Arsenic Exposure Perturbs the Gut Microbiome and Its Metabolic Profile in Mice: An Integrated Metagenomics and Metabolomics Analysis

Citation


As Published

http://dx.doi.org/10.1289/ehp.1307429

Publisher

Environmental Health Perspectives

Version

Final published version

Accessed

Sat Dec 08 20:44:44 EST 2018

Citable Link

http://hdl.handle.net/1721.1/99492

Terms of Use

Article is made available in accordance with the publisher’s policy and may be subject to US copyright law. Please refer to the publisher’s site for terms of use.
Arsenic Exposure Perturbs the Gut Microbiome and Its Metabolic Profile in Mice: An Integrated Metagenomics and Metabolomics Analysis

Kun Lu,1 Ryan Phillip Abo,2 Katherine Ann Schlieper,3 Michelle E. Graffam,3 Stuart Levine,1,2 John S. Wishnok,1 James A. Swenberg,4 Steven R. Tannenbaum,1,5 and James G. Fox1,3

1Department of Biological Engineering, 2Department of Biology, and 3Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA; 4Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; 5Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

BACKGROUND: The human intestine is host to an enormously complex, diverse, and vast microbial community—the gut microbiota. The gut microbiota plays a profound role in metabolic processing, energy production, immune and cognitive development, epithelial homeostasis, and so forth. However, the composition and diversity of the gut microbiome can be readily affected by external factors, which raises the possibility that exposure to toxic environmental chemicals leads to gut microbiome alteration, or dysbiosis. Arsenic exposure affects large human populations worldwide and has been linked to a number of diseases, including cancer, diabetes, and cardiovascular disorders.

OBJECTIVES: We investigated the impact of arsenic exposure on the gut microbiome composition and its metabolic profiles.

METHODS: We used an integrated approach combining 16S rRNA gene sequencing and mass spectrometry–based metabolomics profiling to examine the functional impact of arsenic exposure on the gut microbiome.

RESULTS: 16S rRNA gene sequencing revealed that arsenic significantly perturbed the gut microbiome composition in C57BL/6 mice after exposure to 10 ppm arsenic for 4 weeks in drinking water. Moreover, metabolomics profiling revealed a concurrent effect, with a number of gut microflora–related metabolites being perturbed in multiple biological matrices.

CONCLUSIONS: Arsenic exposure not only alters the gut microbiome community at the abundance level but also substantially disturbs its metabolic profiles at the function level. These findings may provide novel insights regarding perturbations of the gut microbiome and its functions as a potential new mechanism by which arsenic exposure leads to or exacerbates human diseases.


Introduction

The human body is host to 100 trillion gut microbes, approximately 10 times more than all human cells (Ley et al. 2006). Ley et al. (2006) estimated that the approximately 500–1,000 species residing in the human gut encode 100-fold more unique genes than the human genome. The gut microbiota has important functions in metabolic processing, energy production, immune cell development, food digestion, epithelial homeostasis, and so forth (Young et al. 2008). Mounting evidence indicates that dysregulated gut microflora contributes in a significant way to a variety of diseases, including diabetes, obesity, cardiovascular diseases, allergies, inflammatory bowel disease, and so on (Ley et al. 2005; Qin et al. 2012; Wang et al. 2011). For example, obese individuals exhibit a remarkable reduction in the abundance of Bacteroidetes and a relative increase in Firmicutes compared with lean individuals (Turnbaugh et al. 2006). Likewise, a metagenome-wide association study revealed that beneficial butyrate-producing bacteria are less abundant and that opportunistic pathogens are more abundant in individuals with diabetes than in healthy individuals (Qin et al. 2012). The gut microbiome evolves through several transitions during the first years of life and thereafter remains relatively constant if no significant perturbations occur. However, the composition of the gut microbiome is highly diverse, and this diversity can be readily affected by external factors such as environment, diet, bacterial/viral infection, and antibiotics. This raises the possibility that exposure to toxic environmental chemicals leads to gut microbiome alteration (dysbiosis) as a mechanism by which environmental agents exert their detrimental effects on human health.

Arsenic exposure affects large human populations worldwide, with contamination of drinking water by geological sources of inorganic arsenic being the primary route of exposure. Hundreds of millions of people around the world, especially in South and East Asia, drink water with arsenic levels that far exceed the 10-μg/L guideline established or accepted by the World Health Organization (WHO) and the Environmental Protection Agency (EPA) (Hou et al. 2012; Hughes et al. 2011; Ren et al. 2011; Smeester et al. 2011). Numerous studies have reported interactions between the gut microbiome and environmental chemicals such as arsenic, mercury, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls (Choi et al. 2013; Liebert et al. 1997; Pinyayev et al. 2011; Van de Wiele et al. 2005, 2010).

The gut microbiome has profound roles in modulating host metabolism. For example, nondigestible carbohydrates are degraded via
fermentation by the gut bacteria to yield energy for microbial growth and microbial end products that act as energy substrates, inflammation modulators, and signaling molecules (Holmes et al. 2011). Therefore, the reach of the gut microbiome on host metabolism extends well beyond local effects in the gut to diverse remote organ systems, such as liver, brain, adipose, and muscle (Claus et al. 2011; Diaz et al. 2011). Accumulating evidence indicates that metabolic perturbations associated with changes in the gut microbiome composition are important risk factors for developing diseases (Jones et al. 2008; Wang et al. 2011). For example, gut microflora–generated trimethylamine N-oxide from dietary choline and carnitine has been strongly associated with atherosclerosis in animal models and clinic cohorts (Wang et al. 2011). Therefore, it is of particular interest to probe the gut microbiome–related metabolic changes associated with an arsenic-perturbed gut microbiota community. In this aspect, mass spectrometry–based metabolomics profiling is highly attractive because of its high sensitivity, ability to detect molecules with diverse structures, wide dynamic range, quantitative capability, and ease of interfacing with other separation techniques such as liquid chromatography (Lu et al. 2012).

In the present study, we applied an integrated approach combining 16S rRNA gene sequencing and liquid chromatography–mass spectrometry (LC-MS) metabolomics to analyze the effects of arsenic exposure on the gut microbiome and its metabolite profiles. Metagenomics sequencing revealed that arsenic exposure significantly perturbed the gut microbiome composition in C57BL/6 mice. Our nontargeted metabolomics profiling revealed a notable effect of arsenic exposure in these mice, with diverse perturbed metabolites being correlated with gut microbiome changes.

**Materials and Methods**

**Animals and exposure.** Specific-pathogen-free C57BL/6 female mice (~ 6 weeks of age) were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were provided pelleted rodent diet (ProLab 3000; Purina Mills, Gray Summit, MO) and filtered water *ad libitum* and were maintained in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. A total of 20 mice (10 mice/group, body weight = 20 ± 3 g) were housed in static microisocorator cages (5 mice/cage) on heat-treated hardwood bedding, under environmental conditions of 22°C, 40–70% humidity, and a 12:12 hr light:dark cycle. All experiments were approved by the Massachusetts Institute of Technology Committee on Animal Care. The animals were treated humanely and with regard for the alleviation of suffering. Inorganic arsenic (arsenic, 10 ppm) was administered to mice (~ 8 weeks of age) as sodium arsenite (Fisher Scientific, Waltham, MA) in drinking water for 4 weeks. Freshly prepared arsenic-containing water (10 ppm) was provided to mice every Monday and Thursday. Control mice received water alone.

**Analysis of 16S rRNA sequencing data.** The raw mate-paired fastq files were quality-filtered, demultiplexed, and analyzed using Quantitative Insights into Microbial Ecology (QIIME) software (http://qiime.org). For quality filtering, the default parameters of QIIME were maintained in which reads with a minimum Phred quality score of < 20, containing ambiguous base calls and < 113 bp of consecutive high-quality base calls, were discarded. In addition, reads with three consecutive low-quality bases were truncated. The samples sequenced were demultiplexed using 8-bp barcodes, allowing 1.5 errors in the barcode. UCLUST software (http://www.drive5.com/uclust) was used to choose the operational taxonomic units (OTUs) with a threshold of 97% sequence similarity. A representative set of sequences from each OTU was selected for taxonomic identification of each OTU using the Ribosomal Database Project (RDP) classifier (http://rdp.cme.msu.edu). The Greengenes OTUs (4feb2011 build) reference sequences (97% sequence similarity) were used as the training sequences for RDP classifier. A 0.80 confidence threshold was used for taxonomic assignment. The taxonomic assignment of 16S rRNA sequencing data could be achieved at different levels, including phylum, class, order, family and genus. Our analyses were typically conducted at the family level because of the higher confidence in the assignment of taxa based on the sequencing reads; therefore, a significant change at the family level may reflect changes of multiple gut bacteria at genus and species levels. The sequencing data have been deposited in the MG-RAST (metagenomics Rapid Annotation using Subsystem Technology) server (http://metagenomics.anl.gov/).

**Sample processing for metabolomics.** One day before euthanasia of the mice, we collected urine samples using a metabolic cage with dry ice placed around the urine collection vessel to prevent oxidation or degradation of metabolites during the collection period (approximately 16 hr). Fecal pellets were also collected from individual animals. We collected plasma samples during necropsy. Metabolites were isolated from urine using methanol as described previously (Lu et al. 2012). Cold methanol (80 μL) was added to 20 μL urine or plasma. After vortexing for 1 min, the samples were incubated at 4°C for 20 min and then centrifuged for 10 min at 12,000 rpm. The supernatant was collected, dried in a SpeedVac (Savant SCI10A; Thermo Electron, Waltham, MA), and then resuspended in 30 μL 98:2 water:acetonitrile for MS analysis. Metabolite extraction from fecal pellets was conducted in a similar manner. Fecal pellets (25 mg) were dissolved in 400 μL cold methanol solution (50:50 methanol:water), followed by vortexing at maximum speed for 10 min using a flat-bed vortex (MO BIO Laboratories). The supernatant was centrifuged for 10 min at 12,000 rpm, dried in a SpeedVac, and then resuspended in 30 μL 98:2 water:acetonitrile for metabolomics profiling.

**Metabolomics profiling.** We performed LC-MS analyses on a quadrupole-time-of-flight (Q-TOF) 6510 mass spectrometer (Agilent Technologies, Santa Clara, CA) with an electrospray ionization source. The mass spectrometer was interfaced with an Agilent 1200 HPLC system. The Q-TOF was calibrated daily using the standard tuning solution from Agilent Technologies. The typical mass accuracy of the Q-TOF was < 10 ppm. Metabolites were analyzed in the positive mode only over a range of 80–1000 m/z using a C18 T3 reverse-phase column from Waters Corporation (Milford, MA) because of the
higher numbers of detected molecular features (i.e., metabolites), as demonstrated previously (Lu et al. 2012). Metabolomics profiling data were processed as described previously (Lu et al. 2012). MS/MS was generated on the Q-TOF to confirm the identity of perturbed metabolites. The metabolomics data were submitted to the XCMS Online server (https://xcmsonline.scripps.edu/).

Data processing of metabolomics data. Data acquired in Agilent .d format were converted to mzXML using MassHunter Workstation software from Agilent Technologies. Data were filtered by intensity, and only signals with intensities > 1,000 were considered. The converted data were processed using XCMS Online for peak picking, alignment, integration, and extraction of the peak intensities. To profile individual metabolite differences between control and arsenic-treatment groups, a two-tailed Welch’s t-test was used (p < 0.05). The exact masses of molecular features with significant changes were searched against the Human Metabolome Database (HMDB; http://www.hmdb.ca/), METLIN (https://metlin.scripps.edu), and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (http://www.genome.jp/kegg/pathway.html) with a 10 ppm mass accuracy threshold. The matched exact masses were stored and used for the generation of MS/MS data to identify the metabolites.

Statistical analysis of data. Principal component analysis (PCA) was performed to examine intrinsic clusters of metabolomics data. A 95% confidence interval (CI) was used as the threshold to identify potential outliers in all samples. In addition, heat maps were generated using a hierarchical clustering algorithm to visualize the metabolite difference within the data set. Principal coordinate analysis (PCoA) was used to compare the gut microbiome profiles between the control and treatment. The difference in the gut microbiome composition was further assessed using a nonparametric test via Metastats software (http://metastats.cbcb.umd.edu/) as described previously (White et al. 2009). The correlation matrix between the gut microflora–related metabolites and gut bacterial species was generated using Pearson’s correlation coefficient.

Results

Workflow to probe functional changes of the gut microbiome. The experimental workflow combined 16S rRNA gene sequencing and metabolite profiling to examine the impact of arsenic exposure on the gut microbiome and its metabolic profiles (see Supplemental Material, Figure S1). Briefly, DNA was isolated from fecal pellets, amplified by polymerase chain reaction using 16S rRNA–specific primers, followed by 150 × 150 bp paired-end sequencing using the Illumina MiSeq platform. The resultant sequencing reads were processed using the QIIME and Metastats software packages to reveal exposure-induced gut microbiome changes. For metabolomics analysis, metabolites from fecal pellets, urine, and plasma were extracted and analyzed by Q-TOF. Molecular features (i.e., all signals associated with a given analyte) were further processed and statistically analyzed with XCMS software to profile metabolites with significant changes (1.5-fold change, p < 0.05) between the control and arsenic-treated animals. The resultant peak list with exact masses was searched against metabolite databases, including HMDB and METLIN. Next, the matched exact masses and associated retention times were used to generate MS/MS spectra to confirm the metabolite identities, followed by metabolic pathway or function analysis with the KEGG and HMDB databases. Finally, correlations between the gut microbiome changes and shifted metabolome were examined to establish the functional impacts of arsenic exposure on the gut microbiome.

Arsenic-induced gut microbiome changes. Figure 1A shows the identified gut bacteria assigned at the family level from 16S rRNA sequencing reads, with each color representing an individual bacterial family (see also Supplemental Material, Figure S2A and Table S1). In terms of the assignment at the phylum level, Firmicutes (52.79%) and Bacteroidetes (41.57%) were predominant in the gut bacteria of mice, followed by Tenericutes (3%), Actinobacteria (0.18%), Cyanobacteria (0.023%), and Proteobacteria (0.0042%), with 2.41% sequences unmatched with the database (see Supplemental Material, Figure S2B). Our observations and assignments of gut bacteria at phylum level are consistent with previous reports that the gut microbiome consists of only several phyla (Turnbaugh et al. 2006). The taxonomic assignments and fold changes of gut bacterial components that were significantly changed (p < 0.05) are listed in Figure 1B. The difference in the gut microbiome patterns arising from arsenic is readily differentiated using multivariate statistical analysis as shown by the PCoA plot (Figure 1C). The control and treated animals clustered in their own groups, as shown in Figure 1D. In addition, control mice clustered into two subgroups in the PCoA plot (Figure 1C) and UPGMA hierarchical clustering analysis (Figure 1D), which was attributed to individual variations of gut microbiome profiles, as illustrated in Figure 1A.

Correlation between the gut microbiome and metabolites. To explore the functional correlation between the gut microbiome changes and metabolite perturbations, a correlation matrix was generated by calculating the Pearson’s correlation coefficient (Figure 3A). Clear correlations could be identified between the perturbed gut microbiome and altered metabolite profiles (p > 0.5 or < –0.5, p < 0.05). Figure 3B lists several typical gut microflora–related metabolites that are highly correlated with specific gut bacteria to demonstrate the functional correlation between the gut microbiome and metabolites. For example, indolelactic acid, which decreased 11.6-fold in arsenic-treated mice, positively correlates with the B9 family (p.Tenericutes:f.Erysipelotrichiaceae), but negatively correlates with the B8 family (p.Firmicutes:f.Clostridiales Family XIII Incertae Sedis). Likewise, daidzein positively correlates with the B4 (p.Firmicutes:f_Other) and B7 (p.Firmicutes:f_Clostridiales) families, respectively. Phenylpyruvic acid, indole-3-carbinol, and glycocholic acid positively correlate with the B2 (p.Cyanobacteria:f_unassigned), B5 (p.Firmicutes:f_unassigned), and B7 (p.Firmicutes:f_Clostridiales) families, respectively, whereas dihydrodaidzein negatively correlates with the B8 family.
Arsenic perturbs the gut microbiome and its function

Discussion

We used high-throughput 16S rRNA gene sequencing and metabolomics profiling to study the impact of arsenic exposure on the gut microbiome and its metabolic profiles. The data clearly show that arsenic exposure induced a significant change in the gut microbiome composition of mice. In addition, these perturbed gut bacteria were strongly associated with changes of a number of gut microbiota–related metabolites, indicating that arsenic exposure not only disturbs gut bacteria at the abundance level but also substantially alters the metabolomic profile of the gut microbiome, resulting in the disturbance of host metabolism after arsenic exposure. These findings may provide mechanistic insights regarding perturbations of the gut microbiome as a new mechanism of environmental chemical–induced human disease.

Accumulating evidence suggests that metabolic changes associated with gut microbiome perturbations are important risk factors for inducing abnormal tissue functions resulting in diseases such as obesity, insulin resistance, and cardiovascular disease (Jones et al. 2008; Turnbaugh et al. 2006; Wang et al. 2011). The gut microbiome could directly change its metabolic capacity and affect intestinal function locally through microbial products. For example, the gut microbiota has a strong effect on energy homeostasis in the colon, attributed to the use of butyrate produced by gut bacteria as the primary energy source for colonocytes (Donohoe et al. 2011). Likewise, altered gut microbiota can also trigger systemic effects on host metabolism in remote tissues such as liver, brain, adipose, and muscle because the products of microbial metabolism could serve as signaling molecules or act in conjunction with the host on the metabolism of diverse chemicals to affect individual susceptibility to different diseases. For instance, trimethylamine N-oxide, a gut microbiota–generated metabolite, has been identified to be strongly associated with atherosclerosis in a large clinical cohort (Wang et al. 2011). Dietary choline–enhanced atherosclerosis in athero-sclerosis-prone mice, further highlighting the role of perturbations of gut microflora–generated metabolites in disease development.

The gut microbiome plays a key role in the energy metabolism of the host. Nondigestible carbohydrates are degraded via fermentation in the colon by the gut microbiota to yield

![Figure 1](image-url)

**Figure 1.** (A) The gut microbiome composition profiles at the family level in control and arsenic-treated mice revealed by 16S rRNA sequencing (each color represents one bacterial family). (B) The fold changes and taxa assignments of significantly perturbed gut bacteria in arsenic-treated mice compared with controls [Abbreviations: c, class; f, family; o, order; p, phylum; and family abbreviations: B1 (Other;Other;Other); B2 (p_Cyanobacteria;c_Cyanobacteria;o_Streptophyta;f_unassigned); B3 (p_Firmicutes;c_Bacilli;o_Bacillales;Other); B4 (p_Firmicutes;c_Clostridia;Other;Other); B5 (p_Firmicutes;c_Clostridia;o_Clostridiales;f_unassigned); B6 (p_Firmicutes;c_Clostridia;o_Clostridiales;f_Catabacteriaceae); B7 (p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae); B8 (p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiales Family XIII Incertae Sedis); B9 (p_Tenericutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichiaceae). (C) The gut microbiome patterns of control and arsenic-treated mice differentiated by principal coordinate analysis. (D) Hierarchical clustering analysis by UPGMA indicates that controls and arsenic-treated mice clustered in their own groups, with the UPGMA distance tree constructed at a distance of 0.03.
energy for the host and metabolic end products such as short-chain fatty acids. Previous studies have documented that the imbalanced gut microbiome may be associated with human diseases such as obesity and diabetes (Qin et al. 2012; Turnbaugh et al. 2006). For example, Turnbaugh et al. (2006) found that obesity was associated with a large shift in the relative abundance of the specific taxa present, with a statistically significant reduction in Bacteroidetes and a significantly greater proportion of Firmicutes in obese mice (Turnbaugh et al. 2006). Likewise, transplantation of the cecal microbiota from obese mice fed on high-fat diets into germ-free recipients increases adiposity significantly more than transplantation of microbiota from lean mice (Turnbaugh et al. 2006), further highlighting the role of the gut microbiome in affecting the efficiency of harvesting energy and defining obese/lean phenotypes. Cho et al. (2012) also demonstrated the influence of gut microbiome perturbations on tissue adiposity of the host by delineating the effect of antibiotics use in early life. These authors discovered substantial taxonomic changes in the gut microbiome, changes in primary genes responsible for the metabolism of carbohydrates to short-chain fatty acids, and marked alterations in hepatic metabolism of lipids and cholesterol. In the present study, bacteria in the family of Bacteroidetes were not increased by arsenic exposure, whereas four Firmicutes families were significantly decreased. Arsenic is not an obesogen, but rather has anti-obesogenic properties, although the mechanism remains unknown (Paul et al. 2011). Excess accumulation of white adipose tissue in obesity is a risk factor for insulin resistance and the development of diabetes because white adipose tissue regulates energy balance, lipid, and glucose homeostasis, and release a variety of signaling factors that affect insulin sensitivity and inflammation (Rosen and Spiegelman 2006). However, defects in adipogenesis in adipose tissue can also lead to the development of insulin resistance and diabetes (Hou et al. 2012; Vigouroux et al. 2011). We identified several decreased Firmicutes families following

Figure 2. (A) Arsenic exposure perturbed the metabolic profile of fecal samples of mice, with 370 molecular features being significantly changed compared with controls (fold change > 1.5 and \( p < 0.05 \)). (B) Controls were separated from arsenic-treated mice in metabolite profiles by PCA. (C) Hierarchical clustering heat map constructed using molecular features with 1.5-fold changes (\( p < 0.05 \)) shows a consistent clustering pattern within individual groups.
Arsenic perturbs the gut microbiome and its function

**Figure 3.** (A) Correlation plot showing the functional correlation between perturbed gut bacteria families and altered fecal metabolites. (B) Scatter plots illustrating statistical association between the relative abundance of altered gut bacteria families and the mass spectrum intensities of some typical gut microflora–related metabolites, including indole-containing compounds, isofavone metabolites, and bile acids.
arsenic exposure, and a number of Firmicutes species, such as *Enterobacterium*, *Faecalibacterium*, and *Roseburia*, are known to be butyrate pro-
ducers (Tremaroli and Backhed 2012). Thus,
an arsenic-altered gut microbiome may affect
energy harvesting, short-chain fatty acid pro-
duction, and adipogenesis. Another example
to support arsenic exposure impairment of
energy metabolism stems from the observa-
tion that fatty acid carnitines were significantly
reduced in the urine of arsenic-treated mice (see
Supplemental Material, Table S3). Fatty acid
carnitines are used to transport fatty acids into
mitochondria for fatty acid oxidation to gener-
ate metabolic energy; this process may be used
to compensate for insufficient energy harvest
due to disturbed gut microbiota after arsenic
exposure. In agreement with this potential
mechanism, germ-free mice and lean individu-
als also exhibit increased fatty acid oxidation and
decreased lipogenesis (Backhed et al. 2007).

Indole-containing metabolites were signifi-
cantly altered in fecal and urine samples after
arsenic exposure in mice (see Supplemental
Material, Figure S5A). The presence of the
altered indole-containing metabolites is highly
correlated with perturbed gut bacterial families.

For example, 3-indolepropionic acid may
serve as a specific indicator of imbalanced gut
bacteria because a gut bacterial metabolic pro-
cess is needed to synthesize this compound.

A recent study identified *Clostridium sporogenes*
as the only species, among 24 intestinal
microflora tested, to produce 3-indolepropionic
acid (Wikoff et al. 2009). Thus, increased
excretion of 3-indolepropionic acid in urine
(+1.8-fold) may indicate that the abundance of
*Clostridium sporogenes* or species with simi-
lar functions have increased. In fact, among
six significantly perturbed Firmicutes families,
one Firmicutes family significantly increased
in abundance after arsenic exposure—
*p_Firmicutes\_Clostridiales\_family XIII Incertae Sedis, B8
in Figure 1B, with a +2.0-fold change. In par-
ticular, *Clostridium sporogenes* and this increased
gut bacteria family are classified into the same
order of Clostridiales. The formation of indole-
containing metabolites likely occurs via the
generation of indole by the intestinal bacte-
rria that produce the enzymes to catalyze the
conversion of tryptophan to indole, followed
by further enzymatic processing. For example,
absorbed indole could be converted to indoxyl
in the liver, and then sulfated to allow for uri-
nary excretion. An increased amount of indoxyl
in urine (+2.9-fold in arsenic-treated mice) sug-
gests an increased indole production by gut
bacteria, which also supports the production of
increased amounts of other urine indole-related
metabolites such as IPA, indole-3-carboxylic
acid, and indoleacrylic acid. However, increased
urinary excretion of indoxyl may also suggest
that phase II/III biotransformations—including
hydroxylation, sulfation, and glucuronida-
tion—were changed during exposure. In an ele-
gant study, Wikoff et al. (2009) demonstrated a
broad phase II metabolic response of the host
to metabolites generated by the microbiome, as
evidenced by the exclusive presence of numer-
sous sulfated, glycine-conjugated, and glucuro-
nide addsucts in the serum of mice with normal
gut microbiota compared with germ-free mice.

In the present study, we also found several gluc-
uronide metabolites to be significantly reduced
in urine (see Supplemental Material, Table S3),
possibly reflecting an impaired glucuronidation
capacity affected by a perturbed gut micro-
bio as a result of arsenic exposure.

Isoflavone metabolites are also sensitive to
gut microbiome changes (see Supplemental
Material, Figure S5B). Previously, we discov-
ered that daidzein was significantly increased
in the serum of Rag2−/− mice infected with
*Helicobacter hepaticus* (Lu et al. 2012). The
increased amount of daidzein was attrib-
uted to an altered gut microflora induced by
*Helicobacter hepaticus* infection consist-
tent with previous studies that demonstrated
that *Helicobacter* spp. infections perturb
the gut microflora in mice (Whary et al. 2006).

Daidzein is converted by anero-
bacteria in the large intestine to several
metabolites—including dihydrodaidzein,
O-desmethylangolensin, and equol—depend-
ing on the gut bacterial composition of the
host. In the present study, we detected sig-
nificantly decreased daidzein and dihydro-
daidzein but increased O-desmethylangolensin
in feces, suggesting that the formation of
O-desmethylangolensin is favored in
arsenic-treated mice. Of particular interest,
O-desmethylangolensin is positively corre-
lated with a Firmicutes family (p_Firmicutes;
c_Bacillio\_Bacillales;Other, +9.2-fold in
arsenic-treated mice). Hur et al. (2000) sug-
gested that several candidate bacteria are par-
icipating in daidzein metabolism, although
the intestinal bacteria responsible for daid-
zein metabolism in humans have not been
unambiguously identified. Nevertheless,
altered isoflavone metabolites may be used as
biindicators to probe changes in the gut
microbiome composition arising from diverse
environmental factors such as bacterial
infections (Lu et al. 2012) and toxic chemicals.

Bile acids and intermediates were signifi-
cantly perturbed in arsenic-treated mice (see
Supplemental Material, Table S2). Bile acids
are cholesterol derivatives synthesized in the
liver, and they undergo extensive enterohepa-
tic recycling and gut microbial modification,
including deconjugation and dehydroxylation.

Bile acids not only have important functions
in solubilizing cholesterol and facilitating the
absorption of cholesterol, fat-soluble vitamins,
and lipids from the gut, but they also act as
signaling molecules to regulate metabolic
homeostasis by activating diverse nuclear recep-
tors (Nguyen and Boucared 2008). In the pre-
ent study, 7-α-hydroxy-3-oxo-4-cholestenoic
acid, and its degradation product were excreted
in arsenic-treated mice compared with con-
trols. 7-α-hydroxy-3-oxo-4-cholestenol is
involved in the biosynthesis of primary bile
acids (Kanehsa et al. 2012), and an increased
excretion of 7-α-hydroxy-3-oxo-4-cholesterol
and its degradation product may impair the
synthesis and enterohepatic recycling of bile acids. Likewise, lev-
els of glycocholic acid decreased (–2.3-fold)
in fecal samples of arsenic-treated mice. These
altered bile acid species indicate that arsenic
exposure affects the homeostasis of bile acids.

The underlying mechanisms remain elusive;
however, arsenic-induced gut microbiome
perturbations may play a role in this process.

Previous studies demonstrated a large effect
of the gut microflora on primary and secondary
bile acid profiles in tissues of antibiotic-treated
rats (e.g., Swann et al. 2011). Moreover, speci-
cific microbial bile acid co-metabolites present
in peripheral tissues and pathway changes
regulated by farnesoid X receptor indicate a
broad signaling role for bile acids and high-
light the symbiotic microbial influences in
bile acid homeostasis in the host (Swann et al.
2011). Moreover, the role of gut bacteria in
the regulation of bile acids has raised the ques-
tion of whether bile acids modulated by the
gut microbiome are associated with regulation
of the host immune system (Brestoff and Artis
2013). In particular, it has been reported that
chronic arsenic exposure significantly com-
promises the immune response to influenza A
infection (Kozul et al. 2009). Clearly, further
research is needed to shed light on the role of
the gut microbiome and its associated metabo-
lites, including bile acids, in arsenic-induced
impaired immune responses and other diseases.

In the present study, we demonstrated that
arsenic exposure altered the gut microbiome
and associated metabolomic profiles. However,
future studies are warranted to delineate the
mechanistic basis of these perturbations. As
shown by the correlation analysis between
the gut bacteria and metabolites, arsenic exposure
can induce changes in the gut microbiome in
terms of abundance, resulting in a shifted
metabolome in the gut microbiome. Of equal
importance, arsenic exposure may also cause an
altered metabolome by affecting the physiology
of the gut bacteria without changing the spe-
cies and abundance. Therefore, the changes in
the metabolic profiles of the gut microbiome
may not entirely depend on shifts in the spec-
trum of microbes revealed by 16S rRNA gene
sequencing. Such metabolic changes could be
Arsenic perturbs the gut microbiome and its function

identified that some gut bacteria families were highly correlated with altered gut microflora–related metabolites. Taken together, these data indicate that arsenic exposure not only perturbs the gut microbiome at the abundance level but that it also alters metabolic profiles of the gut microbiome, supporting the hypothesis that perturbations of the gut microbiome may serve as a new mechanism by which arsenic exposure leads to or exacerbates human diseases. Furthermore, these modulated gut microflora–related metabolites may be potential biomarkers useful for probing the functional impacts of arsenic and other diverse environmental chemicals on the gut microbiome.

Reference


