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Nutritional status affects the bioaccessibility and speciation of arsenic from soils in a simulator of the human intestinal microbial ecosystem

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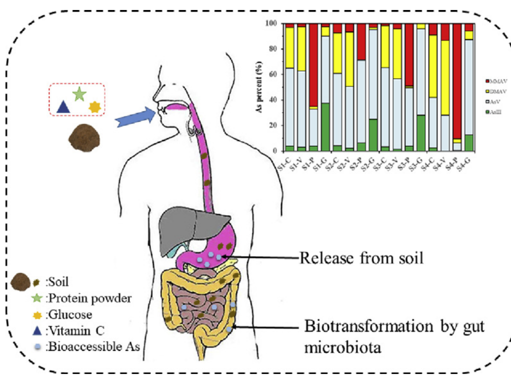
HIGHLIGHTS

- The effects of four nutritional states on arsenic bioaccessibility were different.
- In the colon phase, As bioaccessibility was observably enhanced by protein powder.
- The order of As methylation percentages was protein powder > vitamin C > fasted state > glucose (except S2).
- Large amounts of MMA^V were observed with the addition of protein powder in the colon digest.
- Glucose enhanced the reduction of As (V) in the colon digest.

GRAPHICAL ABSTRACT



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ABSTRACT

Arsenic (As) is a highly toxic contaminant in food and soil. In this study, we investigated the effects of four nutritional states (including a fed state with vitamin C, a fed state with protein powder, a fed state with glucose and a fasted state) on the variability of soil As bioaccessibility and biotransformation using the physiologically based extraction test (PBET) combined with a simulator of the human intestinal microbial ecosystem model (SHIME). The results indicated that the vitamin C and protein powder increased As bioaccessibility in gastric digests. In the colon phase, As bioaccessibility was observably enhanced by protein powder, and it varied under the vitamin C and glucose conditions. Additionally, the order of As methylation percentages in the four nutritional states was protein powder > vitamin C > fasted state > glucose (except S2); As bioaccessibility increased 1.3–13.7% and 15.8–35.4% in treatments of the vitamin C and protein powder, respectively. Meanwhile, large amounts of monomethylarsonic acid (MMA^V) were observed in the colon digest in the protein powder condition. In contrast, As methylation was significantly decreased with the addition of glucose, with a decline of 25.9–45.5%. Additionally, glucose enhanced the reduction of As(V). Therefore, nutritional status is a crucial parameter for the prediction of bioaccessibility and speciation of As when assessing health risks from As following oral exposure.

1. Introduction

Arsenic (As) is a prevalent environmental contaminant. Chronic exposure to As can lead to a variety of cancers and noncancerous diseases, such as skin and internal cancers and cardiovascular effects (Jomova

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et al., 2011). The total level or speciation of As in the immediate matrix (such as water, food, and soil) can be used to estimate potential health risks (Yusa et al., 2018). Arsenic speciation determines its toxicity, which includes inorganic arsenic (iAs), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Yu et al., 2016a). Generally, the order of cytotoxicity is $DMA^{III} > iAs^{III} > MMA^{III} > iAs^{V} > MMA^{V}$, DMA^{V} (Dopp et al., 2005; Naranmandura et al., 2011).

Bioaccessibility is defined as the soluble fraction of contaminants in the gastrointestinal environment of humans. Previous studies demonstrated that bioaccessibility of soil contaminants depended on soil physicochemical properties, *in vitro* tests pH and other parameters (Juhasz et al., 2009; Smith et al., 2014). However, in real environments, diet influenced the bioaccessibility of contaminants. Therefore, dietary background is an important parameter affecting bioaccessibility when assessing health risks from oral exposure to contaminants (Alava et al., 2015).

Vitamins, proteins and carbohydrates are human essential nutrients, which maintain health and promote growth in humans. Vitamin C can act as an antioxidant and is needed for many physiological functions in the human biology. Deficiency of vitamin C can cause some diseases, such as scurvy, infections, anemia and muscle degeneration (Kumar, 2012). Protein is the most fundamental nutrient for humans, and it is hydrolyzed to amino acids that provide nitrogen, hydrocarbon skeletons, and sulfur. Deficiency in protein can cause symptoms in humans (hypertension, anemia, cardiac failure) (Wu, 2016). Hence, protein cannot be replaced by any other nutrients. Glucose is the most important energy source in the body. The human brain relies on glucose as the main source of energy (Mergenthaler et al., 2013).

Some nutritional supplements, such as vitamin, were beneficial to prevent long-term health problems from As exposure (Majumdar et al., 2012). The effects of some nutrients (*e.g.* protein, carbohydrate) on As bioaccessibility from contaminated matrix (*e.g.* soil and food) were studied. Clemente et al. (2017) studied that some nutrients (*e.g.* cysteine) reduced the bioaccessibility of As from rice and seaweed (44.0% and 38%) by using *in vitro* method. Oomen et al. (2002) found that milk powder (including protein and carbohydrate) increased As bioaccessibility from contaminated soils about 7–14% using simulate *in vitro* gastrointestinal method. The previous studies merely concentrated on As bioaccessibility in the gastric and small intestine. However, diet can rapidly alter the structure and activity of the human gut microbiota (David et al., 2014; Derrien & van Hylckama Vlieg, 2015). In a scheme of nutrients consumption, it isn't ignored what extent the nutrients affect As bioaccessibility in the colon where proceed to microbial fermentation processes. Laird et al. (2009) had studied that the effect carbohydrate mixture on As bioaccessibility from contaminated soils in the gastrointestinal and colon phase. But the As speciation largely determine toxicity of As (Yu et al., 2016b), which is important when assessing the human health. It's significant for the human health to investigate the effect of nutritional status on As bioaccessibility and speciation. Additionally, gut microbiota can impact speciation (Van de Wiele et al., 2010; Yin et al., 2015). The simulator of the human intestinal microbial ecosystem (SHIME) simulates the colon microbial community of humans (Van de Wiele et al., 2004b), which combined with an *in vitro* test showed a microbial metabolic potency towards metals (Diaz-Bone & Van de Wiele, 2009). The model showed that human gut microbiota increased As bioaccessibility in contaminated soils (Van de Wiele et al., 2010).

Most studies were focused on the effect of food on As bioaccessibility in the gastrointestinal environment, but research about the effect of nutrients on As bioaccessibility and biotransformation has been limited. In this study, we evaluated the effect of nutrients on As bioaccessibility and its metabolism from four contaminated soil samples using and *in vitro* physiologically based extraction test (PBET) combined with the SHIME model. The experiment included four dietary treatments, including vitamin C, protein powder, glucose and a fasted control. The objectives of this study were to investigate the influence of nutritional status on soil As bioaccessibility in the gastrointestinal and colon phase. Meanwhile, the influence of the four nutrients on As biotransformation was investigated.

2. Materials and methods

2.1. As-contaminated soils

Four surface (0–20 cm depth) soil samples were collected from a range of locations in China. All soil samples were sieved after air-drying, and particle size fraction were <250 μm for the *in vitro* test. Soil physicochemical properties and concentrations of total As were determined according the method described in Yin et al. (2017) and listed in Table 1. In soil, As mobility was mainly controlled by adsorption/desorption processes and co-precipitation with metal oxides (Ehlert et al., 2014; Smeaton et al., 2012). As speciation was also affected by oxalate-extractable Fe and Mn (Yin et al., 2015). Therefore, the concentrations of Fe, Mn, and Al oxides were determined in the study.

The three nutrients (vitamin C, glucose, and protein powder) in the experiment were purchased from Beijing local supermarket, China. The dominant component of the protein powder was protein, which reaches to 98.0%. The purity of vitamin C is 99.0%, and the content of carbohydrates from glucose is 90.1%. The concentration of total As was determined after microwave digestion using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500a, Santa Clara, CA). The results showed that As concentration in the nutrients was lower than the detection limit and therefore negligible.

2.2. Production and characterization of colon microbiota for SHIME

The colon microbial community used in the experiment was cultured and maintained in a modified SHIME model described previously by Van de Wiele et al. (2004b), which consisted of five compartments to simulate the gastric, small intestine, ascending colon, transverse colon, and descending colon at a temperature of 37 °C. The pH of the SHIME reactors was maintained in the ranges of 5.6–5.9, 6.2–6.5 and 6.7–6.9 in the ascending colon, transverse colon and descending colon, respectively. A detailed description of the SHIME is provided by Van de Wiele et al. (2004a). Briefly, fresh fecal microbiota was obtained from an adult volunteer, who had no history of antibiotic treatment for 6 months before the study. The growth medium was inoculated with the microbiota three times per day to provide digested nutrition. The SHIME system was flushed regularly with N_2 to ensure anaerobic conditions. After 3–4 weeks of adaptation, microbial communities tended to be stable. The microbial fermentation activity of the distal colon and community composition were consistent with those of previous SHIME runs and the *in vivo* investigations (Van de Wiele et al., 2004a).

2.3. Estimation of bioaccessible As

In this study, we compared the effect of nutritional status on As bioaccessibility in the gastric, small intestinal and colon phases using the PBET method combined with the SHIME. The PBET method was modified (Rodriguez and Basta, 1999) using the procedure described by Ruby et al. (1996). In brief, soils (0.3 g) were added to 50-mL polypropylene tubes with the gastrointestinal solutions (30 mL) (Yin et al., 2015). Gastric solution was added to each vessel for the fasted treatment. According to reference intake of nutrients in diets for Chinese people (Cheng, 2014), the solution consisted of 0.02 g of vitamin C in a gastric solution adjusted to pH 1.5. For the protein treatment, the solution consisted of 1.0 g of protein powder in a gastric solution adjusted to pH 1.5. For the carbohydrate treatment, the solution consisted of 1.0 g of glucose in a gastric solution adjusted to pH 1.5. Following the small intestinal phase, these digests were transferred into 100 mL anaerobic serum bottles with 30 mL of colon solution from the colon compartment of the dynamic SHIME model (Van de Wiele et al., 2010; Yin et al., 2016;