Supplemental Material to:

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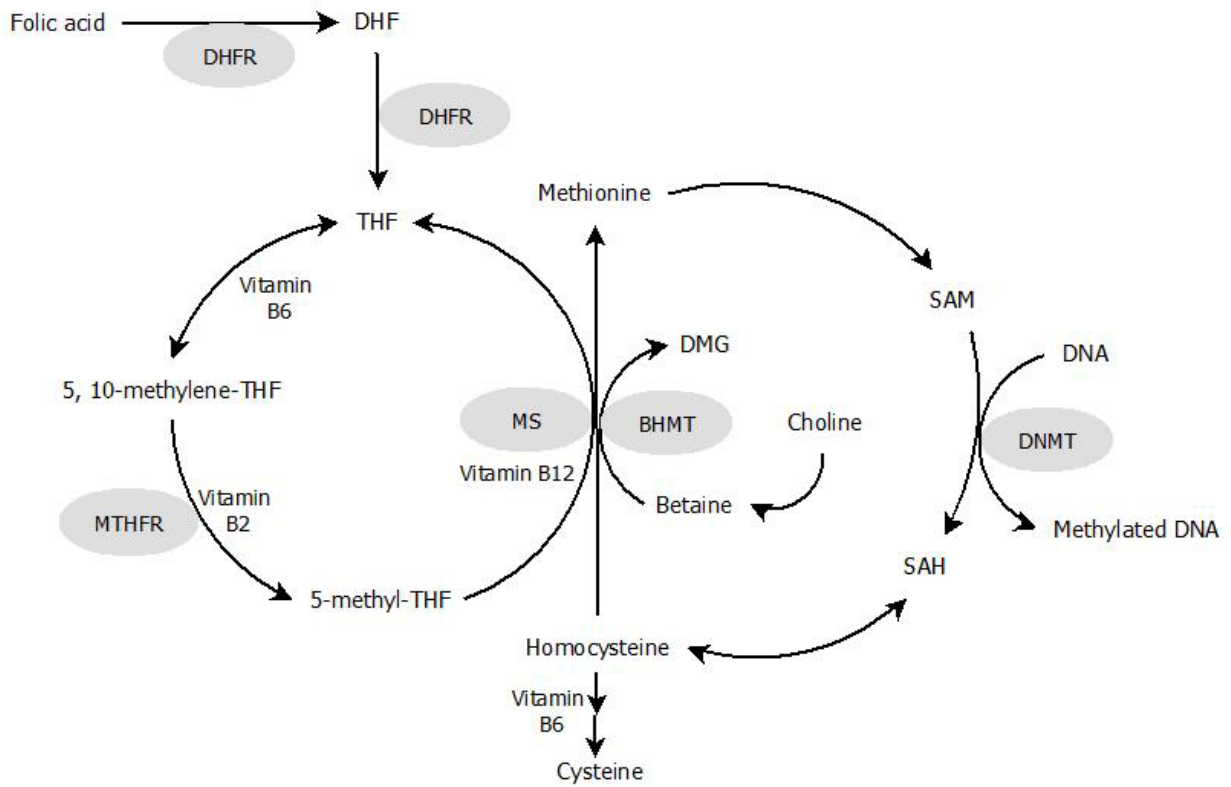
Impact of folic acid fortification on global DNA methylation and one-carbon biomarkers in the Women's Health Initiative Observational Study cohort

Supplementary Figure 1.

Simplified diagram of folate- and choline-mediated DNA methylation reactions. Relevant enzymes are highlighted in gray. Abbreviations: BHMT, betaine homocysteine methyltransferase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; DMG, dimethylglycine; DNMT, DNA methyltransferase; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate.

Supplementary Table 1. Overall R^2 explained by all one-carbon biomarkers according to folic acid (FA) fortification period[1,2]

	Overall unadjusted R^2
Pre-fortification (1994-1995)	0.12
Peri-fortification (1996-1997)	0.12
Post-fortification (1998)	0.19

[1]Unadjusted linear regression models were used by including all variables simultaneously.
[2]n=73 in the pre-fortification period; n=123 in the peri-fortification period; n=45 in the post-fortification period.

Supplementary Table 2. Predictors of baseline leukocyte global DNA methylation according to folic acid (FA) fortification period with all variables included in the statistical model[1, 2, 3]

	FA fortification period								
	Pre- (1994-1995)			Peri- (1996-1997)			Post- (1998)		
	β Coefficient	*P* value	Partial R^2	β Coefficient	*P* value	Partial R^2	β Coefficient	*P* value	Partial R^2
Plasma folate (ng/mL)	-1.16	0.79	0.001	-3.20	0.19	0.015	9.42	0.13	0.066
RBC folate (ng/mL)	0.11	0.53	0.006	-0.13	0.24	0.012	-0.18	0.52	0.012
Plasma vitamin B12 (pg/mL)	0.04	0.84	0.001	-0.08	0.65	0.002	0.00	0.99	0.000
Plasma MMA (nmol/L)	-0.49	0.38	0.012	0.26	0.55	0.003	-0.36	0.64	0.007
Plasma choline (µmol/L)	-44.37	0.06	0.057	-10.37	0.50	0.004	-37.03	0.42	0.019
Plasma betaine (µmol/L)	-1.78	0.75	0.002	-1.83	0.61	0.002	8.60	0.39	0.022
Plasma DMG (µmol/L)	-0.85	0.99	0.000	61.89	0.07	0.029	-89.57	0.46	0.016
Plasma TMAO (µmol/L)	7.10	0.31	0.016	0.72	0.86	0.000	-7.05	0.53	0.012
Plasma Hcy (µmol/L)	16.22	0.51	0.007	11.84	0.52	0.004	72.77	0.08	0.088
Plasma cysteine (µmol/L)	0.08	0.96	0.000	0.06	0.95	0.000	-2.10	0.36	0.025

[1]Unadjusted linear regression models were used by including all variables simultaneously.
[2]Beta (β) coefficient indicates mean increase in DNA methylation per 1000-unit increase in one-carbon biomarker.
[3]n=73 in the pre-fortification period; n=123 in the peri-fortification period; n=45 in the post-fortification period.
Abbreviations used: RBC, red blood cell; MMA, methylmalonic acid; DMG, dimethylglycine; TMAO, trimethylamine *N*-oxide; Hcy, homocysteine.

Supplementary Table 3. Plasma folate concentrations (ng/mL) within the lowest and highest RBC folate groups according to folic acid (FA) fortification period[1]

	Lowest RBC Folate group		Highest RBC Folate group		
	n	Median (IQR)	n	Median (IQR)	*P* value
Pre-fortification (1994-1995)	70	8.5 (5.8-14.6)	49	25.1 (17.3-37.5)	< 0.001
Peri-fortification (1996-1997)	100	9.2 (5.8-13.7)	100	23.0 (17.2-30.3)	< 0.001
Post-fortification (1998)	28	12.8 (9.7-20.0)	51	25.3 (17.5-34.7)	< 0.001

[1]Linear regression models were used to compare median plasma folate concentrations between RBC folate groups and were adjusted for age, BMI, ethnicity, creatinine, and *MTHFR* C677T genotype.

Supplementary Table 4. Folic acid (FA) supplement use (%) within the lowest (n=202) and highest (n=206) RBC folate groups.

FA supplement use	Lowest RBC folate group	Highest RBC folate group
Yes	19.8% (40/202)	75.7% (156/206)
No	80.2% (162/202)	24.3% (50/206)

Supplementary Table 5. RBC folate concentrations (ng/mL) within folic acid (FA) supplement users and non-supplement users according to FA fortification period[1]

	FA supplement users		Non-FA supplement users		
	n	Mean ± SD	n	Mean ± SD	*P* value
Pre-fortification (1994-1995)	56	761 ± 282	66	386 ± 173	< 0.001
Peri-fortification (1996-1997)	103	820 ± 317	101	450 ± 220	< 0.001
Post-fortification (1998)	37	814 ± 276	45	582 ± 247	0.0495

[1]Linear regression models were used to compare mean RBC folate concentrations between FA supplement users and non-supplement users and were adjusted for age, BMI, ethnicity, creatinine, and *MTHFR* C677T genotype.