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Supplementation with Folic Acid, but Not Creatine, Increases Plasma Betaine, Decreases Plasma Dimethylglycine, and Prevents a Decrease in Plasma Choline in Arsenic-Exposed Bangladeshi Adults[1–3]

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Abstract

Background: Folic acid (FA) supplementation facilitates urinary excretion of arsenic, a human carcinogen. A better understanding of interactions between one-carbon metabolism intermediates may improve the ability to design nutrition interventions that further facilitate arsenic excretion.

Objective: The objective was to determine if FA and/or creatine supplementation increase choline and betaine and decrease dimethylglycine (DMG).

Methods: We conducted a secondary analysis of the Folic Acid and Creatine Trial, a randomized trial in arsenic-exposed Bangladeshi adults (n = 605, aged 24–55 y, 50.3% male) who received arsenic-removal water filters. We examined treatment effects of FA and/or creatine supplementation on plasma choline, betaine, and DMG concentrations, measured by LC–tandem mass spectrometry at baseline and at week 12. Group comparisons were between *1*) 400 and 800 μg FA/d (FA400 and FA800, respectively) compared with placebo, *2*) creatine (3 g/d) compared with placebo, and *3*) creatine plus FA400 compared with FA400.

Results: Choline decreased in the placebo group (−6.6%; 95% CI: −10.2%, −2.9%) but did not change in the FA groups (FA400: 2.5%; 95% CI: −0.9%, 6.1%; FA800: 1.4%; 95% CI: −2.5%, 5.5%; $P < 0.05$). Betaine did not change in the placebo group (−3.5%; 95% CI: −9.3%, 2.6%) but increased in the FA groups (FA400: 14.1%; 95% CI: 9.4%, 19.0%; FA800: 13.0%; 95% CI: 7.2%, 19.1%; $P < 0.01$). The decrease in DMG was greater in the FA groups (FA400: −26.7%; 95% CI: −30.9%, −22.2%; FA800: −27.8%; 95% CI: −31.8%, −23.4%) than in the placebo group (−12.3%; 95% CI: −18.1%, −6.2%; $P < 0.01$). The percentage change in choline, betaine, and DMG did not differ between creatine treatment arms and their respective reference groups.

Conclusion: Supplementation for 12 wk with FA, but not creatine, increases plasma betaine, decreases plasma DMG, and prevents a decrease in plasma choline in arsenic-exposed Bangladeshi adults. This trial was registered at clinicaltrials.gov as NCT01050556. *J Nutr* 2016;146:1062–7.

Keywords: folic acid, creatine, choline, betaine, arsenic

Introduction

In Bangladesh, >57 million individuals are exposed chronically to arsenic-contaminated drinking water (1), which has been associated with numerous adverse health outcomes (2). Studies in both animals and human populations have shown that nutrition influences the metabolism (3–8) and toxicity (9–11) of arsenic. For example, folate plays an important role in the biosynthesis of *S*-adenosylmethionine (SAM)[10], the universal methyl donor. Arsenic metabolism, which involves 2 sequential SAM-dependent methylation reactions, can be increased with FA supplementation (12), thereby facilitating arsenic excretion in urine. We previously showed that supplementation with 400 μg folic acid (FA)/d in folate-deficient individuals (13) or 800 μg FA/d in a mixed folate-deficient and -replete population (14) reduces blood arsenic concentrations. The biosynthesis of SAM

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depends on the remethylation of homocysteine to methionine, which can be catalyzed by 1 of 2 enzymes: either by methionine synthase, which receives a methyl group from folate in the form of 5-methyltetrahydrofolate (15), or by betaine homocysteine methyltransferase (BHMT), which utilizes a methyl group from betaine (16), generating methionine and dimethylglycine (DMG). Thus, folate and betaine are highly interrelated because they can be used interchangeably for the remethylation of homocysteine, and methylation via 1 reaction may therefore spare methyl groups from the alternative reaction.

Betaine can be obtained from the diet or derived from the oxidation of choline. Choline can also be obtained from the diet or synthesized endogenously. Approximately 30% of endogenously derived choline is synthesized de novo through a pathway that involves 3 sequential SAM-dependent methylation reactions, which are catalyzed by phosphatidylethanolamine *N*-methyltransferase (PEMT) (17). Therefore, folate and betaine are further interrelated, because folate can facilitate choline synthesis by contributing to the regeneration of SAM. High SAM concentrations also inhibit BHMT activity (18). Thus, by increasing SAM concentrations, folate supplementation may increase choline and betaine concentrations for 3 reasons: *1*) less betaine would be required for the regeneration of SAM, *2*) more SAM would be available for choline synthesis, and *3*) the inhibition of BHMT by SAM would spare betaine and choline.

Although not well studied, choline and betaine could also potentially be spared by reducing SAM-dependent methylation demand. For example, creatine synthesis, which involves the methylation of guanidinoacetate, accounts for the consumption of ~50% of SAM-derived methyl groups (19, 20). Creatine supplementation can downregulate endogenous creatine biosynthesis (21, 22) and may thereby spare SAM, such that less betaine is required for SAM synthesis and more SAM is available for choline synthesis. However, the relations between folate, betaine, choline, and creatine have not been examined in populations with widespread exposure to both arsenic and nutritional deficiencies. A better understanding of these complex relations will improve the ability to design the most effective nutritional interventions in arsenic-exposed populations.

The purpose of this study was therefore to determine if supplementation with *1*) FA (compared with placebo), *2*) creatine alone (compared with placebo), or *3*) creatine + FA (compared with FA alone) increases plasma concentrations of betaine and choline and decreases plasma concentrations of DMG, an indicator of flux through the BHMT pathway, in an arsenic-exposed population in Bangladesh, where the prevalences of folate deficiency and hyperhomocysteinemia are high (23). To do this, we conducted secondary analyses using samples from the Folic Acid and Creatine Trial (FACT), a randomized, placebo-controlled trial that was originally designed to examine whether FA and/or creatine can be used as therapeutic approaches to lower blood arsenic in Bangladeshi adults. The main findings of this trial have been published (14).

Methods

Study region and participants. As described previously (14), FACT participants were selected from the Health Effects of Arsenic Longitudinal Study (HEALS) (24), which is an ongoing, prospective cohort study located in a 35-km^2 area in Araihazar, Bangladesh, where there is widespread exposure to naturally occurring arsenic through drinking water. To participate in the HEALS, participants had to be married, aged between 20 and 65 y, and had to have been drinking from the same well for at least 3 y. For the FACT, 622 Bangladeshi adults were randomly selected from all HEALS participants who had been drinking from wells with water arsenic concentrations >50 μg/L. Individuals were excluded from FACT if they were pregnant, taking nutritional supplements, or had any known health problems. Bangladeshi field staff physicians obtained informed consent, and ethical approval was obtained from the Institutional Review Board of Columbia University Medical Center and from the Bangladesh Medical Research Council.

Study design. The FACT study design has been described previously (14). Briefly, participants were randomly assigned to 5 treatment arms: 400 μg FA/d (FA400; n = 156), 800 μg FA/d (FA800; n = 154), 3 g creatine/d (creatine; n = 104), 3 g creatine + 400 μg FA/d (creatine +FA400; n = 104), or placebo (n = 104). A dose of 400 μg FA/d was selected on the basis of the RDA for FA, and we have previously shown that this dose reduces blood arsenic concentrations in folate-deficient individuals (13). We also selected a second, higher, dose (800 μg FA/d), which lowered blood arsenic concentrations in this mixed population of folate-deficient and -replete individuals (14). For creatine, we selected a dose of 3 g/d, because it is ~1.5 times the average daily requirements for creatine, which are normally met by a combination of dietary creatine and endogenous creatine synthesis (25); 3 g creatine/d is also the dose recommended by the European Food Safety Authority (26).

Participants received their assigned treatments for 12 wk. At 12 wk, half of the participants in the FA treatment arms were switched to placebo. The creatine treatment arm was switched to placebo at week 12. A total of 12 participants were dropped from the study. Due to ethical considerations, participants in all 5 treatment groups also received arsenic-removal water filters (READ-F filter; Brota Services International) at baseline to be used for the duration of the study and thereafter.

Sample collection and handling. Blood was collected in EDTA-coated evacuated tubes and transported at 0°C to the field clinic laboratory in Araihazar within 4 h. Plasma was separated from cells after centrifugation at 3000 × g for 10 min at 4°C. Whole-blood and plasma aliquots were shipped on dry ice to Columbia University and stored at −80°C.

Plasma betaine, choline, and DMG. Plasma betaine, choline, and DMG concentrations were measured for all participants at baseline and at week 12 by LC–tandem MS by using the method of Holm et al. (27), with some modifications, which have been described previously (28). The intra-assay CVs for choline, betaine, and DMG were 2.2%, 2.5%, and 3.6%, respectively. The interassay CVs were 5.8%, 5.6%, and 6.7%, respectively. Five participants were missing measures for choline, betaine, or DMG for at least 1 time point due to insufficient plasma sample.

Blood arsenic. Total blood arsenic concentrations were measured by using a Perkin-Elmer Elan DRC II ICP-MS, equipped with an AS10+ autosampler, as previously described (29). The intra- and interassay CVs were 2.7% and 5.7%, respectively.

Statistical analyses. The outcomes of interest were plasma choline, betaine, and DMG concentrations. Because these variables had right-skewed distributions, a natural logarithmic (ln) transformation was applied to each to meet the assumptions of parametric analytic methods.

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[3] Supplemental Table 1 is available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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[10] Abbreviations used: BHMT, betaine homocysteine methyltransferase; DMG, dimethylglycine; FA, folic acid; FA400, 400-μg/d folic acid group; FA800, 800-μg/d folic acid group; FACT, Folic Acid and Creatine Trial; HEALS, Health Effects of Arsenic Longitudinal Study; PEMT, phosphatidylethanolamine *N*-methyltransferase; SAM, *S*-adenosylmethionine.

The geometric mean (anti-ln mean of ln-transformed variable) of each outcome was calculated at baseline and at week 12 for each treatment group. On the basis of the ratio of the geometric mean at week 12 to the geometric mean at baseline, we also calculated the percentage change in the geometric mean from baseline to week 12 using the following formula: % change = (geometric mean ratio − 1) × 100%. ANOVA was used to detect treatment group differences between the FA800, FA400, and placebo groups in the geometric mean of each outcome at baseline and at week 12 and for the percentage change from baseline to week 12. For any outcome that differed by treatment group, we followed with pairwise group comparisons using *t* tests, with *P* values adjusted for multiple tests with the use of the Bonferroni method. We also compared the geometric mean of each outcome at baseline and at week 12, as well as the percentage change from baseline to week 12, between *1*) the creatine and placebo groups and *2*) the creatine+FA400 group and the FA400 group using 2-sample *t* tests.

The relation between baseline blood arsenic and plasma choline concentrations was also examined by Spearman correlation. Although this study was not powered to formally evaluate sex differences, we examined potential sex differences in exploratory analyses. All analyses were conducted in SAS (version 9.3; SAS Institute). A significance level of 0.05 was used for all statistical tests.

Results

General characteristics of study participants at baseline. General characteristics of the FACT participants have been reported previously for each treatment group (14). These characteristics, as well as baseline plasma choline, betaine, and DMG concentrations, are also presented separately by treatment group in **Supplemental Table 1** for participants with measures of each outcome variable. At baseline, there were no significant differences in any of the covariates between the treatment arms ($P > 0.20$). Participants were between 24 and 55 y old (50.3% male), with BMIs (in kg/m^2) ranging from 13.9 to 31.6. At baseline, plasma choline and betaine concentrations ranged from 6.0 to 20.1 μmol/L and from 12 to 116 μmol/L, respectively, and plasma DMG concentrations ranged from 2 to 106 μmol/L.

Effect of FA supplementation on plasma choline, betaine, and DMG concentrations. The geometric means of choline, betaine, and DMG at baseline and week 12, and the percentage change in the geometric mean from baseline to week 12, are presented with their respective 95% CIs for placebo, FA400, and FA800 groups in **Table 1**.

At week 12, after adjusting for multiple comparisons, choline and betaine concentrations were both significantly higher in the FA400 and FA800 groups than in the placebo group ($P < 0.05$) but did not differ between the 2 FA groups ($P > 0.99$). DMG concentrations at week 12 did not differ significantly between the placebo and FA treatment arms ($P = 0.08$).

Choline decreased significantly from baseline to week 12 in the placebo group (−6.6%; 95% CI: −10.2%, −2.9%). Total blood arsenic concentrations were also positively correlated with choline concentrations at baseline ($\rho = 0.15$, $P < 0.01$; $n = 605$). However, there was no significant change in choline from baseline to week 12 in either of the FA treatment arms (Table 1). The percentage change in choline differed significantly between the placebo and FA groups ($P < 0.05$).

Betaine did not change significantly from baseline to week 12 in the placebo group (Table 1) but did increase significantly in both the FA400 and the FA800 groups, with a similar percentage change at both FA doses (14.1% [95% CI: 9.4%, 19.0%] and 13.0% [95% CI: 7.2%, 19.1%], respectively). The percentage change in betaine differed significantly between the FA and placebo groups ($P < 0.01$). In contrast, DMG decreased significantly in all 3 groups from baseline to week 12 (Table 1). Although DMG concentrations did not differ significantly between the 3 treatment arms at week 12, the percentage decrease in DMG was significantly greater in both FA treatment groups than in the placebo group ($P < 0.01$). The percentage changes in plasma choline, betaine, and DMG did not differ between the FA800 and FA400 groups ($P > 0.99$).

Effects of creatine supplementation on plasma choline, betaine, and DMG concentrations. The geometric means of choline, betaine, and DMG at baseline and week 12, and the percentage change from baseline to week 12, are presented with their respective 95% CIs for the placebo, creatine, FA400, and creatine+FA400 groups in **Table 2**. Group comparisons of interest are between *1*) the creatine and placebo groups and *2*) the creatine+FA400 group and the FA400 group. Although week 12 choline concentrations were higher in the creatine group than in the placebo group ($P = 0.03$), the percentage change in choline did

TABLE 1 Choline, betaine, and DMG at baseline and week 12 by FA treatment group in FACT participants[1]

	Placebo ($n = 101$)	FA400 ($n = 152$)	FA800 ($n = 149$)	P^2
Choline				
Baseline, μmol/L	11.3 (10.8, 11.8)	11.5 (11.1, 11.9)	11.4 (10.9, 11.8)	0.89
Week 12, μmol/L	10.6 (10.1, 11.1)[b]	11.8 (11.3, 12.2)[a]	11.5 (11.1, 12.0)[a]	<0.01
Change, %	−6.6 (−10.2, −2.9)[b]	2.5 (−0.9, 6.1)[a]	1.4 (−2.5, 5.5)[a]	<0.01
Betaine				
Baseline, μmol/L	42.6 (39.3, 46.2)	43.0 (40.8, 45.4)	41.7 (39.1, 44.5)	0.78
Week 12, μmol/L	41.1 (38.1, 44.3)[b]	49.1 (46.5, 51.8)[a]	47.2 (44.2, 50.3)[a]	<0.01
Change, %	−3.5 (−9.3, 2.6)[b]	14.1 (9.4, 19.0)[a]	13.0 (7.2, 19.1)[a]	<0.01
DMG				
Baseline, μmol/L	5.8 (5.2, 6.5)	6.1 (5.6, 6.7)	6.3 (5.7, 6.9)	0.59
Week 12, μmol/L	5.1 (4.6, 5.7)	4.5 (4.2, 4.8)	4.5 (4.2, 4.9)	0.08
Change, %	−12.3 (−18.1, −6.2)[a]	−26.7 (−30.9, −22.2)[b]	−27.8 (−31.8, −23.4)[b]	<0.01

[1] Values are geometric means (95% CIs) or percentage changes (95% CIs) in the geometric mean from baseline to week 12: % change = (geometric mean ratio − 1) × 100%. Labeled means in a row without a common superscript letter differ, $P < 0.05$ (adjusted for multiple comparisons). DMG, dimethylglycine; FA, folic acid; FA400, 400-μg folic acid group; FA800, 800-μg folic acid group, FACT, Folic Acid and Creatine Trial.

[2] Two-sided *P* values were derived by using 1-factor ANOVA.

not differ significantly between the 2 groups ($P = 0.17$). Similarly, whereas week 12 betaine concentrations tended to be higher in the creatine group ($P = 0.06$), the percentage change in betaine did not differ between the creatine and placebo groups ($P = 0.14$). Week 12 DMG concentrations and the percentage change in DMG did not differ between the creatine and placebo groups ($P = 0.12$ and 0.14, respectively). Week 12 concentrations of choline, betaine, and DMG, and the percentage change in each outcome, did not differ between the creatine+FA400 and the FA400 groups (Table 2).

Potential differences by sex. Blood arsenic concentrations were positively and significantly correlated with plasma choline concentrations in women ($\rho = 0.19$, $P < 0.01$; $n = 301$) but not in men ($\rho = 0.06$, $P = 0.32$; $n = 304$), and the percentage decrease in plasma choline observed in the placebo group was greater in women (−9.9%; 95% CI: −14.4%, −5.3%; $n = 50$) than in men (−3.2%; 95% CI: −8.6%, 2.5%; $n = 51$); the difference by sex tended toward significance ($P = 0.07$). Within the FA400 group, the percentage change in choline among women (1.6%; 95% CI: −3.4%, 6.9%; $n = 76$) did not differ ($P = 0.61$) from the percentage change among men (3.4%; 95% CI: −1.2%, 8.3%; $n = 76$). Within the FA800 group, there was also no difference ($P = 0.98$) in the percentage change in choline among women (1.3%; 95% CI: −3.7%, 6.7%; $n = 75$) compared with the percentage change among men (1.5%; 95% CI: −4.4%, 7.7%l $n = 74$). Among women, the percentage change in choline differed significantly between the FA groups and the placebo group ($P < 0.01$). In contrast, the percentage change in choline did not differ significantly between the FA groups and the placebo group (FA400 compared with placebo, $P = 0.08$; FA800 compared with placebo, $P = 0.29$) among men. There were no other notable sex differences.

Discussion

In this population of arsenic-exposed Bangladeshi adults, we observed that supplementation with 2 different doses of FA (400 and 800 μg/d for 12 wk) significantly increased plasma betaine concentrations. A previous study by Melse-Boonstra et al. (30) similarly observed that FA supplementation, at the same doses and for the same duration, increased plasma betaine concentrations in a generally folate-sufficient Dutch population. Specifically, they observed that participants taking FA doses between 400 and 800 μg/d experienced a mean increase in plasma betaine of 15% (30). Despite several differences between the 2 study populations, including the fact that our study population in Bangladesh had been chronically exposed to arsenic-contaminated drinking water and had lower plasma folate concentrations, we observed that 400 and 800 μg FA/d for 12 wk caused almost identical increases in plasma betaine (14% and 13%, respectively). Consistent with Melse-Boonstra et al. (30), we also observed no difference between the effects of 400 compared with 800 μg FA/d on plasma betaine concentrations. However, Melse-Boonstra et al. (30) did observe a dose-response relation between FA supplementation and plasma betaine for FA doses <400 μg/d, suggesting that the effect of FA on plasma betaine concentrations may be saturable.

Unexpectedly, we observed that, among women, total blood arsenic concentrations were positively correlated with plasma choline concentrations at baseline. Furthermore, plasma choline concentrations decreased significantly over time in the placebo group, and this was driven by a decrease among women. Although speculative, because arsenic has been shown to inhibit the expression of *Bhmt* in mice (31, 32), it is possible that reductions in arsenic exposure due to the provision of arsenic-removal filters increased *BHMT* expression in FACT participants; this could lead to an increased flux of betaine through BHMT, causing reductions in betaine and choline and increases in DMG. However, although plasma choline concentrations decreased in the placebo group, plasma betaine concentrations were unaltered, and plasma DMG concentrations decreased. Although the effects of arsenic on choline are not well studied, 2 metabolomics studies showed that choline is one of the metabolites most affected by arsenic exposure (33, 34); consistent with our finding that plasma choline concentrations decreased with the use of arsenic-removal water filters, arsenic exposure induced higher total choline concentrations in the plasma, livers, and kidneys of mice (33) and in the serum and livers of rats (34). In addition, serum glycine concentrations were higher in arsenic-exposed animals (33, 34) and urine DMG concentrations were reduced (34); 1 group therefore hypothesized that arsenic exposure causes a decrease in urinary DMG excretion, such that more DMG can be used for the production of sarcosine, an intermediate in glycine synthesis (34). This could potentially explain the decrease in DMG observed in the placebo group. However,

TABLE 2 Choline, betaine, and DMG by creatine treatment group in FACT participants[1]

	Placebo ($n = 101$)	Creatine ($n = 100$)	Creatine vs. Placebo, P[2]	FA400 ($n = 152$)	Creatine+FA400 ($n = 103$)	Creatine+FA400 vs. FA400, P[2]
Choline						
Baseline, μmol/L	11.3 (10.8, 11.8)	11.7 (11.2, 12.2)	0.29	11.5 (11.1, 11.9)	11.4 (10.9, 11.9)	0.84
Week 12, μmol/L	10.6 (10.1, 11.1)	11.4 (10.9, 11.9)	0.03	11.8 (11.3, 12.2)	11.5 (11.0, 12.0)	0.46
Change, %	−6.6 (−10.2, −2.9)	−2.6 (−7.0, 2.0)	0.17	2.5 (−0.9, 6.1)	0.9 (−3.2, 5.2)	0.55
Betaine						
Baseline, μmol/L	42.6 (39.3, 46.2)	43.9 (40.9, 47.2)	0.57	43.0 (40.8, 45.4)	42.3 (39.1, 45.8)	0.73
Week 12, μmol/L	41.1 (38.1, 44.3)	45.3 (42.2, 48.8)	0.06	49.1 (46.5, 51.8)	45.9 (42.1, 50.1)	0.20
Change, %	−3.5 (−9.3, 2.6)	3.2 (−3.4, 10.3)	0.14	14.1 (9.4, 19.0)	8.5 (2.0, 15.4)	0.19
DMG						
Baseline, μmol/L	5.8 (5.2, 6.5)	6.0 (5.5, 6.5)	0.64	6.1 (5.6, 6.7)	6.6 (6.0, 7.2)	0.27
Week 12, μmol/L	5.1 (4.6, 5.7)	5.7 (5.2, 6.3)	0.12	4.5 (4.2, 4.8)	4.9 (4.5, 5.3)	0.11
Change, %	−12.3 (−18.1, −6.2)	−5.1 (−12.5, 3.0)	0.14	−26.7 (−30.9, −22.2)	−25.6 (−31.3, −19.6)	0.79

[1] Values are geometric means (95% CIs) or percentage changes (95% CIs) in the geometric mean from baseline to week 12: % change = (geometric mean ratio − 1) × 100%. DMG, dimethylglycine; FA400, 400-μg folic acid group; FACT, Folic Acid and Creatine Trial.
[2] Two-sided P values were derived by using a 2-sample t test.

because we did not measure serum sarcosine or glycine concentrations, or DMG concentrations in urine, this remains speculative.

Although the reasons for the observed sex differences in our study are unknown, there are important sex differences in the endogenous synthesis and metabolism of choline (35, 36). For example, estrogen induces the expression of *PEMT* (37). Thus, it is possible that females are particularly sensitive to arsenic-induced alterations in choline. Consistent with our findings in human plasma, a study in female rats also showed that arsenic increases total choline concentrations in the liver (38). However, given that we do not have a comparison group who did not receive arsenic-removal filters, we cannot rule out alternative explanations for the decrease in plasma choline concentrations that occurred in the placebo group. Nevertheless, the potential influence of arsenic on choline concentrations, particularly among women, merits additional study. Plasma choline concentrations did not decrease in groups receiving FA, suggesting that FA treatment prevented the decrease in plasma choline, potentially by a compensatory increase in flux through methionine synthase and a reduction in flux of betaine through BHMT.

In FACT participants, we previously observed that 3 g creatine/d significantly reduced guanidinoacetate concentrations (14), suggesting that this dose of creatine reduced endogenous creatine synthesis. However, although week 12 plasma choline concentrations were higher in the creatine group than in the placebo group, creatine supplementation did not significantly affect the percentage change in any of the outcomes. This may be due to long-range allosteric regulation of SAM concentrations. Although creatine supplementation likely spared SAM by downregulating endogenous creatine synthesis, the resulting increase in SAM concentrations may have downregulated methylenetetrahydrofolate reductase, leading to subsequent reductions in SAM synthesis.

Although FA supplementation reduces blood arsenic concentrations in arsenic-exposed adults (13, 14), with 400 μg FA/d reducing blood arsenic by 14% in individuals with low folate and 800 μg FA/d reducing blood arsenic by 12% in a mixed population of folate-deficient and -replete individuals, optimized multinutrient interventions have the potential to further reduce blood arsenic concentrations over and above that of FA alone. To design such interventions, a better understanding of the complex interactions between nutrients, including those involved in one-carbon metabolism, is needed. The findings of this study suggest that creatine supplementation, either alone or in combination with FA, does not significantly increase plasma betaine or choline concentrations; it also had no effect on blood arsenic concentrations in this population (14), although potential effects on arsenic methylation still need to be evaluated. However, low-dose FA supplementation increased plasma betaine concentrations and maintained plasma choline concentrations in this arsenic-exposed population. Future studies that examine the contributions of choline and betaine to arsenic metabolism will be important for elucidating whether supplementation with these nutrients could further benefit arsenic-exposed populations. One strategy may be to use in silico mathematical models, which incorporate long-range interactions present in the one-carbon metabolic pathway (39). We have used these models successfully in the past to predict the effects of FA supplementation on blood arsenic concentrations (40), and the models could be expanded moving forward to make predictions about the effects of supplementation with multiple micronutrients on arsenic metabolism and blood arsenic concentrations.

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