles of relative concentrations of metabolites, which are end products of genomic, transcriptomic, and proteomic variability and environmental stimulations, provide an integrated, functional measure of cellular biochemistry.[34] Alterations in metabolic profiles can function as useful indicators of exposure−response relationships, provide information on the mechanisms of toxic effects, and give unique insights into overall health.[35] However, investigations of the effects of MCs on human metabolic profiles are scarce, which hinders accurate assessments of dose−effect relationships between chronic exposure to MCs and human health.

Here, for the first time, a molecular epidemiological study based on metabolomics is conducted on humans living near Lake Chao, where dense toxic cyanobacterial blooms occur annually. By integrating human metabolic and biochemical profiles and supplemented with systematic toxicological findings of animal experiments, we aim to decipher molecular responses of humans to MC exposure and to elucidate the related toxicity mechanisms. The present study will provide new sights into human health risks posed by long-term environmental exposure to cyanotoxins.

2. MATERIALS AND METHODS

2.1. Site Selection and Study Populations. Lake Chao (30°25′−31°43′N, 117°16′−117°51′E), located in the middle of Anhui Province between the Yangtze and Huai Rivers, is one of the five famous freshwater lakes in China and plays essential roles in water supply, aquaculture, irrigation, flood prevention, shipping, and tourism. Two large cities in Anhui Province, Hefei and Chaohu, are located on the northwest and eastern shores of the lake, respectively (Figure S1). Over the past few decades, Lake Chao has been increasingly culturally eutrophied and has regularly exhibited blooms of cyanobacteria dominated by the genera *Microcystis* and *Anabaena* (Figure S2). Widespread and persistent contamination of MCs is found in lake water, groundwater, finished water, and various aquatic products.[36−41] These exposures have resulted in concerns about potential adverse effects on the health of local residents. The Zhongmiao town, which is situated in the central area of the north bank of Lake Chao and is near the risk area (the west half of Lake Chao) for cyanobacterial blooms, was selected for sampling (Figures S1 and S2). Most of the water and aquatic products consumed by residents of Zhongmiao come from Lake Chao. In addition, bioaccumulation of MCs has also been detected in crops occasionally irrigated with lake water.[9] Thus, residents of Zhongmiao, due to potential long-term exposure to MCs, were suitable objects to evaluate the health effects of chronic exposure to environmental concentrations of MCs.

Local adult residents (age >18 years) who had lived in Zhongmiao for at least 5 years were invited to enroll in this study. A total of 176 eligible volunteers were recruited in October 2010. Volunteers were asked to complete a questionnaire to ascertain demographic and lifestyle information, including age, sex, marital status, location and length of residence, occupation, smoking status, alcohol intake, source of drinking water, daily water intake, diet especially consumption of aquatic products, disease, and medical history. Participants were defined as smokers if they smoked over 100 cigarettes in their lifetime and still smoked at least one cigarette daily or quit smoking less than 6 months before questionnaire data collection.[28] Participants were classified as alcohol drinkers if they consumed alcohol at least once a week for 6 consecutive months in their lifetimes.[28] In addition, each participant provided 15 mL of blood collected in the morning after an overnight fast and one first-morning-void urine sample. Serum was separated from whole blood by centrifugation at 1000*g* for 15 min. All samples were frozen on the day of collection. Ethical approval for this study was granted by the Ethics Committee of the Institute of Hydrobiology, Chinese Academy of Science, and all subjects provided written informed consent before data collection. Data were anonymized for the present study.

2.2. Quantitative Detection of MCs in Water and Human Serum by UPLC−MS/MS. Five raw lake water samples were collected from Lake Chao, five tap water samples were collected from Zhongmiao in October 2010, and MCs in water, including MC-LR and MC-RR, were detected by using our previously published methods with the limit of detection (LOD) of 30 ng/L.[42] For serum, the method described by Chen et al.[37] was used with modification to more efficiently extract and detect small concentrations of MCs. Details regarding the detection procedures are included in Supporting Information Text S1. The "serum MC-positive" and "serum MC-negative" groups were defined, where the concentrations of MCs detected in human serum were greater and less than the LOD of 1 ng/L, respectively.

2.3. Biochemical Analyses of Human Serum and Urine Samples. Serum biochemistry was assessed by standard spectrophotometric methods using a Synchron Clinical System LX20 (Beckman-Coulter Diagnosis, Fullerton, CA). Biochemical parameters analyzed were as follows: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, total protein, albumin, globulin, albumin/globulin, total bilirubin, direct bilirubin, indirect bilirubin, total biliary acid, cholinesterase, γ-glutamyl transpeptadase, glucose, triglyceride, cholesterol, very low-density lipoprotein, blood urea nitrogen, creatinine, and uric acid. In addition, the estimated glomerular filtration rate (eGFR) was calculated according to two separate equations, which were parameterized for Chinese adults: eGFR (men) = $175 \times SCr^{-1.234} \times age^{-0.179}$ and eGFR (women) = $175 \times SCr^{-1.234} \times age^{-0.179} \times 0.79$.[43] Several urinary biochemical parameters, including bilirubin, pH, blood, protein, nitrite, glucose, specific gravity (SG), microalbumin, and creatinine, were also measured using an automatic urine analyzer (DIRUI H-800, Changchun, China).

2.4. Metabolomic Analysis of Human Serum. *2.4.1. NMR Data Acquisition.* Aliquots (200 μL) of serum were mixed with 400 μL of saline solution containing 50% D_2O as a locking substance. The mixture was centrifuged at 12,000*g* for 10 min at 4 °C, and 550 μL of the supernatant was transferred into 5 mm NMR tubes for NMR experiments. Acquisition of NMR data was accomplished using previously described methods.[29]

2.4.2. Quantification of Free Amino Acids in Human Serum. For each serum sample, an aliquot (400 μL) was mixed with 100 μL of 10% sulfosalicylic acid/90% water solution and

https://doi.org/10.1021/acs.est.2c00973
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

allowed to stand for 60 min at 4 °C. The mixed solutions were centrifuged. Then, 200 μL of the supernatant was diluted into an equal volume of ultrapure water and filtered through a 0.22 μm membrane (JINTENG, China). Filtrates containing free amino acids were analyzed using an A300 amino acid analyzer equipped with an ion-exchange chromatographic column (MembraPure Bodenheim, Germany). A total of 18 amino acids were identified and quantified using external standards, including Asp, Thr, Ser, Glu, Gly, Ala, Cys, Val, Met, Ile, Leu, Tyr, Phe, His, Lys, Arg, Pro, and Orn. The sum of the total concentrations of the 18 amino acids (TAA); 7 essential amino acids (EAA), including Thr, Val, Met, Ile, Leu, Phe, and Lys; and the remaining 11 nonessential amino acids (NEAA) was calculated. Serum amino acid ratios EAA/NEAA and Val/Gly were also calculated.

2.5. Mouse Modeling of Kidney Injury and Metabolic Disturbance Induced by MCs. *2.5.1. Mouse Model.* Specific pathogen-free grade male BALB/c mice, aged 8 weeks and 20−22 g, were obtained from the Vital River Laboratories (Beijing, China). Mice were housed at a certified specific pathogen-free animal facility under controlled conditions (temperature, 21−23 °C; relative humidity, 40−60%; light/dark cycle, 12/12 h). Certified standard rodent chow and sterile water were supplied ad libitum. After adaptation for 2 weeks, mice were randomized into 2 groups of 10 each. Mice in the treated group were exposed to 30 μg MC-LR/kg body mass (bm)/day (MC-LR purity >95%, Enzo Life Sciences, Farmingdale, NY, USA) through oral gavage for 180 days. The dosage was slightly less than the no observed adverse effect level (NOAEL, the reference standard) of 40 μg MC-LR/kg bm/day in mice.[44] Based on the aforementioned NOAEL, the World Health Organization (WHO) proposed the tolerable daily intake (TDI) of 2.4 μg MC-LR for 60 kg adults for protection of human health. The control group was given sterile normal saline. Body mass, food intake, and water consumption of mice were monitored weekly during the experiment. Mice were euthanized by isoflurane inhalation 24 h after the last gavage exposure, and blood was obtained from the orbital vein. The kidney was immediately removed and weighed, and each was divided into two portions; the smaller one was fixed in a buffered aldehyde fixative for histopathological examinations (Supporting Information Text S2), and the other larger one was stored at −80 °C mainly for proteomic analyses.

2.5.2. Measurement of Biochemical Markers of Renal Functions. Serum was obtained by centrifuging blood at 3000 rpm for 20 min at 4 °C. The concentrations of blood urea nitrogen, creatinine, and uric acid in serum were measured to assess renal functions using an automated analyzer (Olympus AU640).

2.5.3. Tandem Mass Tag Quantitative Proteomic Analysis of the Kidney. Tandem mass tag (TMT) quantitative proteomic analysis was conducted to quantify dynamic changes in the proteome of kidney tissues induced by MCs. Protein extraction, trypsin digestion, TMT labeling, HPLC fractionation, and liquid chromatography−tandem mass spectrometry (LC−MS/MS) were performed according to the protocol (Supporting Information Text S3).

2.5.4. Metabolomic Analysis of Mice Serum. Ultra-high-pressure liquid chromatography−mass spectrometry (UPLC−MS)-based metabolomics of mice serum was performed to identify the metabolite changes in mice chronically exposed to MC-LR. Details of sample pretreatment, UPLC-quadrupole time of flight mass spectrometry (UPLC-Q-TOF-MS) analysis, data processing, and statistical analysis are described in Supporting Information Text S4.

2.6. Multivariate Statistical Analysis and Visualization. The data processing, including Mann−Whitney *U*-test and regression analysis of biochemical parameters, metabolomics analysis [NMR spectral preprocessing, support vector machine (SVM) modeling of metabolite discrimination, and screening of amino acid biomarkers], and correlation analysis of amino acid profiles and indicators of renal functions, was elaborated in Supporting Information Text S5. All statistical significances were defined as a p value less than 0.05.

3. RESULTS

3.1. Baseline Subject Information. Among the 176 eligible adult volunteers recruited for this study, 147 participants completed questionnaire interviews and provided samples of both blood and urine. A further three women with genetic disease were excluded. Finally, 144 subjects, consisting of 88 men and 56 women, were enrolled in the present study. No significant differences were found between participants in the serum MC-negative and MC-positive groups regarding age, sex, ethnicity, material status, smoking status, alcohol intake, consumption of aquatic products, or history of liver and kidney diseases. However, significant differences were observed in drinking water sources (Table S1).

3.2. Concentrations of MCs Detected in Environmental Waters and Human Sera. MC-LR and MC-RR were detected in all samples of lake water, and the total concentrations ranged from 0.14 to 0.74 μg MC-LR equivalents (MC-LReq)/L, with a mean of 0.34 μg MC-LReq/L, when a coefficient of 0.2 was used to convert the concentration of MC-RR to MC-LReq.[45] A few MCs were also detected in tap water, ranging from 0.06 to 0.12 μg MC-LReq/L, with a mean of 0.09 μg MC-LReq/L. The concentrations of MCs in both lake water and tap water were less than the safe threshold of 1 μg MC-LR/L in drinking water proposed by the WHO.

For human serum, representative UPLC/MS2 spectra of MC-LR and MC-RR of an anonymous subject are shown in Figure S3. MCs, including MC-LR and/or MC-RR, were detected at concentrations greater than the LOD in 92 of 144 serum samples, with a positive detection rate of 63.9%. The concentrations of MC-LR ranged from 0.002 to 0.078 μg/L, with a median of 0.013 μg/L, while the concentrations of MC-RR ranged from 0.001 to 0.3 μg/L, with a median of 0.010 μg/L. The total concentrations of MCs ranged from 0.001 to 0.31 μg/L, with a median of 0.016 μg/L.

3.3. Abnormalities in Renal Function-related Biochemical Parameters in the Serum and Urine of Humans Exposed to MCs. To define potential health hazards associated with environmental exposure to MCs, biochemistry of both serum and urine, which are commonly used as clinical indicators of health status, was analyzed.

Among the biochemical parameters measured in serum, significantly smaller concentrations of ALB and GGT activities but greater concentrations of BUN were observed in the MC-positive group than in the MC-negative group (Table S2). Linear regression analysis consistently showed that after adjustment for confounding factors, concentrations of ALB (β = −1.07; 95% CI, −2.05 to −0.09; p = 0.033) and GGT activities (β = −35.01; 95% CI, −64.87 to −5.14; p = 0.022) were negatively correlated with concentrations of MCs, and concentrations of BUN were positively correlated (β = 0.96;

https://doi.org/10.1021/acs.est.2c00973
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

95% CI, 0.29 to 1.62; $p = 0.005$) with concentrations of MCs in the serum (Table S3). Compared to those in the MC-negative group, greater rates of abnormalities in serum A/G (34.78 vs 19.23%), BUN (19.57 vs 5.77%), CRE (34.78 vs 9.62%), and eGFR (26.09 vs 11.54%) but lesser rates of abnormalities in GGT (8.70 vs 21.15%) were observed in the serum MC-positive group (Table S4). Meanwhile, upon additional adjustment for multiple confounding factors, serum MCs were shown to be significant risk factors for the abnormal increase in CRE (odd ratios [OR] = 12.30; 95% CI, 1.51−100.34; one-sided $p = 0.010$) and borderline significant risk factors for the abnormal increase in BUN (OR = 5.62; 95% CI, 0.97−32.70; one-sided $p = 0.028$), and decrease in eGFR (OR = 3.44; 95% CI, 0.93−12.66; one-sided $p = 0.032$), all of which are well-established markers of renal damage (Table 1).

In urine, greater SG ($p = 0.005$) and concentrations of microalbumin (ALBu) ($p = 0.04$) were observed in the MC-positive group (Table S5), which showed impaired renal functions. After adjustment for multiple confounding factors, the concentrations of MCs in serum were still significantly correlated with the mean urine SG ($\beta = 0.005$; 95% CI, 0.001−0.008; $p = 0.005$). These urinary results are consistent with those observed in serum and indicate kidney injury.

3.4. Alterations of Serum Metabolic Profiles in Humans Chronically Exposed to MCs. The constructed SVM model had excellent classification and predictive ability (R^2 = 99%, Q^2 = 63%, $p < 0.05$) and displayed obvious metabolic discrimination between the serum MC-positive and MC-negative groups (Figure 1A). By employing the SVM−RFE method, a minimum of 100 metabolic features (bins) was determined to effectively distinguish between the MC-positive and MC-negative groups, with an optimal accuracy of 68.7% (Figure 1B). The 100 top-ranked features were identified, which included 18 discriminatory metabolites (Figure 1C), 14 of which were amino acids and their derivatives [Val, Leu, Ile, Asp, Gln, Glu, Lys, Pro, Phe, His, Thr, Cre, 4-hydroxyphenylacetate (4-HPA), and urea]. Except for a lesser concentration of Val, the concentrations of the other 13 amino acids and derivatives were significantly greater in the serum MC-positive group than in the MC-negative group. In addition to amino acids, reduced concentrations of glucose, choline, and HDL/LDL/VLDL and greater concentrations of lactate were observed in the serum MC-positive group.

Since the metabolomics results suggested measurable changes in the relative amounts of amino acids, the concentrations of 18 common free amino acids in serum were further quantified using an analyzer specific for amino acids. There were no significant differences ($p > 0.05$) in the concentrations of Ser, Gly, Ala, Val, Tyr, His, or Lys in serum or in the ratio of EAA/NEAA and Val/Gly between the two groups. However, significantly greater concentrations of Asp, Thr, Glu, Cys, Met, Ile, Leu, Phe, Arg, Pro, Orn, TAA, EAA, and NEAA were observed in sera from the MC-positive group ($p < 0.05$) (Figure 2A,B). These discriminatory amino acids were highly enriched in the urea cycle, Arg and Pro metabolism, and Gly and Ser metabolism (FDR < 0.05) (Figure 2C). Furthermore, the two top-ranked amino acids Arg and Cys were screened as the most robust biomarkers relevant to the effects of MCs using the ensemble machine learning method. Receiver operating characteristic (ROC) analysis, based on a logistic regression model, showed that the areas under the ROC curve (AUC) for the training and testing sets were 0.72 and 0.70, respectively (Figure 2D). These AUC values suggested good classification performance for Arg and Cys to distinguish between the serum MC-positive and MC-negative groups in both the training and testing sets.

Table 1. Multivariable Logistic Regression for Associations of Abnormalities in Serum Biochemical Parameters with Serum MC Status[a]

serum biochemical parameters	adjusted odd ratios for serum MC-positive[b] (95% CI)	p value
ALT abnormality (yes, no)	0.70 (0.05−10.33)	0.398
AST abnormality (yes, no)	0.70 (0.05−10.33)	0.398
ALP abnormality (yes, no)		
LDH abnormality (yes, no)	0.80 (0.12−5.56)	0.412
TPR abnormality (yes, no)	3.61 (0.77−16.97)	0.054
ALB abnormality (yes, no)		
GLO abnormality (yes, no)	0.85 (0.28−2.58)	0.389
A/G abnormality (yes, no)	1.87 (0.59−5.99)	0.141
TBIL abnormality (yes, no)	1.43 (0.43−4.80)	0.281
DBIL abnormality (yes, no)	0.74 (0.20−2.66)	0.321
IBIL abnormality (yes, no)	0.92 (0.29−2.92)	0.447
TBA abnormality (yes, no)	1.68 (0.14−20.23)	0.340
CHE abnormality (yes, no)	0.93 (0.07−12.12)	0.477
GGT abnormality (yes, no)	0.38 (0.11−1.32)	0.064
GLU abnormality (yes, no)	1.11 (0.38−3.23)	0.422
TG abnormality (yes, no)	0.37 (0.12−1.13)	0.082
CHO abnormality (yes, no)	0.82 (0.05−12.26)	0.443
VLDL abnormality (yes, no)	0.43 (0.15−1.26)	0.062
BUN abnormality (yes, no)	5.62 (0.97−32.70)	0.028*
CRE abnormality (yes, no)	12.30 (1.51−100.34)	0.010*
UA abnormality (yes, no)	0.72 (0.23−2.18)	0.278
eGFR abnormality (yes, no)	3.44 (0.93−12.66)	0.032*

[a]Binary logistical regression analysis was applied, and age, sex, smoking status, and alcohol intake were adjusted as covariates. [b]Status of serum MCs (positive if MC concentration ≥ LOD; otherwise negative); *$p < 0.05$ (one-tailed).

3.5. Correlations between Amino Acid Profiles and Indicators of Renal Functions in Human Serum. Univariate correlation networks between concentrations of individual amino acids and serum indicators of renal functions, ALB, BUN, CRE, and eGFR, are illustrated in Figure 3. In comparison with the serum MC-negative group, eGFR, CRE, and ALB in the MC-positive group exhibited a larger number of amino acid associations ($|r| \geq 0.3$, $p < 0.05$). Compared to wide correlations with numerous amino acids in the MC-negative group, BUN exhibited a unique, positive correlation with Cys, Glu, and Phe and a negative correlation with ALB in

https://doi.org/10.1021/acs.est.2c00973
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

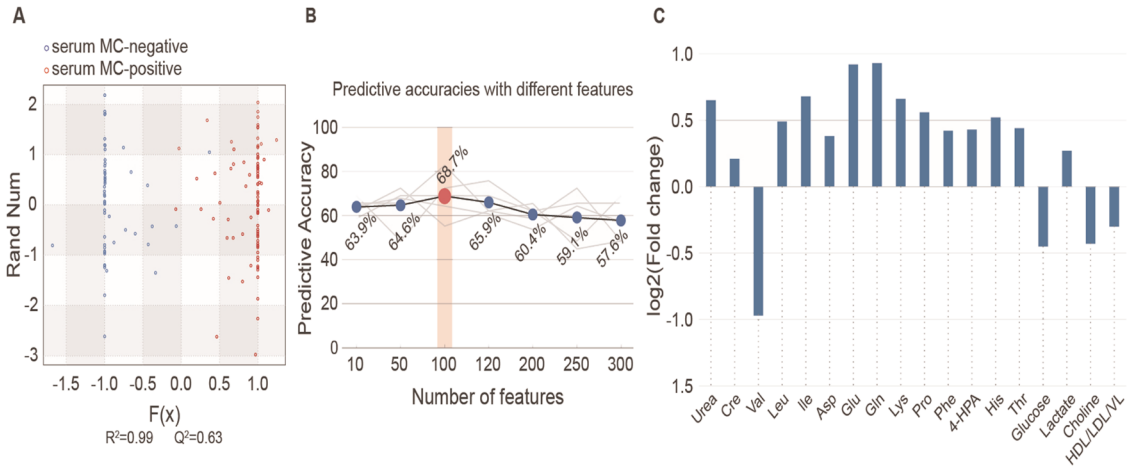


Figure 1. Alterations of the serum metabolic profile in humans chronically exposed to MCs. (A) SVM separate score plot of SVM modeling. Serum metabolic profiles of humans in the serum MC-positive group (red circle) were markedly different from those in the negative group (blue circle). The high value of the classification accuracy rate, R^2, and prediction accuracy, Q^2, using fivefold cross validation confirmed good model quality. (B) Evolution of model prediction accuracy using SVM−RFE for discriminant metabolic feature selection. A minimum of 100 metabolic features (bins) was determined to provide the highest accuracy, 68.7%. (C) Discriminatory metabolites corresponded to the 100 top-ranked features. Fold change was calculated based on the average peak areas of each discriminatory metabolite. Positive values indicate greater concentrations of metabolites in the serum of the MC-positive group compared to the MC-negative group, while negative values indicate the inverse relationship. The Mann−Whitney *U*-test was applied for comparison of average peak areas with a significance level of $p < 0.05$. Abbreviation: 4-HPA, 4-hydroxyphenylacetate.

the MC-positive group. Overall, correlations of individual amino acids with indicators of renal functions in the MC-positive group were markedly differentiated from those in the MC-negative group.

Further correlations between amino acid profiles and indicators of renal functions were investigated using multivariate orthogonal partial least squares (OPLS) regression (Table S6). No OPLS regression model was successfully constructed for the MC-negative group, which indicated that there were no significant correlations between amino acid profiles in the serum and renal functions. However, for the MC-positive group, significant variations were predicted by OPLS regression derived from amino acid profiles for eGFR ($R^2Y = 0.28$, $Q^2 = 0.25$, $p = 0.031$) and CRE ($R^2Y = 0.31$, $Q^2 = 0.18$, $p = 0.005$) but not BUN. Taken together, the results of OPLS regression suggested a strong association between the metabolism of amino acids and impaired renal functions induced by MCs.

3.6. Effects of Chronic Exposure of Mice to MC-LR on the Endogenous Metabolism. To confirm the MC-associated metabolic changes detected in humans, metabolomics of mice serum was also conducted. Clear metabolic separation of the MC-LR-treated group and the control group was evidenced in the score plot of the validated orthogonal partial least squares discriminant analysis (OPLS-DA) model and demonstrated that chronic exposure to MC-LR significantly affected metabolic profiles in mice (Figure S4A,B). A total of 82 significantly discriminant metabolites (VIP > 1, $p < 0.05$) were identified (Table S7), and among these, up to 28 dipeptides remarkably decreased and 6 amino acids, including Asp, Glu, Tau, Arg, *N*-acetyl-L-Ala, and *N*-acetyl-L-Met, significantly elevated. Metabolic enrichment analysis showed that amino acid-relevant metabolisms, including protein digestion and absorption; alanine, aspartate, and glutamate metabolism; taurine and hypotaurine metabolism; arginine biosynthesis; and glycine, serine, and threonine metabolism were most significantly affected by MC-LR exposure (Figure S4C). The dramatic changes in amino acid metabolisms observed in exposed mice were consistent with the metabolomics findings from residents chronically exposed to MCs.

3.7. Effects of Chronic Exposure of Mice to MC-LR on Renal Functions. The concentrations of BUN and CRE in the serum of mice treated with 30 μg MC-LR/kg bm/day were significantly greater than those in the control, while there was no significant difference in the concentrations of UA between the control and treated groups (Table S8).

3.8. Effects of Chronic Exposure of Mice to MC-LR on Renal Histopathology. Oral exposure to 30 μg MC-LR/kg bm/day for 180 days resulted in obvious histological lesions in the renal tissues of mice, including massive dilation of proximal convoluted tubules, vacuolar degeneration in epithelial cells, and glomerulomegaly (Figure S5A,B). TEM examinations showed evidence of fibrous intimal thickening of the glomerular basement membrane (GBM) and podocyte fusion (Figure S5C,D). Correspondingly, Sirius Red staining of kidney sections revealed significantly greater deposition of collagen, forming a fibrotic "scar" (Figure S5E−G).

3.9. Effects of Chronic Exposure of Mice to MC-LR on the Renal Proteome. To further decipher the molecular mechanisms involved in nephrotoxicity caused by chronic exposure to small doses of MC-LR, the renal proteome was analyzed. Compared with the control group, a total of 90 proteins were dysregulated (adjusted $p < 0.05$, absolute value of fold change >1.2) in mice exposed to 30 μg MC-LR/kg bm/day. Of the significantly altered proteins, 57 were upregulated, and 33 were downregulated (Figure S5H). Accordingly, a gene ontology term analysis designating functional annotation showed that amino acid/collagen-activated responses were the most enriched (Figure S5I). Specifically, the top-enriched terms in the cellular component category were all related to the collagen network or trimmers. The molecular function category showed that the collagen-implicated extracellular matrix structural constituent, structural molecule activity, and

https://doi.org/10.1021/acs.est.2c00973
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

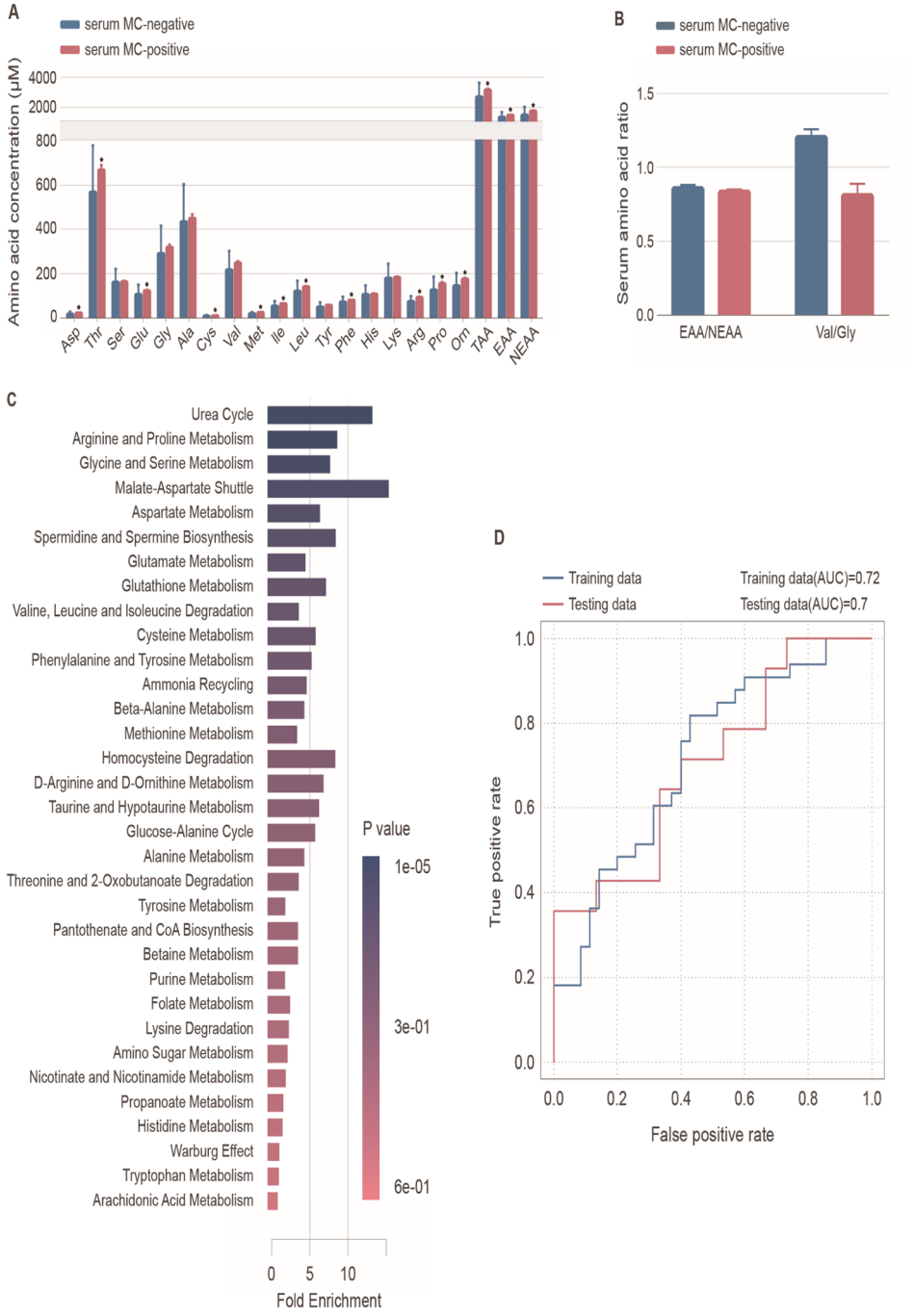


Figure 2. Conspicuous alterations of the amino acid profile in humans chronically exposed to MCs. (A) Concentrations of serum amino acids and (B) amino acid ratios. Concentration data are presented as the means ± S.E., and ratios are dimensionless (n = 52 biological replicates for the serum MC-negative group, n = 92 for the serum MC-positive group). The Mannn−Whitney U-test was applied, and * designates a significant difference at the 95% confidence level. (C) Metabolite set enrichment analysis showing the most highly altered amino acids in the serum MC-positive group. All metabolic pathways were arranged according to scores from the enrichment analysis (y-axis) and from topology analysis (x-axis). (D) Evaluation of the classification performance of screened amino acid biomarkers by ROC analysis derived from the logistic regression model. Blue and red lines represent the training set and the testing set, respectively. The area under the ROC curve (AUC) is the area under the curve. Abbreviations: TAA, total amino acids; EAA, essential amino acids; NEAA, nonessential amino acids.

platelet-derived growth factor binding were the most top-enriched terms. Collagen-activated signaling pathways, cellular response to amino acid stimuli, response to amino acids, and collagen fibril organization were among the most enriched biological processes. Correspondingly, the expression of various collagens and laminins (Col4a1, Col4a2, Col4a3, Col1a1, Col1a2, Col5a2, Col24a1, Lamc1, Cav1, Myh11, Itga7, and Nid1) was significantly upregulated by MC-LR treatment (Figure S5J).

4. DISCUSSION

By using sensitive metabolomics, the present molecular epidemiological study showed that exposure to small amounts of MCs was associated with remarkable disturbance in the

https://doi.org/10.1021/acs.est.2c00973
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

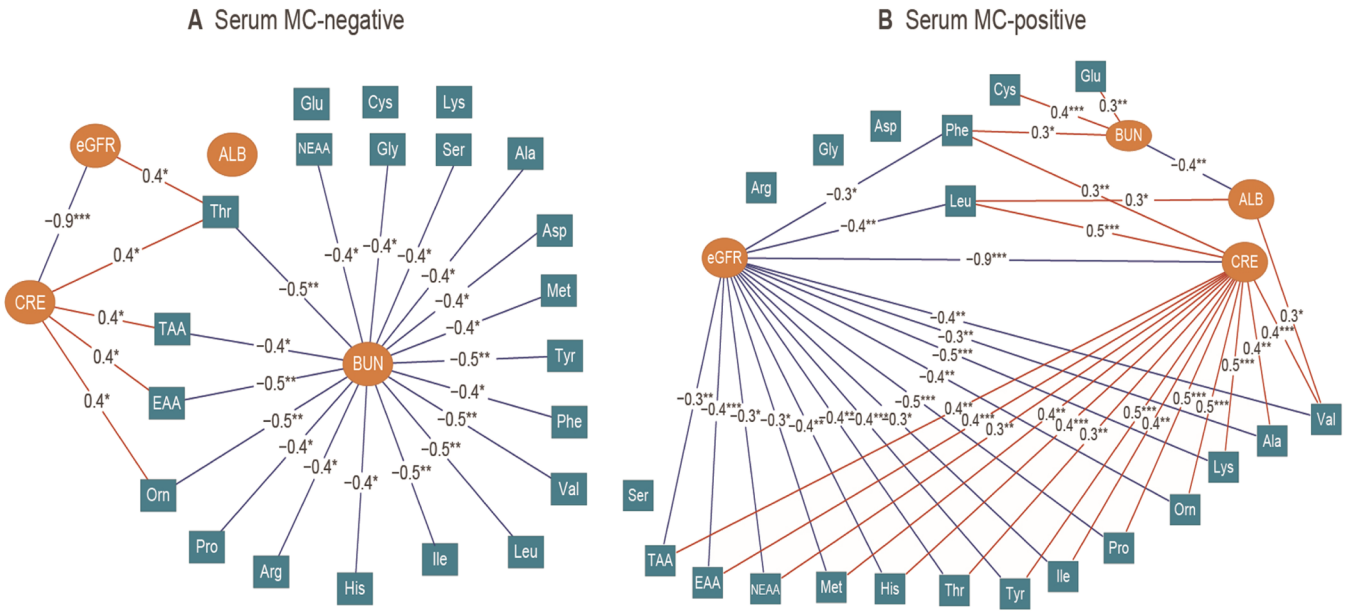


Figure 3. Integrated correlation networks among individual amino acids and biochemical parameters of renal functions in the (A) serum MC-negative group (n = 52) and (B) serum MC-positive group (n = 92). A cutoff value of 0.3 was applied to the absolute value of the coefficient $|r|$ to display the correlations. Serum amino acids and biochemical parameters of renal functions correspond to blue rectangle nodes and orange ellipse nodes, respectively. Edges are coded according to the correlation value: positive and negative correlations are displayed in red and in blue, respectively. *, **, and *** indicate a statistically significant correlation at 95, 99, and 99.9% confidence levels, respectively.

amino acid metabolism and substantial risks of renal impairment in humans living near cyanobacteria-dominated Lake Chao. This observation was strengthened by an animal experiment, in which prolonged exposure of mice to MCs less than the NOAEL also caused dysfunctions of the amino acid metabolism and kidney injury.

4.1. Metabolic and Biochemical Mechanisms Underlying Renal Impairment in Humans Chronically Exposed to Small Doses of MCs. In this study, MC-associated renal impairment in humans was indicated by aberrant changes in serum BUN, CRE, eGFR, and urinary ALBu. In our previous investigation on fishermen exposed to high levels of cyanotoxins, principal component and classifying analysis demonstrated that the first component had high, positive weights for renal functional indicators, including BUN, CRE, UA, and A/G.[37] A cross-sectional study on 5493 people in rural areas, Southwest China, by measuring the external exposure dose of MC-LR, showed that exposure to MC-LR is a significant risk factor for renal function impairment.[28] Thus, the results of the present study together with previous reports suggest that chronic exposure to MCs may induce renal function impairment in humans. The detailed mechanisms remain to be further studied.

In the present study, alterations of serum amino acid profiles in humans exposed to MCs were conspicuous, which was confirmed by the remarkable changes of the amino acid metabolism in mice exposed to MC-LR. Greater concentrations of end metabolic products of the urea cycle, urea, and core intermediates Arg and Orn, which could be converted into Pro and creatine, respectively, and the primary amino substrates Glu and Asp, collectively suggested enhancement in the synthesis of urea. Urea is the major metabolic end product of amino groups derived from amino acids, and its production changes in parallel with the degradation of dietary and endogenous proteins.[46] In the present study, there was no change in the ratios of EAA/NEAA or Val/Gly, which are often well correlated with nutritional status.[47−49] Therefore, greater concentrations of various amino acids from which urea is formed are likely due to accelerated degradation of endogenous proteins in response to exposure to MCs. This view was supported by the animal experiment as exposure of mice to MC-LR caused a remarkable decrease in numerous dipeptides and an increase in multiple amino acids.

There are several probable causes for this response. First, lesser concentrations of glucose and greater concentrations of lactate were observed in human serum, which suggested that exposure to MCs results in a deficiency of glucose. This stimulates the release of glucogenic amino acids from endogenous proteins, including Asp, Thr, Glu, Cys, Met, Ile, Arg, Phe, and Pro (Figure 4(①,②)).[50,51] Second, significantly greater concentrations of Cys and Glu and their close association with BUN in the serum MC-positive group indicated increases in the need for GSH synthesis. GSH plays a key role in the detoxification of MCs and the regulation of oxidative stress, an inherent characteristic response to the toxic effects of MCs.[52] Cys, a sulfur-containing semiessential amino acid, can be synthesized from Met and Ser via *trans*-sulfuration and/or from the breakdown of proteins. Degradation of proteins is a source of supply of the substrates Cys, Glu, Gly, Met, and Ser for GSH synthesis and thus favors detoxification of MCs and antioxidation through phase II conjugation (Figure 4(③A)). Third, an association between greater concentrations of amino acids and reduced renal capacities as indicated by eGFR was identified only in the serum MC-positive group. Released amino acids from endogenous proteins are likely to be partly involved in substrate turnover for collagen synthesis, subsequently contributing to deposition of renal collagen, thickening of GBM, and reduction of GFR (Figure 4(④)).[53] Fibrous intimal thickening of the GBM and increases in the production of collagen proteins were also demonstrated in mice exposed to MCs (Figure S5).

https://doi.org/10.1021/acs.est.2c00973
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

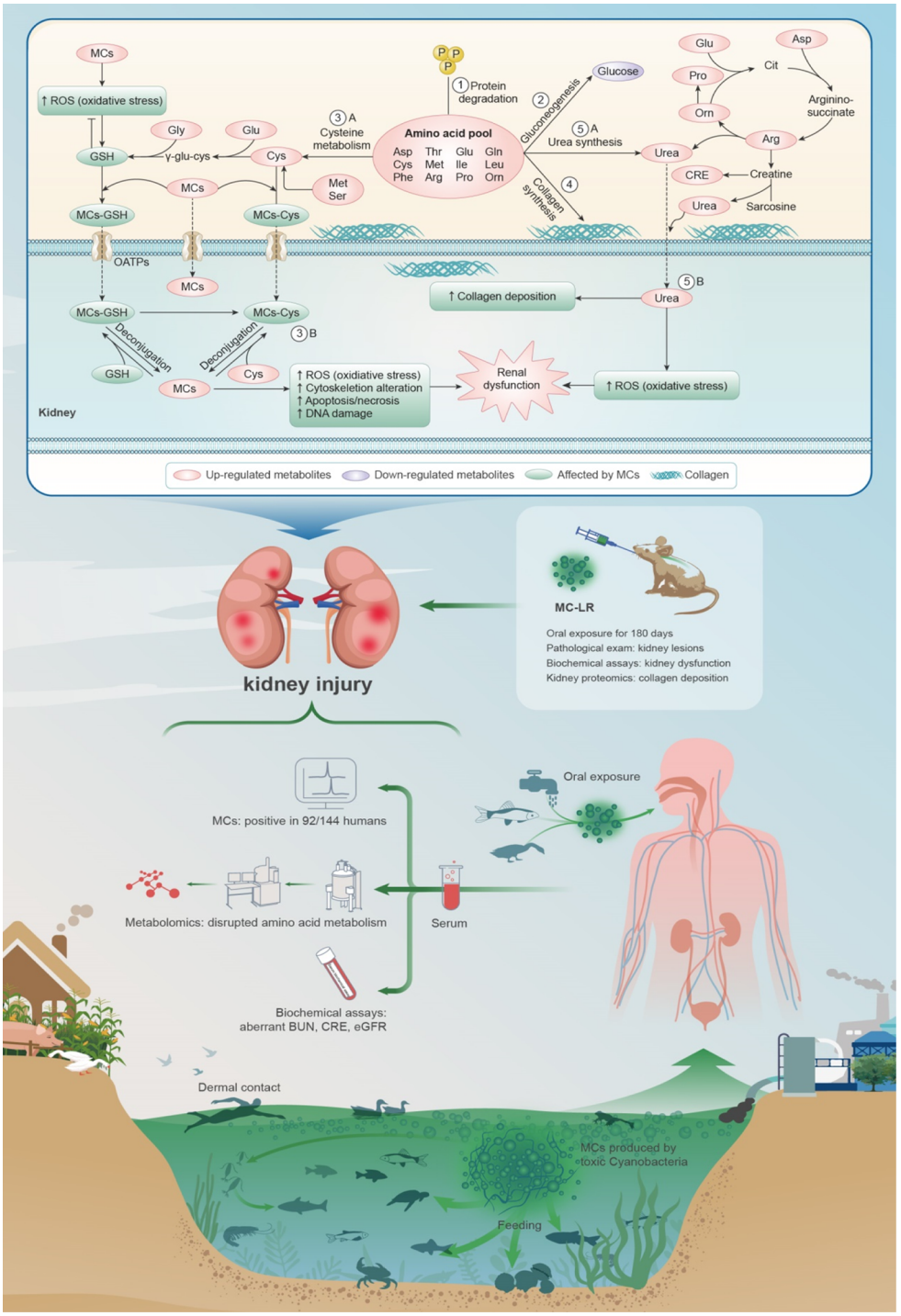


Figure 4. Disturbance in amino acid profiles and its role in renal injury associated with MCs: ① MCs enhance the degradation of endogenous proteins and result in greater concentrations of amino acids in serum. ② Glycogenic amino acids are implicated in gluconeogenesis in response to serum glucose deficiency induced by MCs. ③ (A) Long-term exposure to MCs causes GSH deficiency and stimulates GSH synthesis from Cys, Glu, and Gly for detoxification of MCs and antioxidation through phase II conjugations. ③ (B) Conjugates of MCs with Cys (MCs-Cys) are organotropic to the kidney and tend to be reversibly deconjugated to release MCs, which prolongs exposure of the kidney to MCs and thus aggravates renal toxicity. ④ Various amino acids are likely to be implicated in collagen synthesis, and excessive accumulation of collagens causes deposition of collagen and reduction of renal filtration functions. ⑤ (A) Increased availabilities of amino acids are accompanied by enhanced urea synthesis to dispose off the excessive nitrogen. ⑤ (B) Excessive production of urea promotes ROS enhancement, drives collagen deposition, and accelerates kidney dysfunction. Metabolites colored pink and purple are up- and downregulated, respectively, as determined from the present metabolomic data. Abbreviations: MCs, microcystins; ROS, reactive oxygen species; GSH, glutathione; Cys, cysteine; MCs-GSH/Cys, glutathione/cysteine conjugate of MCs; Cit, citrulline.

Dysfunction of the amino acid metabolism seems to play important roles in MC-associated renal injury. With accelerated protein degradation, increased amino acid availabilities are accompanied by enhanced production of urea to dispose

https://doi.org/10.1021/acs.est.2c00973
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

off excessive nitrogen (Figure 4(⑤A)). Urea, a critical indicator of renal functions, is excreted from the kidney and recognized as a bona fide uremic toxin.[54] Urea induces reactive oxygen species,[55] which sharpens MC-induced oxidative stress and in turn exacerbates kidney dysfunction. Excessive production of urea also alters the structure and function of the kidney by carbamylation of proteins and stimulates fibrogenesis (Figure 4(⑤B)).[54,56] Amino acids are likely to be involved in collagen synthesis, which causes collagen deposition in the glomerulus and a reduction in GFR, as previously discussed (Figure 4(④)). Furthermore, conjugates of MCs with Cys (MCs-Cys) are organotropic to the kidney and thus predominantly accumulate in this organ.[52,57] This organotropism of MCs-Cys is primarily due to effective transportation of MCs and their conjugates (MCs-GSH and MCs-Cys) into the kidney by the OATP superfamily located in cell membranes of the kidney as well as efficient conversion of MCs-GSH to MCs-Cys in the kidney.[52,57−59] In both mammals and aquatic organisms, MCs-Cys and MCs-GSH can also reversibly be deconjugated to effectively release the more potent parent MCs.[60−62] Organotropism of MCs-Cys to the kidney and reversible deconjugation characteristics can prolong exposure of the kidney to MCs and MCs-Cys and thus could aggravate renal toxicity via probable multipathway processes, such as actions of enhanced oxidative stress, cytoskeletal changes, apoptosis, and necrosis, as previously discussed (Figure 4(③B)).

4.2. Animal Experimental Evidence on Nephrotoxicity of MCs at a Dose Less Than the NOAEL. To mimic the toxic effects of environmental exposure to MCs on the residents, in the present study, mice were chronically exposed to 30 μg MC-LR/kg bm/day, a dose less than the NOAEL of 40 μg MC-LR/kg bm/day. NOAEL did not cause pathological changes in the liver over a 13-week period of exposure and is the basis for defining the corresponding safe standard, TDI, and the drinking-water guideline value of 1 μg MC-LR/L by the WHO.[44,63,64] In our animal experiment, BUN and CRE in the serum of mice were significantly enhanced by MC-LR exposure, which was consistent with the findings from residents chronically exposed to MCs. Distinct histopathological changes, including renal tubular dilation, vacuolar degeneration in epithelial cells, collagen deposition, GBM thickening, and podocyte fusion, were also observed in mice exposed to MC-LR. Remarkably, these findings confirmed that chronic, oral exposure to MC-LR at a dose less than the NOAEL could cause renal dysfunctions and lesions in mice.

Renal proteomic expression profiling in mice further revealed the molecular changes underlying renal impairment and identified collagen/amino acid-activated molecular responses as the most striking changes in response to exposure to MC-LR (Figure S5I−J). Collagen deposition observed in the present study is a characteristic feature of chronic kidney disease, and collagens, especially types I, III, and IV, are predominant in the pathological fibrillar matrix.[65] A coherent increase in the protein expression of collagens I (Col1a1 and Col1a2), IV (Col4a1, Col4a2, and Col4a3), V (Col5a2), and XXIV (Col24a1) indicated stimulated collagen synthesis from amino acids and induced fibrotic matrix expansion to form a destructive fibrotic "scar", resulting in a diminished kidney volume and compromised perfusion.[53] It is known that increased protein expression of collagen IV, the major constituent of the GBM, enhances the formation of extensively cross-linked oligomeric networks together with laminins (Lamc1) and proteoglycans (Nid1) and thus thickens the GBM.[66]

4.3. Health Safety of the Current TDI Value Proposed by WHO. In the present study, MCs in the serum of the exposed residents were relatively small, with a median concentration of 0.016 μg/L. However, such a burden of MCs in serum was also associated with renal impairment. In our previous study conducted in the same regions, the median concentration of MCs in the sera of fishermen was as great as 0.227 μg/L[37] since fishermen were more frequently exposed to MCs by skin contact during fishing operations and by eating contaminated aquatic products and drinking contaminated lake water every day. Assuming that the rates of absorption, metabolism, and elimination of MCs in humans were constant, the EDI for residents living near Lake Chao in the present study was approximately 7% of the EDI (2.2−3.9 μg MC-LReq/day) for fishermen on Lake Chao in our previous study.[37] That is, the external exposure doses of MCs expressed as EDI for residents were 0.15−0.27 μg MC-LReq/day. Even taking into account fluctuations introduced by various uncertainties, the EDI value for residents should be much less than the TDI (2.4 μg MC-LR/day for 60 kg adults) recommended by the WHO. Thus, molecular epidemiological findings reported here, supplemented with the experimental results of mice, strongly indicated that prolonged exposure to MCs at doses less than the TDI could result in substantial impairments of the kidney, therefore questioning the safety of the current TDI suggested by the WHO.

In summary, relatively small burdens of MCs were detected in the serum of residents living near Lake Chao and were closely associated with significant disruptions of the amino acid metabolism and risks of renal impairment. Animal experiments confirmed that prolonged exposure to MCs could cause metabolic disturbance and kidney injury in mice. The current TDI value recommended by the WHO needs to be revised timely. The present study, for the first time, provides molecular epidemiologic evidence of human metabolic responses to cyanotoxins at naturally occurring concentrations, closing an important gap between metabolic changes and adverse health outcomes, and contributes to a deep understanding of health risks posed by environmental exposure to cyanotoxins.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c00973.

Quantitative detection of MCs in human serum; pathological examinations of kidney tissues; TMT proteomic analysis of renal tissues; metabolomics of mice serum; statistical analysis and visualization; geographic location of the Zhongmiao town; coverage of algal blooms in Lake Chao; demographic characteristics and serum biochemical parameters of the participants; and linear regression and OPLS regression models (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Ping Xie − *Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China; Institute for Ecological*

https://doi.org/10.1021/acs.est.2c00973
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Research and Pollution Control of Plateau Lakes, School of Ecology and Environmental Science, Yunnan University, Kunming 650091, China; Email: xieping@ihb.ac.cn

Authors

Jun He − *Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China*; orcid.org/0000-0001-8759-1429

Jun Chen − *Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China*

Feng Chen − *Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China*

Liang Chen − *Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China; Institute for Ecological Research and Pollution Control of Plateau Lakes, School of Ecology and Environmental Science, Yunnan University, Kunming 650091, China*; orcid.org/0000-0001-6548-5000

John P. Giesy − *Toxicology Center, University of Saskatchewan, Saskatoon, Saskatoon S7N 5B3, Canada; Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, Saskatoon S7N 5B4, Canada; Zoology Department, Institute for Integrative Toxicology, Michigan State University, East Lansing, Michigan 48824, United States; Department of Environmental Sciences, Baylor University, Waco 76706 Texas, United States*

Yuming Guo − *School of Public Health and Preventive Medicine, Monash University, Melbourne 3004, Australia*

Gaodao Liang − *Wuhan Centers for Disease Control and Prevention, Wuhan 430072, China*

Xuwei Deng − *Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China*

Wenjing Wang − *Key Laboratory of Coastal Zone Environmental Processes, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.2c00973

Author Contributions

♦J.H. and J.C. authors contributed equally to this work.

Funding

This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB31000000); the National Natural Science Foundation of China (grant nos 31770555 and 31600423); and the State Key Laboratory of Freshwater Ecology and Biotechnology (2019FBZ03).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the Wuhan Institute of Physics and Mathematics, Chinese Academy of Science, for use of their Bruker NMR spectrometers. The authors thank Hongbing Liu, Juan Wang, Jun Men, and Sizhe Chen for help with the experiment and data processing.

■ ABBREVIATIONS

ALT	alanine aminotransferase
AST	aspartate aminotransferase
ALP	alkaline phosphatase
LDH	lactate dehydrogenase
TPR	total protein
ALB	albumin
GLO	globulin
A/G	albumin/globulin
TBIL	total bilirubin
DBIL	direct bilirubin
IBIL	indirect bilirubin
TBA	total biliary acid
CHE	cholinesterase
GGT	γ-glutamyl transpeptadase
GLU	glucose
TG	triglyceride
CHO	cholesterol
VLDL	very low-density lipoprotein
BUN	urea nitrogen
CRE	creatinine
UA	uric acid
eGFR	estimated glomerular filtration rate.

■ REFERENCES

(1) Paerl, H. W.; Otten, T. G. Blooms bite the hand that feeds them. *Science* **2013**, *342*, 433−434.

(2) Ho, J. C.; Michalak, A. M.; Pahlevan, N. Widespread global increase in intense lake phytoplankton blooms since the 1980s. *Nature* **2019**, *574*, 667−670.

(3) Huisman, J.; Codd, G. A.; Paerl, H. W.; Ibelings, B. W.; Verspagen, J. M. H.; Visser, P. M. Cyanobacterial blooms. *Nat. Rev. Microbiol.* **2018**, *16*, 471−483.

(4) Svirčev, Z.; Drobac, D.; Tokodi, N.; Mijović, B.; Codd, G. A.; Meriluoto, J. Toxicology of microcystins with reference to cases of human intoxications and epidemiological investigations of exposures to cyanobacteria and cyanotoxins. *Arch. Toxicol.* **2017**, *91*, 621−650.

(5) Svirčev, Z.; Lalić, D.; Bojadžija Savić, G.; Tokodi, N.; Drobac Backović, D.; Chen, L.; Meriluoto, J.; Codd, G. A. Global geographical and historical overview of cyanotoxin distribution and cyanobacterial poisonings. *Arch. Toxicol.* **2019**, *93*, 2429−2481.

(6) Meriluoto, J.; Spoof, L.; Codd, G. A. *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis*; John Wiley & Sons, 2017; pp 1−118.

(7) Zuo, J.; Huo, T.; Du, X.; Yang, Q.; Wu, Q.; Shen, J.; Liu, C.; Hung, T.-C.; Yan, W.; Li, G. The joint effect of parental exposure to microcystin-LR and polystyrene nanoplastics on the growth of zebrafish offspring. *J. Hazard. Mater.* **2021**, *410*, 124677.

(8) Zhang, H.; Li, B.; Liu, Y.; Chuan, H.; Liu, Y.; Xie, P. Immunoassay technology: Research progress in microcystin-LR detection in water samples. *J. Hazard. Mater.* **2022**, *424*, 127406.

(9) Lee, J.; Lee, S.; Jiang, X. Cyanobacterial toxins in freshwater and food: important sources of exposure to humans. *Annu. Rev. Food Sci. Technol.* **2017**, *8*, 281−304.

(10) Xiang, L.; Li, Y.-W.; Liu, B.-L.; Zhao, H.-M.; Li, H.; Cai, Q.-Y.; Mo, C.-H.; Wong, M.-H.; Li, Q. X. High ecological and human health risks from microcystins in vegetable fields in southern China. *Environ. Int.* **2019**, *133*, 105142.

https://doi.org/10.1021/acs.est.2c00973
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

(11) Wiśniewska, K.; Lewandowska, A. U.; Śliwińska-Wilczewska, S. The importance of cyanobacteria and microalgae present in aerosols to human health and the environment − Review study. *Environ. Int.* **2019**, *131*, 104964.
(12) Backer, L.; Landsberg, J.; Miller, M.; Keel, K.; Taylor, T. Canine cyanotoxin poisonings in the United States (1920s−2012): Review of suspected and confirmed cases from three data sources. *Toxins* **2013**, *5*, 1597−1628.
(13) Stewart, I.; Seawright, A. A.; Shaw, G. R. Cyanobacterial poisoning in livestock, wild mammals and birds-an overview. In *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*, Hudnell, H. K., Ed.; Springer Science & Business Media, 2008; Vol. *619*, pp 613−637.
(14) Bautista, A. C.; Moore, C. E.; Lin, Y.; Cline, M. G.; Benitah, N.; Puschner, B. Hepatopathy following consumption of a commercially available blue-green algae dietary supplement in a dog. *BMC Vet. Res.* **2015**, *11*, 136.
(15) Lopez-Rodas, V.; Maneiro, E.; Lanzarot, M. P.; Perdigones, N.; Costas, E. Mass wildlife mortality due to cyanobacteria in the Donana National Park, Spain. *Vet. Rec.* **2008**, *162*, 317−318.
(16) Wang, H.; Xu, C.; Liu, Y.; Jeppesen, E.; Svenning, J.-C.; Wu, J.; Zhang, W.; Zhou, T.; Wang, P.; Nangombe, S.; Ma, J.; Duan, H.; Fang, J.; Xie, P. From unusual suspect to serial killer: Cyanotoxins boosted by climate change may jeopardize megafauna. *Innovation* **2021**, *2*, 100092.
(17) Azevedo, S. M. F. O.; Carmichael, W. W.; Jochimsen, E. M.; Rinehart, K. L.; Lau, S.; Shaw, G. R.; Eaglesham, G. K. Human intoxication by microcystins during renal dialysis treatment in Caruaru–Brazil. *Toxicology* **2002**, *181−182*, 441−446.
(18) Funari, E.; Testai, E. Human health risk assessment related to cyanotoxins exposure. *Crit. Rev. Toxicol.* **2008**, *38*, 97−125.
(19) Hilborn, E. D.; Roberts, V. A.; Backer, L.; DeConno, E.; Egan, J. S.; Hyde, J. B.; Nicholas, D. C.; Wiegert, E. J.; Billing, L. M.; Diorio, M. Algal bloom−associated disease outbreaks among users of freshwater lakes—United States, 2009−2010. *Morb. Mortal. Wkly. Rep.* **2014**, *63*, 11.
(20) Yu, S. Drinking water and primary liver cancer. *Primary Liver Cancer*; Springer-Verleg, 1989; pp 20−30.
(21) Yu, S.; Zhao, N.; Zi, X. The relationship between cyanotoxin (Microcystin, MC) in pond-ditch water and primary liver cancer in China. *Zhonghua Zhongliu Zazhi* **2001**, *23*, 96.
(22) Ueno, Y.; Nagata, S.; Tsutsumi, T.; Hasegawa, A.; Watanabe, M. F.; Park, H.-D.; Chen, G.-C.; Chen, G.; Yu, S.-Z. Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. *Carcinogenesis* **1996**, *17*, 1317−1321.
(23) Svirčev, Z.; Drobac, D.; Tokodi, N.; Vidović, M.; Simeunović, J.; Miladinov, M.; Baltić, V. Epidemiology of primary liver cancer in Serbia and possible connection with cyanobacterial blooms. *J. Environ. Sci. Health, Part C: Environ. Carcinog. Rev.* **2013**, *31*, 181−200.
(24) Zhou, L.; Yu, H.; Chen, K. Relationship between microcystin in drinking water and colorectal cancer. *Biomed. Environ. Sci.* **2002**, *15*, 166.
(25) Xu, M. Microcystins in drinking water and mortality of major cancer in a city along Taihu Lake. *Chin. J. Prev. Control Chronic Non-Commun. Dis.* **2003**, *3*, 10.
(26) Li, Y.; Chen, J.-a.; Zhao, Q.; Pu, C.; Qiu, Z.; Zhang, R.; Shu, W. A cross-sectional investigation of chronic exposure to microcystin in relationship to childhood liver damage in the Three Gorges Reservoir region, China. *Environ. Health Perspect.* **2011**, *119*, 1483−1488.
(27) Liu, W.; Wang, L.; Yang, X.; Zeng, H.; Zhang, R.; Pu, C.; Zheng, C.; Tan, Y.; Luo, Y.; Feng, X.; Tian, Y.; Xiao, G.; Wang, J.; Huang, Y.; Luo, J.; Feng, L.; Wang, F.; Yuan, C.; Yao, Y.; Qiu, Z.; Chen, J.-a.; Wu, L.; Nong, Q.; Lin, H.; Shu, W. Environmental microcystin exposure increases liver injury risk induced by hepatitis B virus combined with aflatoxin: A cross-sectional study in southwest China. *Environ. Sci. Technol.* **2017**, *51*, 6367−6378.
(28) Lin, H.; Liu, W.; Zeng, H.; Pu, C.; Zhang, R.; Qiu, Z.; Chen, J.-a.; Wang, L.; Tan, Y.; Zheng, C.; Yang, X.; Tian, Y.; Huang, Y.; Luo, J.; Luo, Y.; Feng, X.; Xiao, G.; Feng, L.; Li, H.; Wang, F.; Yuan, C.; Wang, J.; Zhou, Z.; Wei, T.; Zuo, Y.; Wu, L.; He, L.; Guo, Y.; Shu, W. Determination of environmental exposure to microcystin and aflatoxin as a risk for renal function based on 5493 rural people in Southwest China. *Environ. Sci. Technol.* **2016**, *50*, 5346−5356.
(29) He, J.; Li, G.; Chen, J.; Lin, J.; Zeng, C.; Chen, J.; Deng, J.; Xie, P. Prolonged exposure to low-dose microcystin induces nonalcoholic steatohepatitis in mice: a systems toxicology study. *Arch. Toxicol.* **2017**, *91*, 465−480.
(30) Birungi, G.; Li, S. F. Y. Investigation of the effect of exposure to non cytotoxic amounts of microcystins. *Metabolomics* **2011**, *7*, 485−499.
(31) Chen, L.; Hu, Y.; He, J.; Chen, J.; Giesy, J. P.; Xie, P. Responses of the proteome and metabolome in livers of zebrafish exposed chronically to environmentally relevant concentrations of microcystin-LR. *Environ. Sci. Technol.* **2017**, *51*, 596−607.
(32) Pavagadhi, S.; Natera, S.; Roessner, U.; Balasubramanian, R. Insights into lipidomic perturbations in zebrafish tissues upon exposure to microcystin-LR and microcystin-RR. *Environ. Sci. Technol.* **2013**, *47*, 14376−14384.
(33) Zhang, Z.; Zhang, X.-X.; Wu, B.; Yin, J.; Yu, Y.; Yang, L. Comprehensive insights into microcystin-LR effects on hepatic lipid metabolism using cross-omics technologies. *J. Hazard. Mater.* **2016**, *315*, 126−134.
(34) Fiehn, O. Metabolomics—the link between genotypes and phenotypes. *Functional Genomics*; Springer, 2002; pp 155−171.
(35) Everson, T. M.; Marsit, C. J. *Using Molecular-Omics in Environmental Epidemiology to Gain Unique Insights into Environmental Health*; ISEE Conference Abstracts, 2018.
(36) Li, D.; Kong, F.; Yang, Z.; Zhang, M.; Shi, X. Studies on the abundance of toxic and non-toxic Microcystis population of Lake Chaohu in summer and winter. *Acta Sci. Circumstantiae* **2011**, *31*, 2672.
(37) Chen, J.; Xie, P.; Li, L.; Xu, J. First identification of the hepatotoxic microcystins in the eerum of a chronically exposed human population together with endication of hepatocellular damage. *Toxicol. Sci.* **2009**, *108*, 81−89.
(38) Peng, L.; Liu, Y.; Chen, W.; Liu, L.; Kent, M.; Song, L. Health risks associated with consumption of microcystin-contaminated fish and shellfish in three Chinese lakes: significance for freshwater aquacultures. *Ecotoxicol. Environ. Saf.* **2010**, *73*, 1804−1811.
(39) Chen, J.; Xie, P. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in two freshwater shrimps, Palaemon modestus and Macrobrachium nipponensis, from a large shallow, eutrophic lake of the subtropical China. *Toxicon* **2005**, *45*, 615−625.
(40) Shang, L.; Feng, M.; Xu, X.; Liu, F.; Ke, F.; Li, W. Co-occurrence of microcystins and taste-and-odor compounds in drinking water source and their removal in a full-scale drinking water treatment plant. *Toxins* **2018**, *10*, 26.
(41) Yang, Z.; Kong, F.; Zhang, M. Groundwater contamination by microcystin from toxic cyanobacteria blooms in Lake Chaohu, China. *Environ. Monit. Assess.* **2016**, *188*, 280.
(42) Tao, M.; Xie, P.; Chen, J.; Qin, B.; Zhang, D.; Niu, Y.; Zhang, M.; Wang, Q.; Wu, L. Use of a generalized additive model to investigate key abiotic factors affecting microcystin cellular quotas in heavy bloom areas of Lake Taihu. *PLoS One* **2012**, *7*, No. e32020.
(43) Ma, Y.-C.; Zuo, L.; Chen, J.-H.; Luo, Q.; Yu, X.-Q.; Li, Y.; Xu, J.-S.; Huang, S.-M.; Wang, L.-N.; Huang, W.; Wang, M.; Xu, G.-B.; Wang, H.-Y. Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. *J. Am. Soc. Nephrol.* **2006**, *17*, 2937−2944.
(44) Fawell, J. K.; Mitchell, R. E.; Everett, D. J.; Hill, R. E. The toxicity of cyanobacterial toxins in the mouse: I Microcystin-LR. *Hum. Exp. Toxicol.* **1999**, *18*, 162−167.

https://doi.org/10.1021/acs.est.2c00973
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

(45) Gupta, N.; Pant, S. C.; Vijayaraghavan, R.; Rao, P. V. Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. *Toxicology* **2003**, *188*, 285.
(46) Weiner, I. D.; Mitch, W. E.; Sands, J. M. Urea and ammonia metabolism and the control of renal nitrogen excretion. *Clin. J. Am. Soc. Nephrol.* **2015**, *10*, 1444−1458.
(47) Arroyave, G. Comparative sensitivity of specific amino acid ratios versus "Essential to Nonessential" amino acid ratio. *Am. J. Clin. Nutr.* **1970**, *23*, 703−706.
(48) Laidlaw, S. A.; Berg, R. L.; Kopple, J. D.; Naito, H.; Walker, W. G.; Walser, M. Patterns of fasting plasma amino acid levels in chronic renal insufficiency: Results from the feasibility phase of the modification of diet in renal disease study. *Am. J. Kidney Dis.* **1994**, *23*, 504−513.
(49) Duranton, F.; Lundin, U.; Gayrard, N.; Mischak, H.; Aparicio, M.; Mourad, G.; Daurès, J.-P.; Weinberger, K. M.; Argilés, À. Plasma and urinary amino acid metabolomic profiling in patients with different levels of kidney function. *Clin. J. Am. Soc. Nephrol.* **2014**, *9*, 37−45.
(50) Hayamizu, K. Amino acids and energy metabolism: An overview. In *Sustained Energy for Enhanced Human Functions and Activity*; Bagchi, D., Ed.; Academic Press, 2017; pp 339−349.
(51) Legouis, D.; Faivre, A.; Cippà, P. E.; de Seigneux, S. Renal gluconeogenesis: an underestimated role of the kidney in systemic glucose metabolism. *Nephrol., Dial., Transplant.* **2020**, gfaa302.
(52) He, J.; Chen, J.; Xie, P.; Zhang, D.; Li, G.; Wu, L.; Zhang, W.; Guo, X.; Li, S. Quantitatively evaluating detoxification of the hepatotoxic microcystins through the glutathione and cysteine pathway in the cyanobacteria-eating bighead carp. *Aquat. Toxicol.* **2012**, *116−117*, 61−68.
(53) Eddy, A. A. Overview of the cellular and molecular basis of kidney fibrosis. *Kidney. Int. Suppl.* **2014**, *4*, 2−8.
(54) Lau, W. L.; Vaziri, N. D. Urea, a true uremic toxin: the empire strikes back. *Clin. Sci.* **2016**, *131*, 3−12.
(55) D'Apolito, M.; Du, X.; Pisanelli, D.; Pettoello-Mantovani, M.; Campanozzi, A.; Giacco, F.; Maffione, A. B.; Colia, A. L.; Brownlee, M.; Giardino, I. Urea-induced ROS cause endothelial dysfunction in chronic renal failure. *Atherosclerosis* **2015**, *239*, 393−400.
(56) Shaykh, M.; Pegoraro, A. A.; Mo, W.; Arruda, J. A. L.; Dunea, G.; Singh, A. K. Carbamylated proteins activate glomerular mesangial cells and stimulate collagen deposition. *J. Lab. Clin. Med.* **1999**, *133*, 302−308.
(57) Zhang, D.; Xie, P.; Chen, J.; Dai, M.; Qiu, T.; Liu, Y.; Liang, G. Determination of microcystin-LR and its metabolites in snail (Bellamya aeruginosa), shrimp (Macrobrachium nipponensis) and silver carp (Hypophthalmichthys molitrix) from Lake Taihu, China. *Chemosphere* **2009**, *76*, 974−981.
(58) Ito, E.; Takai, A.; Kondo, F.; Masui, H.; Imanishi, S.; Harada, K.-i. Comparison of protein phosphatase inhibitory activity and apparent toxicity of microcystins and related compounds. *Toxicon* **2002**, *40*, 1017−1025.
(59) Chen, J.; Xie, P.; Zhang, D.; Lei, H. In situ studies on the distribution patterns and dynamics of microcystins in a biomanipulation fish - bighead carp (Aristichthys nobilis). *Environ. Pollut.* **2007**, *147*, 150−157.
(60) Li, W.; He, J.; Chen, J.; Xie, P. Excretion pattern and dynamics of glutathione detoxification of microcystins in Sprague Dawley rat. *Chemosphere* **2018**, *191*, 357−364.
(61) Miles, C. O.; Sandvik, M.; Nonga, H. E.; Ballot, A.; Wilkins, A. L.; Rise, F.; Jaabaek, J. A. H.; Loader, J. I. Conjugation of microcystins with thiols is reversible: Base-catalyzed deconjugation for chemical analysis. *Chem. Res. Toxicol.* **2016**, *29*, 860−870.
(62) Li, W.; Chen, J.; Xie, P.; He, J.; Guo, X.; Tuo, X.; Zhang, W.; Wu, L. Rapid conversion and reversible conjugation of glutathione detoxification of microcystins in bighead carp (Aristichthys nobilis). *Aquat. Toxicol.* **2014**, *147*, 18−25.
(63) WHO *Cyanobacterial Toxins: Microcystin-LR Guidelines for Drinking-Water Quality*; World Health Organization Geneva, 1998; pp 95−110.
(64) WHO *Cyanobacterial Toxins: Microcystins*, 2020.
(65) Duffield, J. S. Cellular and molecular mechanisms in kidney fibrosis. *J. Clin. Invest.* **2014**, *124*, 2299−2306.
(66) Quinlan, C.; Rheault, M. N. Genetic basis of type IV collagen disorders of the kidney. *Clin. J. Am. Soc. Nephrol.* **2021**, *16*, 1101−1109.

https://doi.org/10.1021/acs.est.2c00973
Environ. Sci. Technol. XXXX, XXX, XXX−XXX