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Article

Metabolomics Analysis of Chronic Exposure to Dimethylarsenic Acid in Mice and Toxicity Assessment of Organic Arsenic in Food

Nan Jing, Jing Peng, Xin Yang, Xinzheng Wang, Qian Liu, Hongli Wang, Weihua Li, Fangting Dong,* Kun He,* and Na Wang*

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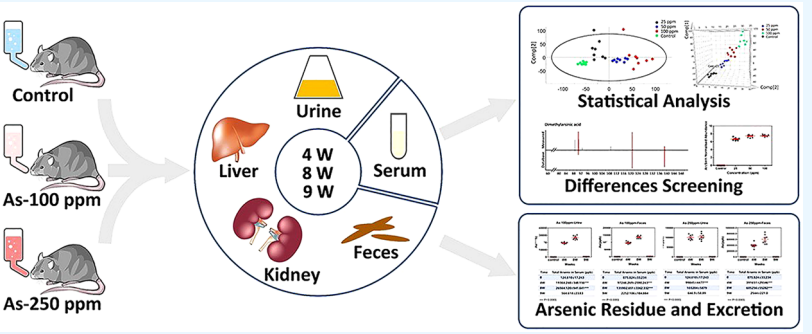

ABSTRACT: Dimethylarsenic acid is a natural organic arsenic in seafood and one of the important metabolites of inorganic arsenic, which is generally considered to have low or no toxicity. However, due to the controversy of the toxicity of organic arsenic, the food safety standard of organic arsenic has not been established until now, and the effects of organic arsenic on chronic toxicity and the overall metabolic level of animals are rarely reported. In our study, 64 female C57BL/6 mice were exposed to different concentrations of dimethylarsenic acid with water intake. Fifteen metabolites in serum were detected to be altered with the increase of arsenic concentration and exposure time. Dimethylarsenic acid exposure significantly affected the overall metabolic level of mice, and the related effects were not recovered shortly after the suspension of arsenic intake. Although arsenic was excreted largely in urine and feces, continued dimethylarsenic acid exposure could still lead to arsenic accumulation in the liver and kidneys and cause mild nephritis in mice.

INTRODUCTION

Arsenic is a natural element in the earth's crust. Arsenic and its related compounds have been identified as carcinogens by the International Agency for Research on Cancer (IARC).[1,2] Seawater contains a high concentration of arsenic (about 1−2 μg As/L),[3,4] mostly in the form of pentavalent arsenic.[5] Marine animals and plants accumulate arsenic from different sources, and there is different arsenic speciation in their bodies. Marine plants, such as algae, ingest inorganic arsenic directly from seawater and convert it into arsenosugar and methylated arsenic compounds through a series of metabolic processes.[6−9] Most marine animals do not ingest arsenic directly from seawater but accumulate it through the food chain.[10−15] Arsenic mainly exists in marine animals in the form of arsenic betaine, arsenic choline, and other methylated forms.[16−20]

At present, organic arsenic in seafood is generally considered to be safe and nontoxic to humans.[21−23] Studies have shown that human ingestion of arsenic sugar in algae can be metabolized into methylated arsenic through the liver and kidney and excreted in urine.[24,25] Arsenic betaine and other methylated forms of arsenic in seafood are currently recognized as the minimum toxic arsenic compound that can be directly excreted from the body.[26−28] However, cases of acute poisoning from seafood consumption may occur sometimes, and several studies have shown that arsenic metabolism varies greatly among individuals. Meanwhile, researchers believe that arsenic can cause damage as it is transformed in the body; the potential health risks of organic arsenic should be of concern.[29−32] Therefore, a systematic study of the metabolic process of organic arsenic in animals has great significance for the in-depth identification of arsenic toxicity in seafood and guarantee of food safety.

The ultra-performance liquid chromatograph−tandem mass spectrometer (UPLC-MS/MS) is a useful tool to separate and analyze complex biochemistry samples such as blood and

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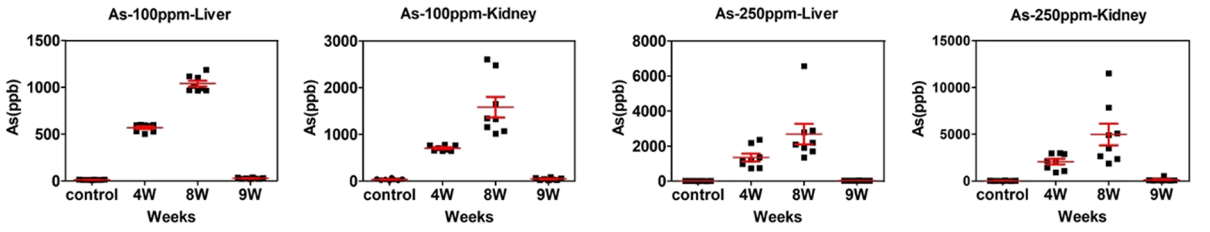


Time	Total Arsenic in Serum (ppb)
0	14.274±0.608
4W	568.722±14.370***
8W	1040.059±30.157***
9W	28.543±2.365

*** P<0.0001

Time	Total Arsenic in Serum (ppb)
0	35.197±6.591
4W	707.057±20.774***
8W	1583.289±221.170***
9W	46.942±7.920

*** P<0.0001

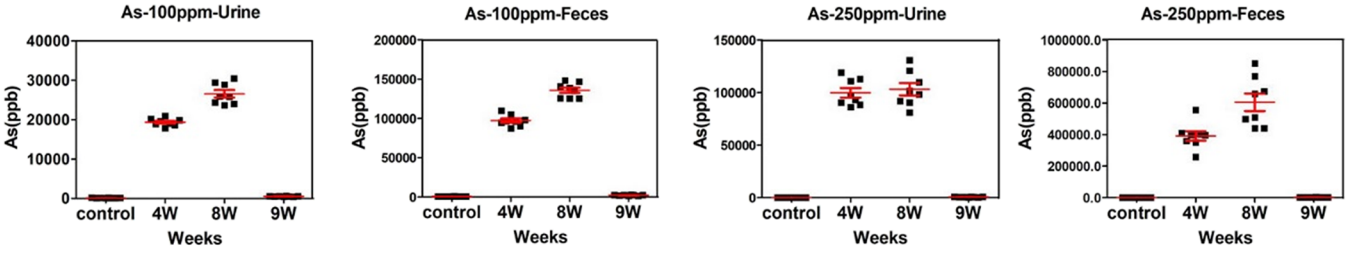
Time	Total Arsenic in Serum (ppb)
0	14.274±0.608
4W	1356±217.1***
8W	2689±583.1***
9W	37.45±0.9978

*** P<0.0001

Time	Total Arsenic in Serum (ppb)
0	35.197±6.591
4W	2064±300.6***
8W	4966±1156***
9W	136.4±59.14

*** P<0.0001

Figure 1. Changes of total arsenic content in the kidney and liver of mice that continuously ingested different concentrations of dimethylarsenate acid.



Time	Total Arsenic in Serum (ppb)
0	124.610±17.243
4W	19364.248±348.156***
8W	26564.120±941.641***
9W	564.618±23.83

*** P<0.0001

Time	Total Arsenic in Serum (ppb)
0	875.824±33.234
4W	97246.269±2590.243***
8W	135902.651±3362.332***
9W	2252.106±184.884

*** P<0.0001

Time	Total Arsenic in Serum (ppb)
0	124.610±17.243
4W	99845±4477***
8W	103204±5879
9W	646.9±58.89

*** P<0.0001

Time	Total Arsenic in Serum (ppb)
0	875.824±33.234
4W	391651±29346***
8W	605256±55282***
9W	2544±221.8

*** P<0.0001

Figure 2. Changes of total arsenic content in urine and feces of mice that continuously ingested different concentrations of dimethylarsenate acid.

urine.[33] In this work, serum metabolite changes in mice were detected by UPLC-MS/MS after long-term exposure to dimethylarsenic acid. A total of 596 serum metabolites were identified, and it was found that 15 metabolites changed significantly with the extension of exposure time and exposure concentration. Statistical analysis showed that dimethylarsenic acid caused significant changes in the overall metabolic level of mice, and the related changes did not recover after short-term cessation of exposure. Mild nephritis was found during sustained dimethylarsenic acid exposure, suggesting that the safety of long-term chronic intake of organic arsenic from seafood should be considered.

RESULTS AND DISCUSSION

Arsenic Excretion and Retention Analysis. The liver, kidney, urine, and feces samples of each mouse with different exposure times and concentrations were examined by ICP-MS to investigate arsenic excretion and retention (Figure 1). The results showed that the concentration of total arsenic in the liver and kidney was extremely low in the control group. With the extension of arsenic intake time and the increase of arsenic concentration, the total arsenic accumulation increased significantly in the liver and kidney. When the concentration of arsenic was 100 ppm in drinking water, the total arsenic content in the kidney was slightly higher than that in the liver, but when the concentration of arsenic reached 250 ppm, the total arsenic content in the kidney was significantly higher than that in the liver, possibly due to the excretion of a large amount of arsenic in the urine. Furthermore, with the increase of arsenic exposure time and concentration, the individual difference of total arsenic content in the liver and kidney of mice gradually increased.

One week after the suspension of arsenic exposure (9W), the total arsenic content in the liver and kidney of two experimental groups showed a trend of precipitous decline. Although the total arsenic content in the liver and kidney was different from that in the control samples, statistical analysis showed that the total arsenic content in the liver and kidney in experimental samples was not significantly different from the control samples after a week of arsenic suspension ($P > 0.05$). The results suggested that the intake of dimethylarsenic acid was hardly retained in the body, and the arsenic content in the liver and kidney returned to the normal level in a short period with no statistical difference after the suspension of exposure.

After ingestion of dimethylarsenate acid, arsenic was excreted mainly in urine and feces. Our results showed a persistently high arsenic excretion in urine and feces (Figure 2). When the concentration of arsenic was 100 ppm in drinking water, the content of arsenic increased gradually in urine and feces with the extension of exposure time, and there was little individual difference in the group. When the concentration of arsenic reached 250 ppm, the increase trend of arsenic excretion in urine was significantly slower than that in the 100 ppm group, and the arsenic excretion in feces continued to increase with time. ICP-MS detection of urine

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Table 1. List of Differential Metabolites after Dimethylarsenic Acid Exposure

metabolite name	change	UPLC column	retention time (min)	*m*/*z*	ion mode	database
4'-dihydroabscisic acid	↑	HSS T3	10.6185	265.1446	−	HMDB
[3-(4-methoxyphenyl)propoxy]sulfonic acid	↑	HSS T3	8.5881	245.0493	−	HMDB
2,8-dihydroxyquinoline-beta-D-glucuronide	↑	HSS T3	7.2092	336.0719	−	HMDB
1-[(5-amino-5-carboxypentyl)amino]-1-deoxyfructose	↑	BEH amide	12.5615	309.1650	+	HMDB
S-adenosylmethionine	↑	BEH amide	11.4903	339.1431	+	METLIN
ethyl glucuronide	↑	BEH amide	5.1636	221.0661	−	HMDB
MG(18:1(9Z)/0:0/0:0)	↓	HSS T3	13.2837	357.2991	+	METLIN
LysoPC(22:5(4Z,7Z,10Z,13Z,16Z))	↓	HSS T3	13.3985	570.3516	+	HMDB
N-acetylmannosamine	↓	BEH amide	3.3548	222.0963	+	HMDB
medicanine	↓	BEH amide	5.8884	160.096	+	HMDB
2-buten-1-ol	↓	HSS T3	7.4346	73.0646	+	HMDB
dihyroxy-1*H*-indole glucuronide I	↓	HSS T3	6.0131	324.0722	−	HMDB
thiomorpholine 3-carboxylate	↓	HSS T3	4.0289	148.0418	+	HMDB
3-indoleacetonitrile	↓	BEH amide	3.1516	157.0753	+	HMDB
dimethylarsenic acid	↑	HSS T3	7.7475	194.0807	+	METLIN

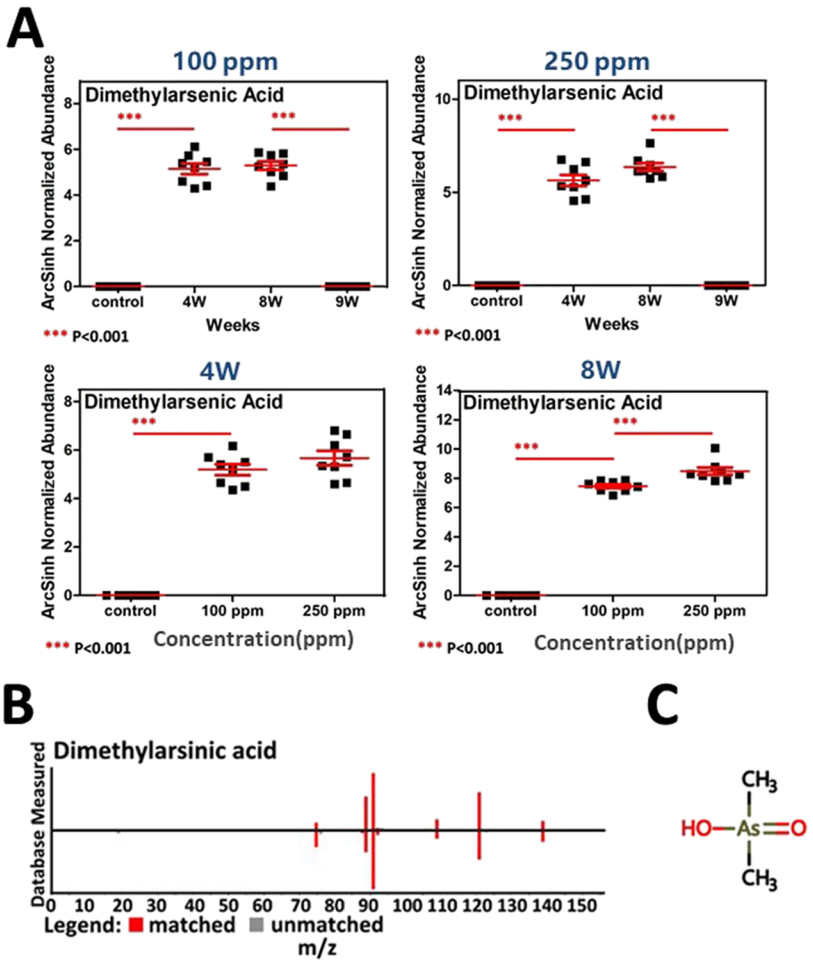

Figure 3. (A) Changes of serum dimethylarsenic acid content with exposure time and concentration; (B) the detected MS spectrum of dimethylarsenic acid matched with the METLIN database; and (C) structural formula of dimethylarsenic acid.

and feces samples showed that arsenic excreted in feces was significantly higher than that in urine.

In addition, the urine and feces kept the high arsenic excretion under continuous exposure to a high concentration (250 ppm) of dimethylarsenic acid. Moreover, the concentration even exceeded the intake of arsenic in drinking water, indicating that the body has a strong ability to get rid of the organic arsenic. One week after the suspension of arsenic exposure (9W), the total arsenic content in urine and feces showed a precipitous decline and quickly returned to a low level that had no statistical difference compared with the control ($P > 0.05$).

Screening for Differential Serum Metabolites from Chronic Exposure to Dimethylarsenate Acid. UPLC-MS/MS was used to separate and analyze the serum metabolism of mice exposed to dimethylarsenic acid. More than 15,000 MS

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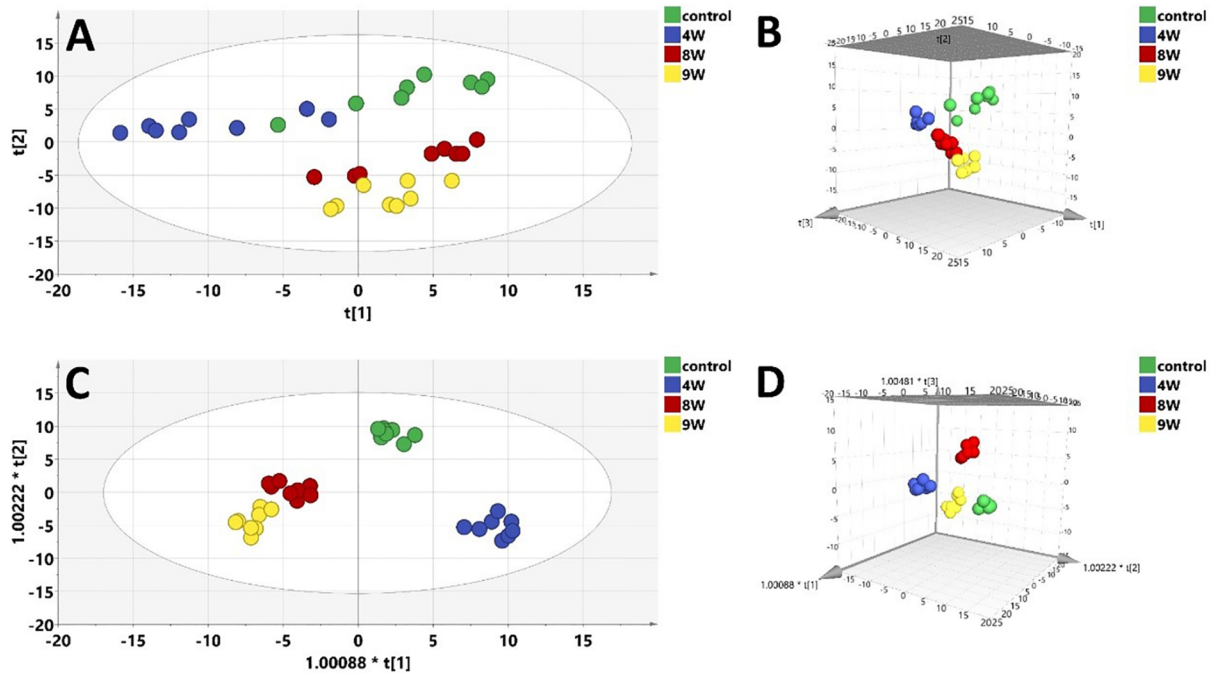

Figure 4. Results of statistical analysis (100 ppm dimethylarsenic acid in drinking water). (A) PCA score plot in 2D plane; (B) PCA score plot in 3D space; (C) OPLS-DA score plot in 2D plane; and (D) OPLS-DA score plot in 3D space.

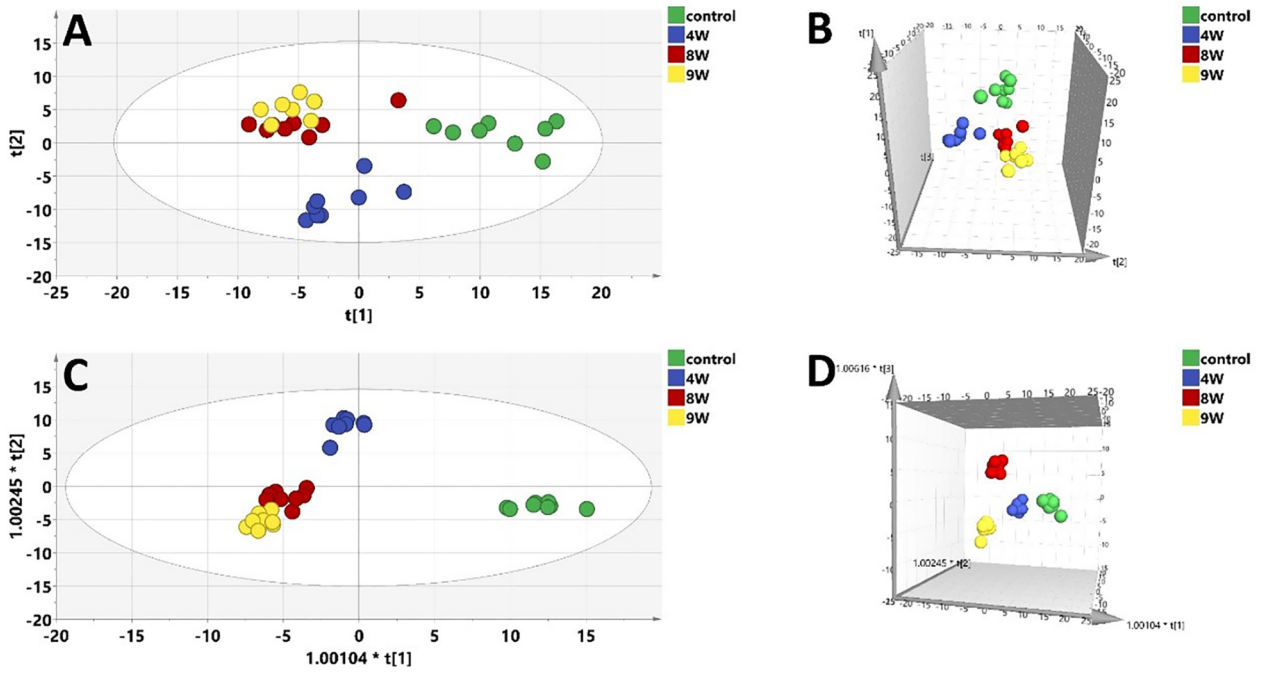

Figure 5. Results of statistical analysis (250 ppm dimethylarsenic acid in drinking water). (A) PCA score plot in 2D plane; (B) PCA score plot in 3D space; (C) OPLS-DA score plot in 2D plane; and (D) OPLS-DA score plot in 3D space.

features were detected, which contained a large amount of metabolite information. A total of 596 structurally determined metabolites were identified in mouse serum. The changes of metabolites at the same exposure concentration with different times and at the same exposure time with different concentrations were investigated, respectively, and a total of 15 metabolites with significant changes were detected (Table 1).

Among them, seven metabolites were upregulated or the overall change showed an upregulated trend, including ethyl glucuronide, *S*-adenosylmethionine, 1-[(5-amino-5-carboxypentyl)amino]-1-deoxyfructose, 2,8-dihydroxyquinoline-beta-D-glucuronide, [3-(4-methoxyphenyl)propoxy]-sulfonic acid, 4′-dihydroabscisic acid, and dimethylarsenic acid. And eight metabolites were downregulated or the overall change showed a downregulated trend, including 3-indoleacetonitrile, thiomorpholine 3-carboxylate, dihyroxy-1*H*-indole glucuronide I, 2-buten-1-ol, medicanine, *N*-acetylmannosamine, LysoPC(22:5(4Z,7Z,10Z,13Z,16Z)), and MG(18:1-(9Z)/0:0/0:0). The structural formula, MS spectrum, and

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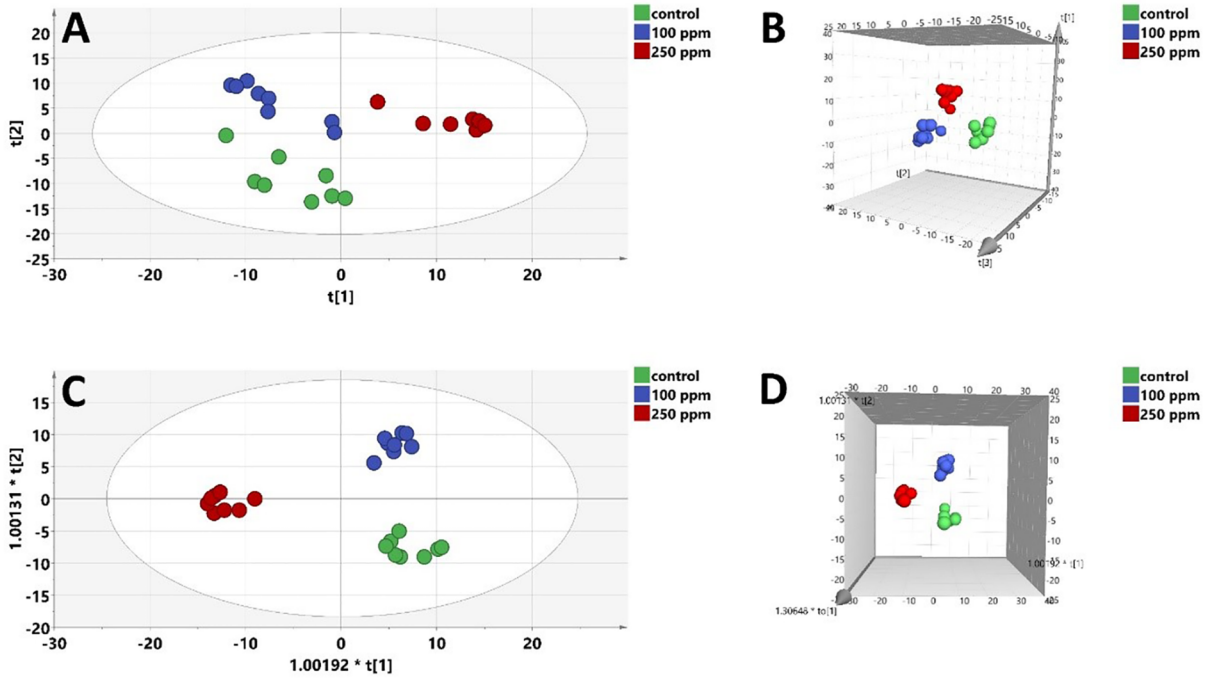

Figure 6. Results of statistical analysis (4 weeks for dimethylarsenic acid exposure). (A) PCA score plot in 2D plane; (B) PCA score plot in 3D space; (C) OPLS-DA score plot in 2D plane; and (D) OPLS-DA score plot in 3D space.

variation of these differential metabolites are shown in the Supporting Information in Figures S2−S15. Serum metabolites' chromatograms with different approaches are shown in the Supporting Information in Figure S20.

Dimethylarsenic Acid in Serum. According to the literature, arsenic is excreted in large amounts in the urine and feces and rarely remains in the blood.[34,35] However, dimethylarsenic acid was still found in serum by UPLC-MS/MS metabolomic tests.[33] Figure 3 shows that with the increase of arsenic concentration and the extension of exposure time, the content of dimethylarsenic acid in serum was increased significantly.

One week after the suspension of arsenic exposure (9W), the content of dimethylarsenic acid dropped rapidly, indicating that the increase of serum arsenic caused by water intake relies on continuous exposure. As a result, organic arsenic exposure has strong concealment, bringing great difficulties to clinical detection.

Statistical Analysis. All MS/MS data of the identified metabolites were sent to SIMCA 14.1 for statistical analysis to investigate the effect of organic arsenic exposure on the overall metabolic level of mice.[33] In the experimental groups, mice exposed to arsenic for 4 and 8 weeks and those studied 1 week after the suspension of arsenic exposure were recorded as 4W, 8W, and 9W, respectively.

As shown in Figure 4, although the 4W and control groups showed significant differences, they could not be completely distinguished in the 2D plot by PCA when the concentration of dimethylarsenic acid in drinking water was 100 ppm. However, when the exposure time reached 8 weeks, the control and 8W groups could be effectively distinguished by PCA; meanwhile, the 4W and 8W groups could also be effectively distinguished by PCA, suggesting that with the prolongation of arsenic exposure time, the overall metabolic level of mice changes significantly. One week after suspending arsenic exposure, PCA could not effectively distinguish the 9W and 8W groups in both 2D (Figure 4A) and 3D (Figure 4B) score plots but significantly distinguished the control group, indicating that the metabolic level of mice was not effectively recovered after arsenic suspension and that the effects of dimethylarsenic acid on the body were continuing.

Further OPLS-DA analysis showed that the control group and all experimental groups could be effectively distinguished (Figure 4C,D). But even though OPLS-DA was a supervised analysis mode, the 8W and 9W groups could only be marginally distinguished, which further prove that dimethylarsenic acid still affects the overall metabolic level after arsenic suspension.

When the exposure concentration of dimethylarsenic acid reached 250 ppm (Figure 5), the mice in all the experimental groups were effectively distinguished from the control group by PCA, but the 9W and 8W groups were still not effectively distinguished (Figure 5A). In the 3D score plot (Figure 5B), there was still no effective differentiation between these two groups (8W and 9W), indicating that the effects of dimethylarsenic acid on body metabolism could continue until arsenic suspension. OPLS-DA results showed that when the arsenic concentration in drinking water reached 250 ppm (Figure 5C,D), there was still partial crossover in the 2D score plot between 8W and 9W, indicating that the metabolic level of these two groups was very similar, which further confirmed that long-term exposure to dimethylarsenic acid still had effects on the body even after the suspension of arsenic.

Compared with 100 ppm, when the exposure concentration of dimethylarsenic acid reached 250 ppm, the distinction between 8W and 9W in the OPLS-DA score plot was significantly reduced, suggesting that the influence of dimethylarsenic acid on the body was related to the concentration, and the recovery of body metabolism level was very low after arsenic suspension.

As shown in Figure 6, when dimethylarsenic acid was continuously exposed for 4 weeks, PCA and OPLS-DA could

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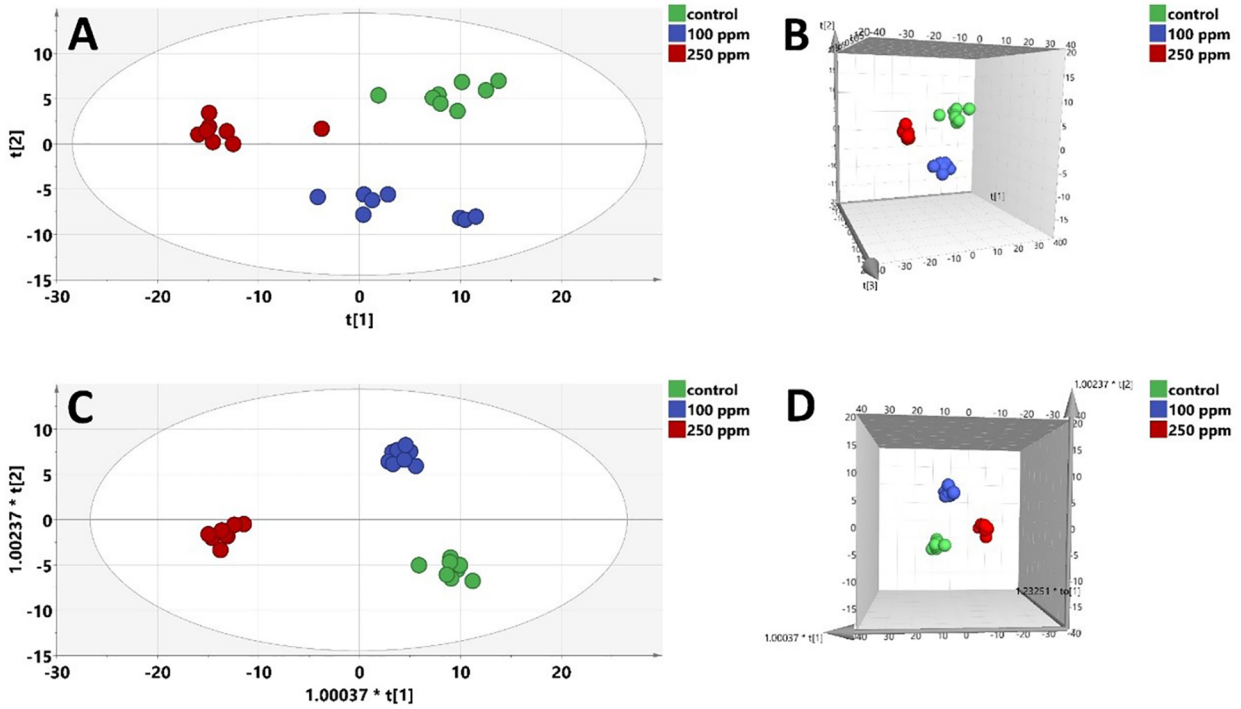

Figure 7. Results of statistical analysis (8 weeks for dimethylarsenic acid exposure). (A) PCA score plot in 2D plane; (B) PCA score plot in 3D space; (C) OPLS-DA score plot in 2D plane; and (D) OPLS-DA score plot in 3D space.

effectively distinguish the control group from the two concentration groups (100 and 250 ppm), indicating that the level of organic arsenic on body metabolism increased with the increase of exposure concentration. When the exposure time reached 8 weeks (Figure 7), the difference between the control group and the experimental groups with different concentrations was significantly higher than that at 4 weeks, suggesting that the impact of dimethylarsenic acid on the body is further deepened with the exposure time increases.

For OLPS-DA, the reliability of the model was evaluated by the permutation test, and the number of permutations for each variable was set to 200. The corresponding results are shown in the Supporting Information in Figures S16−S18. For every group, the *y*-intercept of the fitting line of R^2 and Q^2 is significantly lower than the value of R^2 and Q^2 under the corresponding principal component (the high point on the right of the line). The *y*-intercept of R^2-point and Q^2-point regression lines for each group were lower than 0.66 and −0.4, respectively.[33] In this work, the validity of the OPLS-DA model was confirmed by the permutation test.

Renal Injury Investigation. Urinary biochemical analysis was used to evaluate the renal injury of the mice. Since the urine volume of a single mouse was insufficient for the analysis, urine biochemical analysis was performed on eight mice in each group.

As shown in Table 2, when mice were exposed to dimethylarsenic acid for 4 weeks, urine protein in the 100 ppm group increased compared with the control group, while nitrite and cast indexes did not change significantly. When the exposure time reached 8 weeks, the urine leukocyte (LEU) index of the 100 ppm group increased significantly, suggesting that long-term exposure of organic arsenic may cause nephritis. However, 1 week after the suspension of arsenic exposure, urine LEU quickly returned to the normal level, and other changes of biochemical indicators showed no obvious difference compared with the control, indicating that the degree of nephritis induced by organic arsenic is low and may not cause substantial kidney damage or that the related damage could be repaired by the body after suspending arsenic.

Table 2. Results of the Urine Biochemical Test

	4W		8W		9W
test items	control	100 ppm	control	100 ppm	100 ppm
glucose (GLU)	-	-	-	-	-
protein (PRO)	1.0	3.0	1.0	1.0	0.5
bilirubin (BIL)	-	-	-	-	-
urobilinogen (URO)	normal	normal	normal	normal	normal
pH	8.5	8.5	8.5	8.5	8.5
occult blood (BLD)	-	-	-	-	-
ketone (KET)	1+	2+	1+	1+	1+
nitrite (NIT)	-	1+	-	2+	2+
leukocyte (LEU)	-	-	-	25	-
casts	1+	2+	2+	2+	2+

When the concentration of arsenic exposure reached 250 ppm, the mice in the experimental group developed oliguria problems, and the collected urine samples were not sufficient to support urinary biochemical analysis, suggesting a possible renal injury in mice. To further investigate the kidney damage caused by dimethylarsenic acid, renal pathological sections of mice were examined (in Supporting Information Figure S19), and it was found that when the concentration of dimethylarsenic acid reached 250 ppm, the mice showed glomerular mesangial hyperplasia. With the extension of arsenic exposure time, the mice showed glomerular cyst enlargement at the eighth week, suggesting kidney injury.

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CONCLUSIONS

In this study, 64 C57BL/6 female mice were chronically exposed to dimethylarsenic acid with different concentrations, and the changes of serum metabolites were detected by UPLC-MS/MS. The results showed that a total of 596 serum metabolites were identified, and 15 metabolites were found to be closely related to the exposure concentration and exposure time. Statistical analysis showed that organic arsenic intake had a significant effect on the overall metabolic level of mice, and the effects of long-term chronic dimethylarsenic acid on metabolism cannot be recovered in a short time. Although the total arsenic levels in the kidney and liver continually increased with the ingestion of dimethylarsenic acid, a large quantity of arsenic could be excreted in urine and feces. After discontinuing intake, the total arsenic content in the liver and kidney rapidly decreased to a level that is similar to the control. Sustained intake of a high concentration of dimethylarsenic acid could lead to mild nephritis in mice; thus, the potential health risks of organic arsenic should be taken into consideration seriously.

MATERIALS AND METHODS

Instruments, Chemicals, and Reagents. Serum samples were analyzed by a UPLC system (Shimadzu, Japan, LC-30AD) coupled with a TripleTOF mass spectrometer (AB Sciex, USA, 6600 hybrid Q-TOF). The total arsenic concentration in serum was investigated by ICP-MS (Thermo Fisher, USA, iCAP Q). DXL-XS-1 mouse metabolic cages (Lab Animal Technology Develop Co., Beijing, China) were used for urine and feces collection.

MS data and serum compounds were analyzed or identified by the Progenesis QI software (Waters, USA). The statistical analysis software was SIMCA 14.1 (MKS Umetrics).

Dimethylarsenic acid ($C_2H_7AsO_4{\cdot}3H_2O$) was purchased from Acros Organics (Belgium). Acetonitrile, methanol, nitric acid, and formic acid were purchased from Thermo Fisher Scientific Inc.

Animal Experiments. Sixty-four C57BL/6 female mice (18−22 g) were purchased from Charles River Experimental Animal Technology Co., Ltd. (Beijing, China). These mice were randomly assigned to experimental and control groups. Each experimental group included 24 arsenic-treated mice, and the control group included 16 nontreated mice. Mice were housed in polycarbonate cages. Each cage contained four C57BL/6 female mice. Those mice were kept in a stable environment (12 h light/dark cycle, 22 °C, 40−70% humidity) and fed with standard food. After 1 week of domestication, the arsenic-treated groups were exposed to 100 and 250 ppm arsenic in drinking water, and the control group was fed pure water.

After continuous feeding to the fourth and eighth week, eight mice from each experimental group and the control group were transferred to the metabolic cage (one mouse per cage), and feces and urine were collected for 24 h. During the sample collection, all mice were deprived of food but drank water normally. After sampling, isoflurane was used for general anesthesia of mice, and the mice serum was acquired from the eyes. Finally, mice liver and kidney were obtained.

After the eighth week, the last eight mice in each experimental group stopped feeding arsenic-containing water and were fed with ordinary pure water for 1 week. Then the mice were transferred to the metabolic cage (one mouse per cage), and urine and feces were collected for 24 h. During the sample collection, all mice were deprived of food but drank water normally. After sampling, all mice were anesthetized by isoflurane, and the serum was sampled from mice eyes. Finally, mice were dissected to obtain the liver and kidney.

The serum of mice was stored at −80 °C for subsequent detection and analysis. A part of the liver and kidney samples from each mouse was fixed in paraformaldehyde, and the other part was stored at −80 °C for ICP-MS testing. All animal experiments in this article were approved by the IACUC of the National Center for Biomedical Analysis (IACUC-DWZX-2020-763).

Serum Preparation. The treatment of serum samples was similar to our previous work.[33] First, to remove proteins in serum, 400 μL methanol/acetonitrile solution (1:1, v:v) was added into 100 μL serum. Second, the solution was vortexed for 30 s, incubated for 1 h at −20 °C, and then centrifuged at 15,000 rpm for 15 min. Third, the supernatant was collected and dried in a SpeedVac (FreeZone, Labconco, USA). Finally, a 50% methanol solution that contains 1‰ formic acid was added into the dry samples for further analysis.

ICP-MS Analysis. For urine, samples were diluted with 2% nitric acid by the gravimetric method and filtered through a 0.45 μm membrane. Liver, kidney, and feces samples were accurately weighed to three decimal places, digested by microwave with 10 mL concentrated nitric acid, and diluted with the appropriate amount of deionized water. The total arsenic content was identified by ICP-MS, the power was 1500 W, and the pump speed was 50 rpm. The cooling air flow of ICP-MS was 12 L/min, and the auxiliary and atomized gas flows were both 1.5 L/min.

UPLC-MS/MS Analysis. LC-30AD UPLC coupled with a hybrid Q-TOF MS was used to detect serum samples. To perform the compounds' separation effectively, an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm, Waters Corporation, Milford, USA and Ireland) and an ACQUITY UPLC BEH amide column (2.1 × 100 mm, 1.7 μm, Waters Corporation, Milford, USA) were both used. For the T3 column, the aqueous phase of the mobile phases was (A) water with 0.1% formic acid (FA), and the organic phase was (B) acetonitrile also with 0.1% FA. The gradient was set as follows: 0−1.5 min, 1% B; 1.5−13.0 min, 1−99% B; 13.0−16.5 min, 99% B; and 16.6−20.0 min, 1% B.[33] For the BEH amide column, the organic phase of the mobile phases was (C) 10% water/acetonitrile with 0.1% FA and 10 mM ammonium acetate, and the water phase was (D) water with 0.1% FA and 10 mM ammonium acetate. The gradient was set as follows: 0−1.0 min, 5% D; 1.0−12.0 min, 5%−55% D; 12.1−15.0 min, 55% D; and 15.1−20.0 min, 5% D.[33] The temperature of both T3 and amide columns was maintained at 40 °C, and the rate of mobile phases was 0.3 mL/min. The temperature of the sample chamber was maintained at 10 °C, and the injection volume was 3 μL. To ensure the accuracy of sample analysis, calibration was assigned to every nine samples during the whole test (the related methods and results are in Figure S1 of the Supporting Information).

The TripleTOF 6600 MS was operated in data dependent analysis (DDA) mode with positive or negative ion modes. The m/z scan range of both precursor and fragment ion was set as 50−1250 Da. The accumulation time of MS1 and MS2 was 150 and 30 ms, respectively. The CE voltage was in series set at 15, 30, and 45 V. Gas 1 and gas 2 were 50 Pa, and curtain gas was 35 Pa. The ion spray voltage floating (ISVF) of

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positive ion mode was 5000 V (ESI+), and the ISVF was −4500 V in negative ion mode. The desolvation gas temperature and source temperature were both set at 500 °C. Other parameters of testing are collision energy of 30 ± 15 eV and declustering potential (DP) of ±80 V.

Identification of Metabolites in Serum. Progenesis QI was used for MS/MS data analysis, including peak picking, alignment, and integration. The identification process of metabolites included three steps. First, we searched the METLIN and HMDB libraries with raw data. The version of the METLIN library we used was 1.0.6499.51447, and the precursor and fragment search tolerances were 10 and 20 ppm. For HMDB, the precursor and fragment search tolerances were both 12 ppm, and the retention time tolerance was within 0.5 min. Second, for each compound, we selected its possible identifications by setting up a standard that the score ≥ 50, q value < 0.05, and ANOVA (p) < 0.05. Finally, compounds were identified manually according to the MS/MS matching situation. The metabolites' differences were analyzed by a two-tailed Welch's t test with false discovery rate (FDR) correction. The significantly changed metabolites (ANOVA (p) < 0.05, q value < 0.05, max fold change > 2, and VIP > 1) were considered to be the differential metabolites caused by arsenic exposure.

Statistical Analysis. To distinguish differences of serum samples that were exposed to the different concentrations of arsenic, orthogonal partial least squares discrimination analysis (OPLS-DA) and principal component analysis (PCA) were used. The reliability of the statistical model was verified by the permutation test. SIMCA 14.1 was used for all statistical analyses.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c03806.

> Quality control of the UPLC-MS/MS test; the information of differential metabolites and the corresponding changes after arsenic exposure; permutation test results for groups; renal pathological section results after arsenic exposure; serum metabolites' chromatograms with different approaches; and examples of the identified metabolite compared with standard (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Fangting Dong − *National Center for Biomedical Analysis, Beijing 100850, China*; Phone: 86-10-66931526; Email: dft@proteomics.cn

Kun He − *National Center for Biomedical Analysis, Beijing 100850, China*; Phone: 86-10-66930306; Email: hk@proteomics.cn

Na Wang − *National Center for Biomedical Analysis, Beijing 100850, China*; orcid.org/0000-0002-5678-1323; Phone: 86-10-66930306; Email: nwang@ncba.ac.cn

Authors

Nan Jing − *National Center for Biomedical Analysis, Beijing 100850, China*; orcid.org/0000-0001-9837-5705

Jing Peng − *National Center for Biomedical Analysis, Beijing 100850, China*

Xin Yang − *National Center for Biomedical Analysis, Beijing 100850, China*

Xinzheng Wang − *National Center for Biomedical Analysis, Beijing 100850, China*

Qian Liu − *National Center for Biomedical Analysis, Beijing 100850, China*

Hongli Wang − *National Center for Biomedical Analysis, Beijing 100850, China*

Weihua Li − *National Center for Biomedical Analysis, Beijing 100850, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c03806

Notes

The authors declare no competing financial interest.

[&]N.J. and J.P. are co-first authors.

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