ONE-CARBON METABOLISM INDICES, FOLIC ACID SUPPLEMENTATION, AND HISTONE MODIFICATIONS IN BANGLADESHI ADULTS

Howe CG[1], Liu X[2], Hall MN[3], Ilievski V[1], Caudill M[4], Parvez F[1], Siddique AB[5], Shahriar H[5], Uddin N[5], Islam T[5], Graziano JH[1], Gamble MV[1]

Departments of [1]Environmental Health Sciences, [2]Biostatistics, [3]Epidemiology, Mailman School of Public Health, Columbia University, New York, New York; [4]Division of Nutritional Sciences, Cornell University, Ithaca, New York; [5]Columbia University Arsenic Project in Bangladesh, Dhaka, Bangladesh

INTRODUCTION

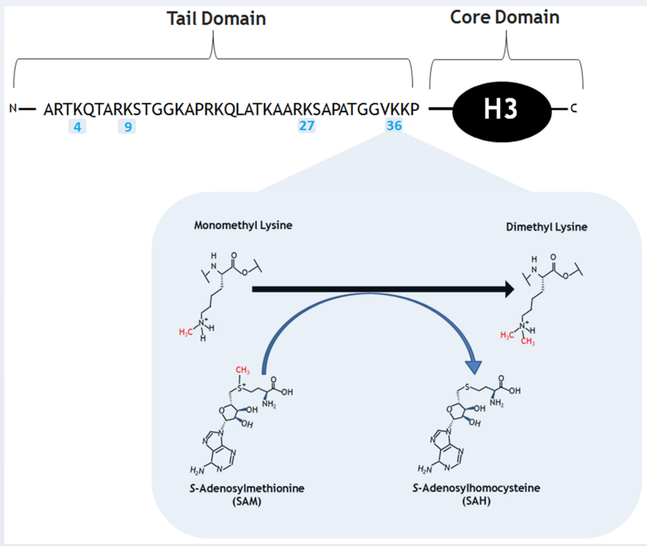
Post-translational histone modifications (PTHMs), such as methylation of lysine residues on histone H3, regulate chromatin structure such that DNA is more or less accessible for cellular processes. Up to 3 methyl groups can be added to histone lysines by lysine histone methyltransferases (KHMTs), and each methylation reaction depends on a methyl donation from *S*-adenosylmethionine (SAM) and is inhibited by *S*-adenosylhomocysteine (SAH) (**Fig. 1**).

In experimental models, nutritional methyl donors, such as folate and choline, influence PTHMs [1-7]. However, few human studies have examined the effects of one-carbon metabolism (OCM) indices on global (%) PTHMs.

The purpose of this study was to examine how OCM indices, including folate, B12, and choline, and plasma homocysteine (Hcys), an indicator of intracellular methylation capacity [8], affect three %PTHMs (H3K36me2, H3K36me3, and H3K79me2) in Bangladeshi adults exposed chronically to arsenic (As)-contaminated drinking water. This study also offered the unique opportunity to determine if supplementation with the U.S. recommended dietary allowance for folic acid (FA) (400 µg/day) for 12 weeks alters these %PTHMs.

Potential differences by sex were evaluated, because we have previously observed that As influences epigenetic marks, including %PTHMs, in a sex-dependent manner [9-11], and others have observed that concurrent exposure to a methyl deficient diet and As induces sex-specific alterations in global DNA methylation patterns in mice [12]. The effects of nutritional methyl donors on %PTHMs in populations chronically exposed to As are unknown.

Fig. 1. SAM-dependent methylation of Histone H3



Blue numbers indicate H3 lysine residues that are commonly methylated. Lysine 79 is located in the H3 core domain. The inset depicts the methylation of lysine 36 from its monomethyl form (H3K36me) to its dimethyl form (H3K36me2) (methyl groups shown in red). Each methylation reaction depends on a methyl donation from *S* adenosylmethionine (SAM) and is inhibited by *S*-adenosylhomocysteine (SAH).

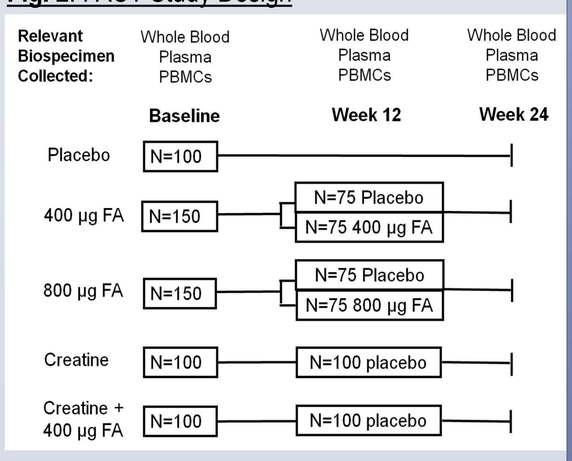
HYPOTHESES

- Red blood cell (RBC) folate and plasma folate, B12, and choline, are associated with higher levels of %PTHMs, while plasma Hcys is associated with lower levels of %PTHMs
- 12 weeks supplementation with FA (400 µg/day), compared with placebo, increases %PTHMs

STUDY DESIGN, METHODS, AND PARTICIPANTS

The Folic Acid and Creatine Trial (FACT) is a randomized, placebo-controlled, trial of FA and creatine supplementation in ~600 Bangladeshi adults who had been drinking from wells with As ≥ 50 µg/L (**Fig. 2**). All participants received As-removal water filters at baseline. We measured %PTHMs in peripheral blood mononuclear cells (PBMCs) collected from a subset (N=324) of FACT participants at baseline and from the placebo and 400 µg FA groups at baseline and week 12.

Fig. 2. FACT Study Design



Plasma Folate and B12, RBC Folate: Measured by radioimmunoassay. RBC folate was adjusted by [%Hematocrit/100].

Plasma Choline: Measured by LC-MS/MS.

Plasma Hcys: Measured by HPLC with fluorescence detection.

%PTHMs: Histones were isolated from PBMCs by acid-extraction. %PTHMs (%H3K36me2, %H3K36me3, and %H3K79me2) were measured by sandwich ELISA.

Statistical Methods: Associations between OCM indices and %PTHMs were determined using generalized linear models with an inverse link (%H3K36me2) or linear regression models (%H3K36me3, %H3K79me2). OCM indices, %H3K36me3, and %H3K79me2 were natural log-transformed. An inverse-transformation was applied to %H3K36me2. Differences by sex were determined by the Wald test. Differences in the intra-person change in %PTHMs between the 400 µg FA and placebo groups were determined by the Wilcoxon test.

Table 1. Characteristics of Study Participants by Sex

	Men (N=162)	Women (N=162)	
Characteristic	**Median (range)**	**Median (range)**	***P*** [a]
Age (y)	42 (25-54)	37 (24-54)	<0.01
BMI (kg/m^2)[c]	18.7 (15.4-27.9)	20.0 (13.9-31.6)	<0.01
bSe (µg/L)	135 (90-191)	132 (74-203)	0.16
RBC Folate (nmol/L)[d]	434 (155-1150)	461 (148-3800)	0.34
Plasma Folate (nmol/L)	12 (3-120)	13 (4-42)	0.10
Plasma B12 (pmol/L)	217 (58-610)	213 (58-871)	0.49
Plasma Choline (µmol/L)	11.9 (6.0-20.0)	10.7 (6.0-19.5)	<0.01
Plasma Betaine (µmol/L)	47.0 (20.9-89.4)	37.3 (14.3-98.4)	<0.01
Plasma Hcys (µmol/L)	14 (6-102)	9 (4-56)	<0.01
%H3K36me2[e]	1.45 (0.68-4.00)	1.43 (1.00-6.87)	0.66
%H3K36me3[f]	1.56 (0.48-4.09)	1.63 (0.52-6.44)	0.10
%H3K79me2[g]	1.26 (0.29-9.46)	1.29 (0.29-9.41)	0.96
Folate Deficient[h] (%)	28.4	17.9	0.03
B12 Deficient[i] (%)	24.1	24.7	0.90
Hhcys (%)[j]	62.4	19.1	<0.01
Ever Smoker (%)	56.2	1.2	<0.01[b]
Education > 5 y (%)	21.0	24.1	0.51
Own TV (%)	32.7	43.8	0.04

[a]*P* for difference by sex calculated by a Wilcoxon or Chi square test for continuous or categorical variables, respectively

[b]*P* for difference by sex calculated by Fisher's Exact Test

[c]N=160 for men, N=155 for women

[d]N=125 for men, N=125 for women

[e]N=159 for men, N=159 for women

[f]N=154 for men, N=152 for women

[g]N=162 for men, N=159 for women

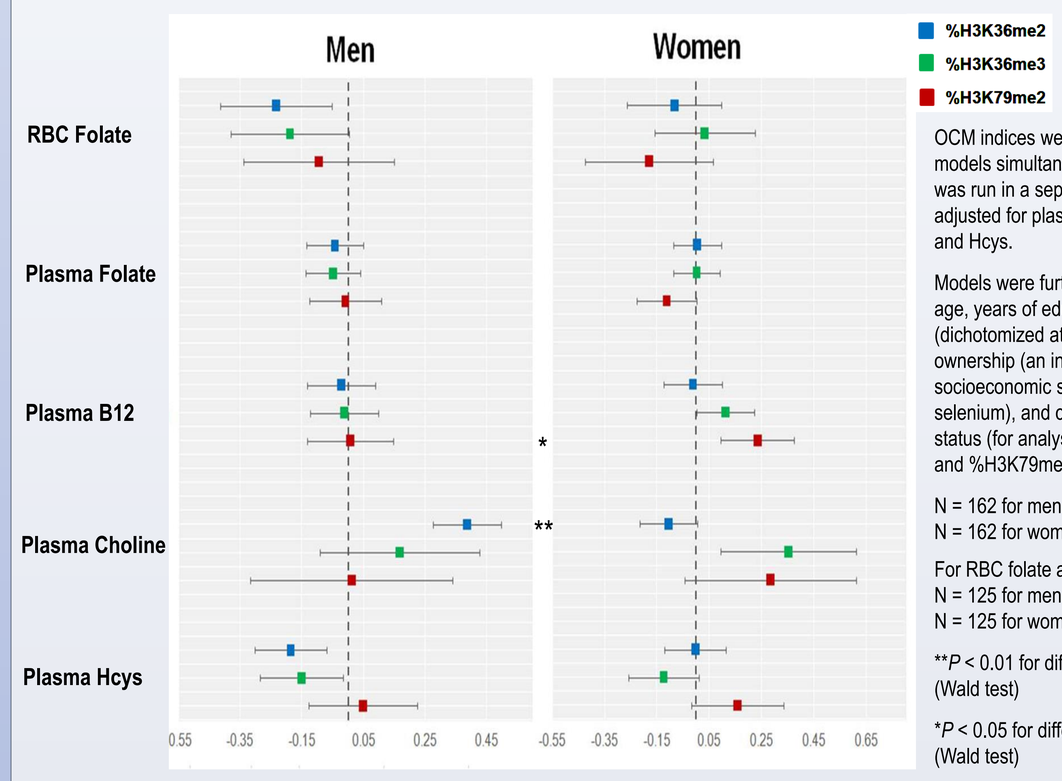
[h]Plasma folate < 9 nmol/L

[i]Plasma B12 < 151 pmol/L

[j]Plasma Hcys > 13 µmol/L

RESULTS

Fig. 3. Associations (β and 95% CI) between OCM indices and %PTHMs



OCM indices were included in models simultaneously. RBC folate was run in a separate model and adjusted for plasma B12, choline, and Hcys.

Models were further adjusted for age, years of education (dichotomized at 5 years), TV ownership (an indicator of socioeconomic status), log(blood selenium), and cigarette smoking status (for analyses of %H3K36me3 and %H3K79me2 in men)

N = 162 for men
N = 162 for women

For RBC folate analyses:
N = 125 for men
N = 125 for women

***P* < 0.01 for difference by sex (Wald test)

**P* < 0.05 for difference by sex (Wald test)

Table 2. Median (IQR) Change in %PTHMs from Baseline to Week 12 by Treatment Arm

	Placebo		400 µg FA		
%PTHM	**Median (IQR)**	**N**	**Median (IQR)**	**N**	***P*[a]**
%H3K36me2	-0.15 (-0.43, 0.11)	56	-0.05 (-0.39, 0.11)	103	0.39
%H3K36me3	0.02 (-0.23, 0.30)	55	0.02 (-0.28, 0.27)	98	0.77
%H3K79me2	-0.05 (-0.24, 0.04)	56	-0.06 (-0.28, 0.14)	97	0.80

IQR = Interquartile range

[a]Wilcoxon rank sum test for difference in intra-person change in %PTHM between placebo and 400 µg FA groups.

SUMMARY

•Men (but not women) with higher plasma choline and lower plasma Hcys had higher %H3K36me2 (*P* < 0.05). There was also a trend for increased %H3K36me3 with higher plasma choline and lower plasma Hcys that was observed in both men and women.

•Women (but not men) with higher plasma B12 had higher %H3K36me3 and %H3K79me2 (*P* < 0.05)

•Plasma folate and FA treatment were not associated with alterations in %PTHMs. However, %H3K36me2 and %H3K36me3 were lower (P < 0.05 and P < 0.10, respectively) in men with higher RBC folate (a marker of long-term folate status)

CONCLUSIONS

•Effects of OCM indices on %PTHMs differed by sex. This may be due to differing ranges of nutritional indices in men vs. women or the fact that some histone demethylases form complexes with androgen receptor [13] and others are dosage-sensitive regulators that reside on the Y chromosome [14].

•The inverse relationships between RBC folate and %PTHMs in men may reflect the potential dual role of folate in regulating %PTHMs; in addition to being a methyl donor, there is evidence that folate may facilitate histone demethylation by accepting the removed one-carbon group [15].

•Consistent with the cross-sectional findings for plasma folate, supplementation with 400 µg FA for 12 weeks did not influence %PTHMs in PBMCs. However, we cannot rule out the possibility that other %PTHMs, or other target tissues, may have been affected, or that the dose or duration was insufficient to alter %PTHMs.

•%PTHMs were differentially affected by OCM indices. This has been observed by other groups [3, 7], and suggests that some KHMTs may be more sensitive than others to alterations in the methyl pool.

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CONTACT INFORMATION

Caitlin Howe, Ph.D. Candidate, E-mail: cgh2121@cumc.columbia.edu