Supplemental Table 1. Information on laboratory method used to measure biomarkers of water-soluble vitamin status in adults ≥20 y during all of part of NHANES 2003–2006[1].

Biomarker	Matrix	Survey cycle	Laboratory method	Imprecision	LOD[2]
FOL	Serum	2003–2006	Bio-Rad radioassay	2.5–8.7% at 1.60–15.1 µg/L	0.1 µg/L
FOL	Whole blood	2003–2006	Bio-Rad radioassay	1.6–4.8% at 63.6–631 µg/L	20 µg/L RBC
PLP	Serum	2005–2006	HPLC-fluorescence	2.5–8.3% at 9.95–63.7 nmol/L	0.3 nmol/L
4PA	Serum	2005–2006	HPLC-fluorescence	2.4–9.7% at 10.5–50.6 nmol/L	0.3 nmol/L
B-12	Serum	2003–2006	Bio-Rad radioassay	1.7–4.4% at 358–916 ng/L	20 ng/L
tHcy	Plasma (EDTA)	2003–2004	Abbott FPIA	2.3–4.6% at 6.51–26.5 µmol/L	0.35 µmol/L
MMA	Plasma (EDTA)	2003–2004	GC-MS	2.8–6.8% at 138–10200 nmol/L	50 nmol/L
VIC	Serum	2003–2006	HPLC-electrochemical	1.7–7.6% at 13.1–126 µmol/L	0.68 µmol/L

[1] 4PA, 4-pyridoxic acid; B12, total cobalamin; FOL, folate; FPIA, fluorescence polarization immunoassay; MMA, methylmalonic acid; PLP, pyridoxal-5'-phosphate; tHcy, total homocysteine; VIC, ascorbic acid. SI conversion factors are as follows: FOL, ×2.266 (nmol/L) and B12, ×0.738 (pmol/L).

[2] LOD, limit of detection

Supplemental Table 2. Sample sizes for water-soluble vitamin status biomarkers by sociodemographic and lifestyle variables for adults ≥20 y, NHANES 2003–2006[1,2].

	S-FOL	RBC-FOL	PLP	4PA	B-12	tHcy	MMA	VIC
Age (y)								
20–39	3242	3262	1699	1698	3214	3267	1496	3233
40–59	2649	2649	1381	1381	2629	2651	1230	2635
≥60	3053	3069	1409	1409	3035	3081	1612	3024
Sex								
Males	4301	4313	2155	2154	4271	4329	2095	4296
Females	4643	4667	2334	2334	4607	4670	2243	4596
Race-ethnicity								
Mexican American	1803	1811	912	911	1798	1814	866	1799
Non-Hispanic black	1845	1875	1001	1001	1841	1871	836	1840
Non-Hispanic white	4658	4652	2266	2266	4605	4670	2316	4616
Education								
<High school	2535	2558	1245	1245	2516	2562	1268	2529
High school	2185	2189	1059	1059	2167	2200	1094	2169
>High school	4212	4220	2180	2179	4183	4224	1968	4182
PIR[3]								
Low	3476	3499	1672	1672	3451	3507	1754	3455
Medium	2256	2271	1160	1159	2235	2269	1067	2246
High	2767	2760	1456	1456	2750	2772	1271	2749

Online Supporting Material

	S-FOL	RBC-FOL	PLP	4PA	B-12	tHcy	MMA	VIC
Supplement use[4]								
No	4286	4322	2215	2215	4259	4322	2019	4278
Yes	4646	4646	2271	2270	4607	4665	2310	4602
Smoking[5]								
No	6591	6572	3333	3332	6548	6602	3175	6562
Yes	2327	2320	1143	1143	2305	2332	1128	2324
Alcohol consumption[6]								
No drinks	2993	3011	1475	1475	2971	3015	1472	2970
<1 (not 0)	4313	4319	2172	2171	4276	4330	2088	4291
1–<2	543	549	284	284	540	547	253	541
≥2	426	426	231	231	423	429	196	423
BMI[7]								
Underweight	136	138	73	73	136	137	64	134
Normal weight	2576	2593	1263	1263	2557	2595	1276	2552
Overweight	3069	3074	1518	1518	3052	3092	1503	3053
Obese	3003	3015	1563	1562	2976	3014	1406	2996
Physical activity[8]								
None reported	3315	3339	1612	1612	3289	3343	1675	3299
0–<500	1977	1984	994	994	1960	1985	946	1963
500–<1000	1086	1090	551	551	1077	1093	518	1082
≥1000	2240	2239	1176	1175	2228	2249	1034	2226

Online Supporting Material

[1] 4PA, 4-pyridoxic acid; B-12, vitamin B-12 (total cobalamin); MMA, methylmalonic acid; PLP, pyridoxal-5'-phosphate; RBC-FOL, RBC folate; S-FOL, serum folate; tHcy, total homocysteine; VIC, vitamin C (ascorbic acid)

[2] PLP and 4PA data only available for NHANES 2005–2006; MMA data only available for NHANES 2003–2004

[3] PIR, family poverty income ratio: 0–1.85 (low); >1.85–3.5 (medium); >3.5 (high)

[4] "Supplement user" defined as participant who reported taking any dietary supplement within the past 30 d

[5] "Smoker" defined by serum cotinine concentration >10 μg/L

[6] Alcohol consumption: calculated as average daily consumption [(quantity x frequency) / 365.25]; 1 drink ≈ 15 g ethanol

[7] BMI (kg/m^2) definitions: <18.5 (underweight); 18.5–<25 (normal weight); 25–<30 (overweight); and ≥30 (obese)

[8] Physical activity: calculated as total metabolic equivalent task (MET)-min/wk from self-reported leisure time physical activities

Supplemental Table 3. Descriptive information of the respondent characteristics by variable for adults ≥20 y, NHANES 2003–2006

Variable	Category	2003–2006[1]	2003–2004[1]	2005–2006[1]
Age, y	20–39	38.4	38.8	38.0
	40–59	38.8	38.5	39.0
	≥60	22.8	22.7	23.0
Sex	Male	48.0	48.0	48.1
	Female	52.0	52.1	51.9
Race-ethnicity	Mexican American	7.9	7.8	8.0
	Non-Hispanic black	11.4	11.2	11.5
	Non-Hispanic white	72	72.1	71.8
	Other Hispanic	3.5	3.6	3.4
	Other (including multiracial)	5.4	5.4	5.4
Education	≤High school	44.2	45.5	42.8
	>High school	55.9	54.5	57.2
PIR[2]	Low	29.3	31.4	27.4
	Middle	28.0	27.6	28.4
	High	42.7	41.1	44.3
Supplement use[3]	No	45.9	46.0	45.9
	Yes	54.1	54.0	54.2
Smoking status[4]	No	71.2	70.2	72.1
	Yes	28.9	29.8	27.9
Alcohol consumption[5]	No drinks	29.4	30.6	28.2
	<1 (not 0)	56.8	56.6	57.0
	1–<2	7.9	7.6	8.1
	≥2	6.0	5.2	6.7
BMI[6]	Underweight	1.8	1.7	1.8
	Normal weight	31.6	32.0	31.2
	Overweight	33.4	34.1	32.8
	Obese	33.3	32.2	34.3
Physical activity[7]	None reported	32.1	33.0	31.3
	0–<500	24.2	25.0	23.4
	500–<1000	14.0	13.6	14.3
	≥1000	29.7	28.4	31.1

[1] Estimates provided are weighted percent (%)

[2] PIR, family poverty income ratio: 0–1.85 (low); >1.85–3.5 (medium); >3.5 (high)

[3] "Supplement user" defined as participant who reported taking any dietary supplement within the past 30 d

[4] "Smoker" defined by serum cotinine concentration >10 μg/L

[5] Alcohol consumption: average daily consumption [(quantity x frequency) / 365.25]; 1 drink ≈ 15 g ethanol

[6] BMI (kg/m^2) definitions: <18.5 (underweight); 18.5–<25 (normal weight); 25–<30 (overweight); and ≥30 (obese)

[7] Physical activity: calculated as total metabolic equivalent task (MET)-min/wk from self-reported leisure time physical activities

Supplemental Table 4. *Beta* coefficients from chunk-wise modeling approach for water-soluble vitamin status biomarkers by sociodemographic and lifestyle variables for adults ≥20 y, NHANES 2003–2006[1,2]

	S-FOL	RBC-FOL	PLP	4PA	B-12	tHcy	MMA	VIC
Age: continuous, every 10 y increase								
Model 1	0.10*	0.07*	-0.00	0.19*	0.02*	0.08*	0.09*	2.68*
Model 2	0.09*	0.06*	0.01	0.18*	0.02*	0.08*	0.09*	2.81*
Model 3	0.07*	0.04*	-0.02*	0.14*	0.01*	0.09*	0.09*	1.85*
Sex: males vs. females								
Model 1	-0.13*	-0.07*	0.18*	-0.02	-0.01	0.18*	0.04*	-8.92*
Model 2	-0.12*	-0.07*	0.17*	-0.00	-0.01	0.19*	0.06*	-8.87*
Model 3	-0.06*	-0.04*	0.24*	0.09*	0.04*	0.16*	0.05*	-5.66*
Race-ethnicity[3]: MA vs. NHW								
Model 1	-0.23*	-0.15*	-0.11*	-0.51*	0.10*	-0.17*	-0.25*	-3.56*
Model 2	-0.07*	-0.05*	0.06	-0.18*	0.14*	-0.12*	-0.21*	4.39*
Model 3	-0.06*	-0.05*	0.08	-0.14*	0.14*	-0.11*	-0.24*	5.34*
Race-ethnicity[3]: NHB vs. NHW								
Model 1	-0.27*	-0.29*	-0.31*	-0.54*	0.12*	-0.02	-0.24*	-4.59*
Model 2	-0.20*	-0.24*	-0.21*	-0.39*	0.15*	0.01	-0.23*	-0.93
Model 3	-0.14*	-0.22*	-0.08	-0.27*	0.18*	0.00	-0.25*	3.39*
PIR[4]: continuous								
Model 1	0.04*	0.03*	0.11*	0.11*	0.00	-0.01*	-0.00	2.51*
Model 2	0.03*	0.02*	0.08*	0.06*	0.01*	-0.02*	-0.02*	2.02*
Model 3	0.01	0.00	0.04*	0.03*	0.00	-0.01*	-0.01*	0.85*

Online Supporting Material

	S-FOL	RBC-FOL	PLP	4PA	B-12	tHcy	MMA	VIC
Education: ≤high school vs. >high school								
Model 1	-0.10*	-0.07*	-0.27*	-0.21*	-0.01	0.06*	0.03	-7.62*
Model 2	-0.08*	-0.06*	-0.19*	-0.15*	-0.02	0.02	0.01	-6.41*
Model 3	-0.00	-0.01	-0.02	-0.01	0.03	-0.00	-0.02	-1.80*
Supplement use[5]: yes vs. no								
Model 1	0.44*	0.30*	0.61*	0.87*	0.18*	-0.05*	-0.03	20.9*
Model 3	0.33*	0.22*	0.58*	0.72*	0.19*	-0.09*	-0.13*	16.2*
Smoking[6]: yes vs. no								
Model 1	0.28*	0.21*	0.33*	0.37*	0.10*	-0.09*	0.01	16.6*
Model 3	0.16[3]	0.13*	0.32*	0.20*	0.07*	-0.08*	0.01	11.0*
Alcohol consumption[7]: continuous, ln + 1								
Model 1	-0.12*	-0.05*	0.21*	-0.02	-0.06*	0.08*	-0.06*	-5.11*
Model 3	-0.04*	0.02	0.15*	-0.00	-0.05*	0.05*	-0.03	-1.16
BMI: continuous, ln								
Model 1	-0.19*	0.15*	-0.70*	-0.33*	-0.18*	0.05*	-0.02	-23.6*
Model 3	-0.19*	0.17*	-0.61*	-0.34*	-0.20*	-0.00	-0.08	-23.7*
Physical activity[8]: continuous, ln + 1								
Model 1	0.01*	0.01*	0.06*	0.03*	0.01*	-0.01*	-0.02*	1.39*
Model 3	0.01*	0.00*	0.02*	0.01	0.00	-0.00	-0.01*	0.87*
Sample size (*n*)								
Model 2	8493	8523	4287	4286	8430	8541	4086	8444
Model 3	7482	7460	3804	3803	7424	7494	3558	7456

R^2 value (%)								
Model 2	14[&]	15[&]	6[&]	13[&]	2[&]	27[&]	13[&]	8[&]
Model 3	26[&]	26[&]	23[&]	25[&]	7[&]	29[&]	15[&]	22[&]

[1] Model 1, simple linear regression; model 2, multiple linear regression by adjusting for sociodemographic variables; model 3, multiple linear regression by adjusting for sociodemographic and lifestyle variables; change in covariate was carried out while holding any other variables in the model constant

[2] For all biomarkers except serum vitamin C, the dependent variable was the natural log-transformed biomarker concentration; for vitamin C, the dependent variable was the untransformed concentration; 4PA (nmol/L), 4-pyridoxic acid; B-12 (ng/L), vitamin B-12 (total cobalamin); MMA (nmol/L), methylmalonic acid; PLP (nmol/L), pyridoxal-5'-phosphate; RBC-FOL (µg/L), RBC folate; S-FOL (µg/L), serum folate; tHcy (µmol/L), total homocysteine; VIC (µmol/L), vitamin C (ascorbic acid); PLP and 4PA data only available for NHANES 2005–2006; MMA data only available for NHANES 2003–2004

[3] MA, Mexican American; NHB, non-Hispanic black; NHW, non-Hispanic white

[4] PIR, family poverty income ratio

[5] "Supplement user" defined as participant who reported taking any dietary supplement within the past 30 d

[6] "Smoker" defined by serum cotinine concentration >10 µg/L

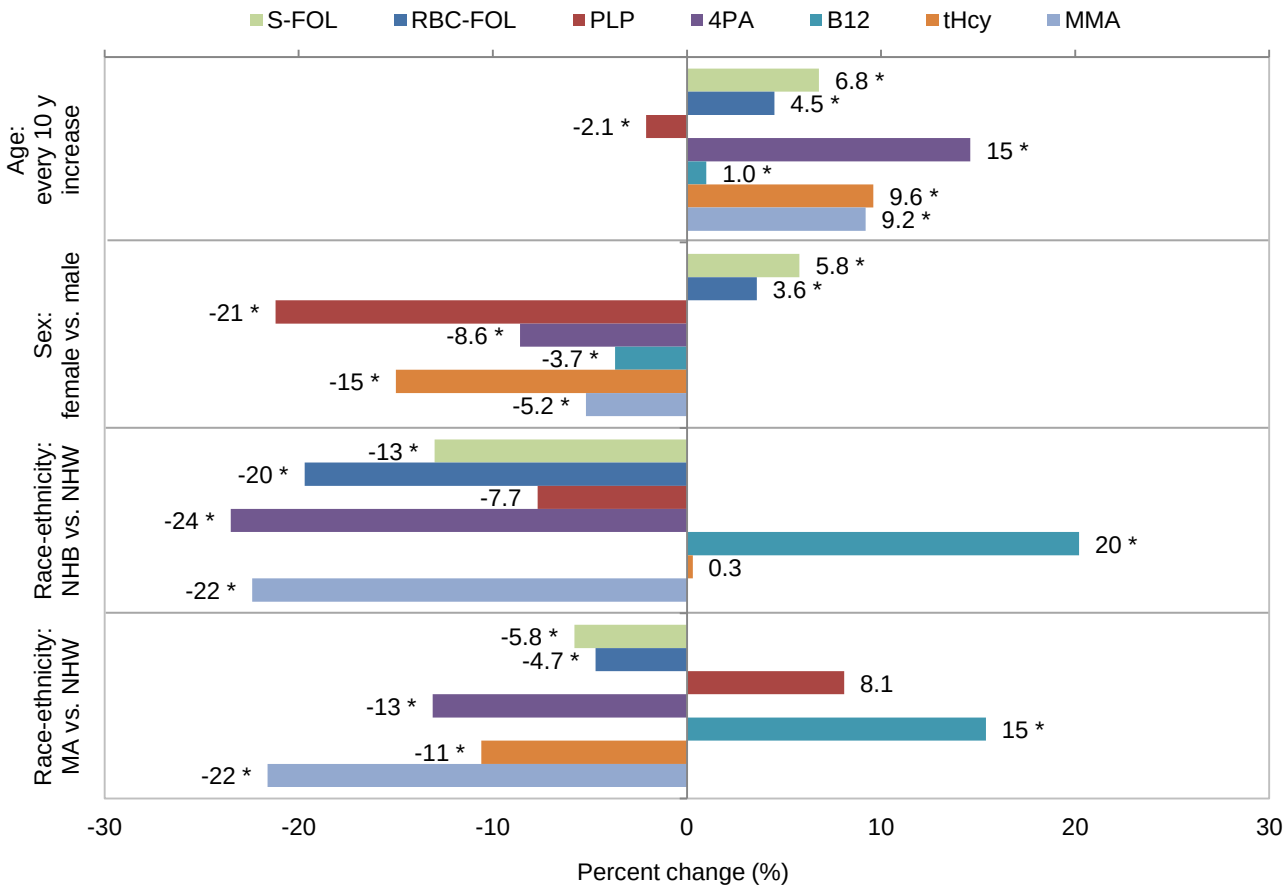
[7] Alcohol consumption: calculated as average daily consumption [(quantity x frequency) / 365.25]; 1 drink ≈ 15 g ethanol

[8] Physical activity: calculated as total metabolic equivalent task (MET)-min/wk from self-reported leisure time physical activities

[*] Wald F *P*-value <0.05; *beta* coefficient is significantly different from zero

[&] Satterthwaite adjusted F *P*-value for chunk test <0.05; testing whether at least one *beta* coefficient for the set of variables in the chunk is significantly different from zero

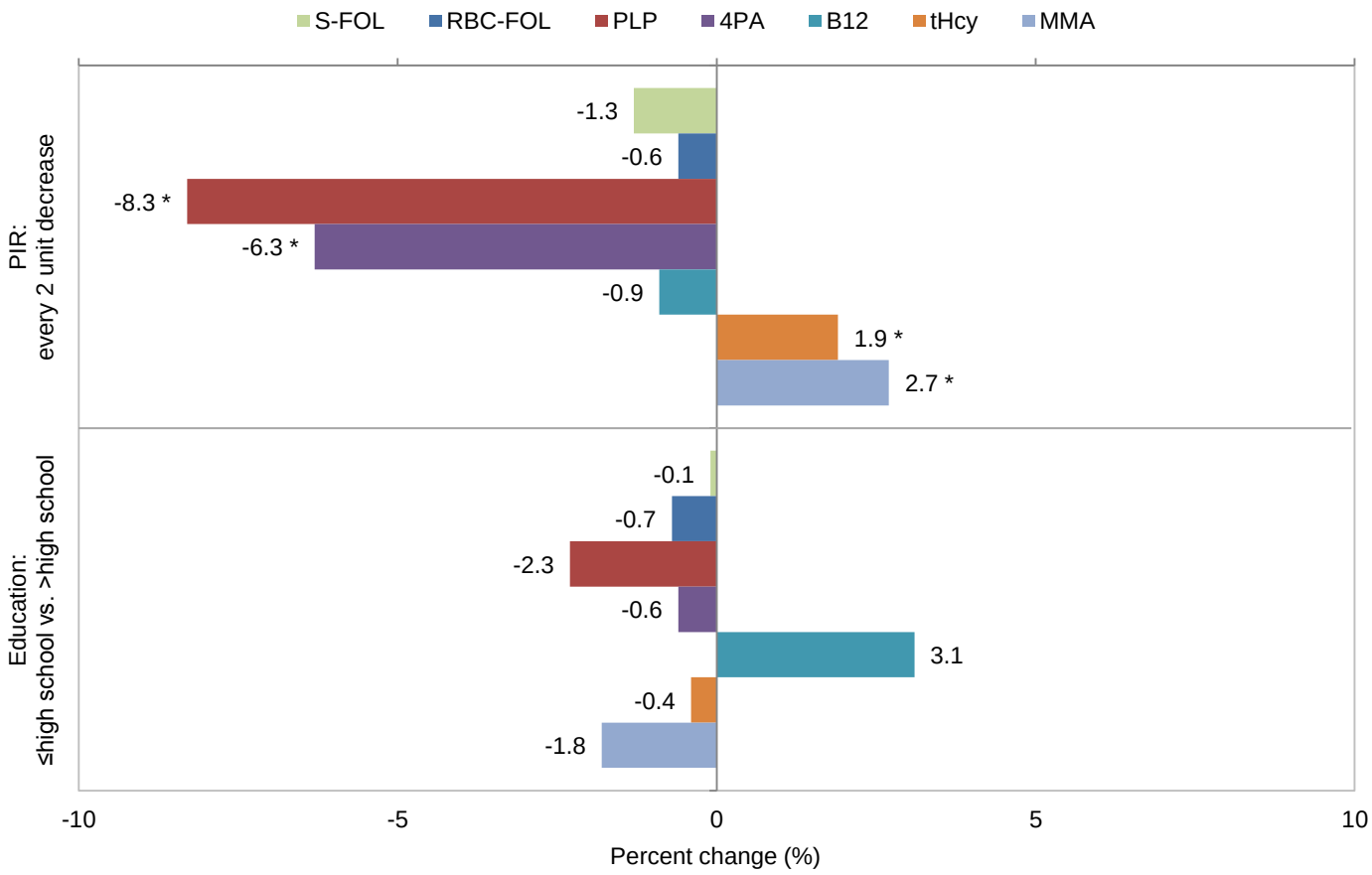
Supplemental Figure 1. Estimated changes in biomarker concentrations of B vitamin status with changes in the demographic variables age, gender, and race-ethnicity using data for adults ≥20 y, NHANES 2003–2006.



Changes derived from a multiple linear regression model adjusting for sociodemographic and lifestyle variables (model 3); change in a covariate was carried out while holding any other variables in the model constant; asterisk accompanying the percent change in the data label indicates significance (P <0.05)

4PA, 4-pyridoxic acid; B12, vitamin B12 (total cobalamin); MA, Mexican American; MMA, methylmalonic acid; NHB, non-Hispanic black; NHW, non-Hispanic white; PLP, pyridoxal-5'-phosphate; RBC-FOL, RBC folate; S-FOL, serum folate; tHcy, total homocysteine

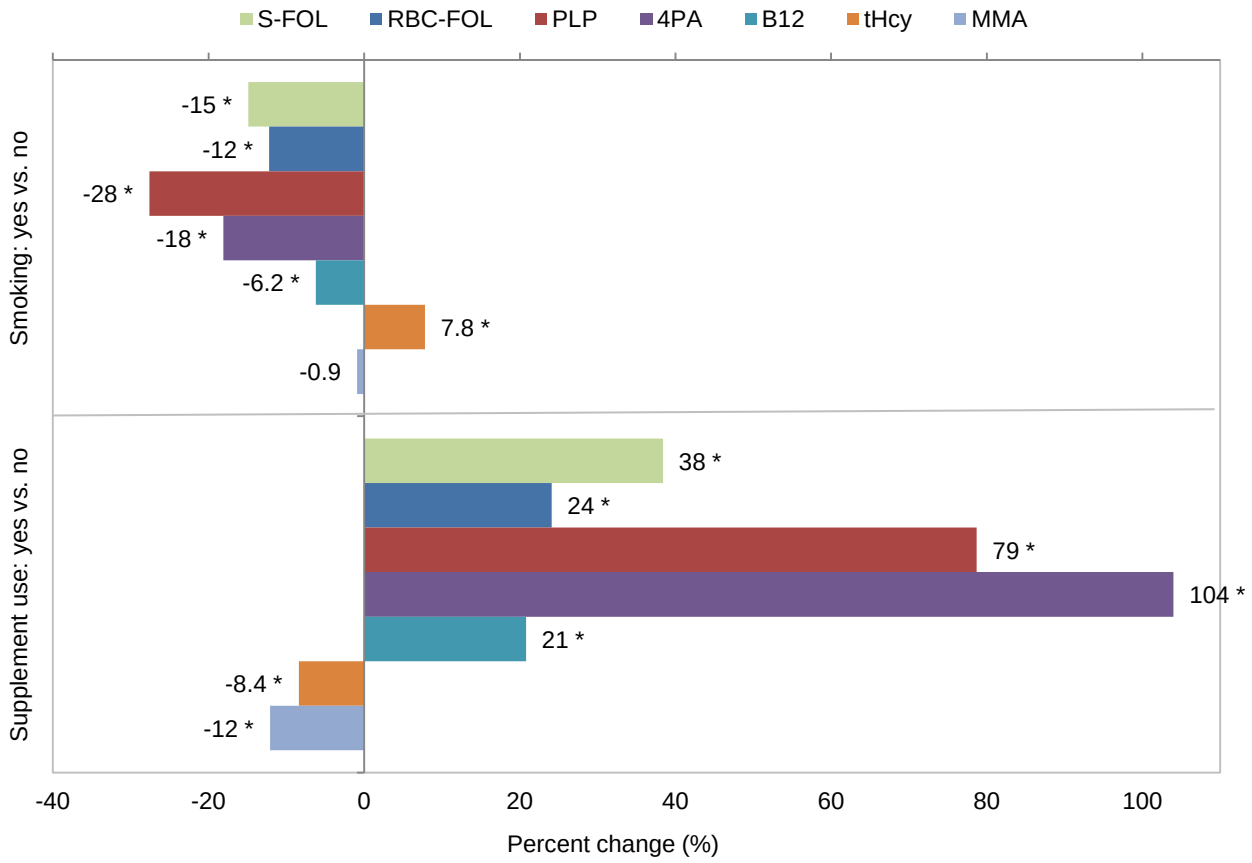
Supplemental Figure 2. Estimated changes in biomarker concentrations of B vitamin status with changes in the socioeconomic variables PIR and education using data for adults ≥20 y, NHANES 2003–2006.



Changes derived from a multiple linear regression model adjusting for sociodemographic and lifestyle variables (model 3); change in a covariate was carried out while holding any other variables in the model constant; asterisk accompanying the percent change in the data label indicates significance (P <0.05)

4PA, 4-pyridoxic acid; B12, vitamin B12 (total cobalamin); MMA, methylmalonic acid; PIR, family poverty income ratio; PLP, pyridoxal-5'-phosphate; RBC-FOL, RBC folate; S-FOL, serum folate; tHcy, total homocysteine

Supplemental Figure 3. Estimated changes in biomarker concentrations of B vitamin status with changes in the lifestyle variables smoking and dietary supplement use using data for adults ≥20 y, NHANES 2003–2006.



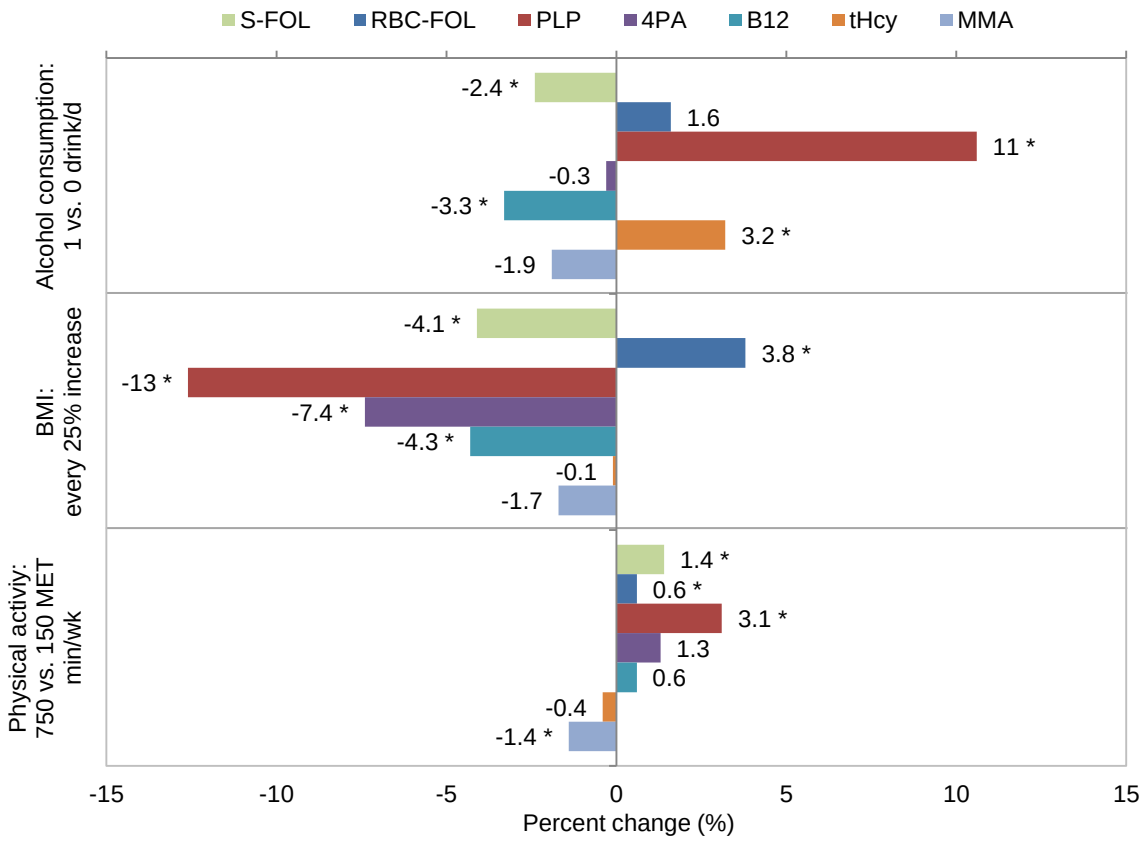
Changes derived from a multiple linear regression model adjusting for sociodemographic and lifestyle variables (model 3); change in a covariate was carried out while holding any other variables in the model constant; asterisk accompanying the percent change in the data label indicates significance (P <0.05)

"Smoker" defined by serum cotinine concentration >10 µg/L

"Supplement user" defined as participant who reported taking a dietary supplement within the past 30 d

4PA, 4-pyridoxic acid; B12, vitamin B12 (total cobalamin); MMA, methylmalonic acid; PLP, pyridoxal-5'-phosphate; RBC-FOL, RBC folate; S-FOL, serum folate; tHcy, total homocysteine

Supplemental Figure 4. Estimated changes in biomarker concentrations of B vitamin status with changes in the lifestyle variables alcohol consumption, BMI, and physical activity using data for adults ≥20 y, NHANES 2003–2006.



Changes derived from a multiple linear regression model adjusting for sociodemographic and lifestyle variables (model 3); change in a covariate was carried out while holding any other variables in the model constant; asterisk accompanying the percent change in the data label indicates significance (P <0.05)

Alcohol consumption calculated as average daily consumption [(quantity x frequency) / 365.25]; 1 drink ≈ 15 g ethanol

A 25% increase in BMI is comparable to a change from being normal weight to overweight

Physical activity calculated as total metabolic equivalent task (MET)-min/wk from self-reported leisure time physical activities

4PA, 4-pyridoxic acid; B12, vitamin B12 (total cobalamin); MMA, methylmalonic acid; PLP, pyridoxal-5'-phosphate; RBC-FOL, RBC folate; S-FOL, serum folate; tHcy, total homocysteine