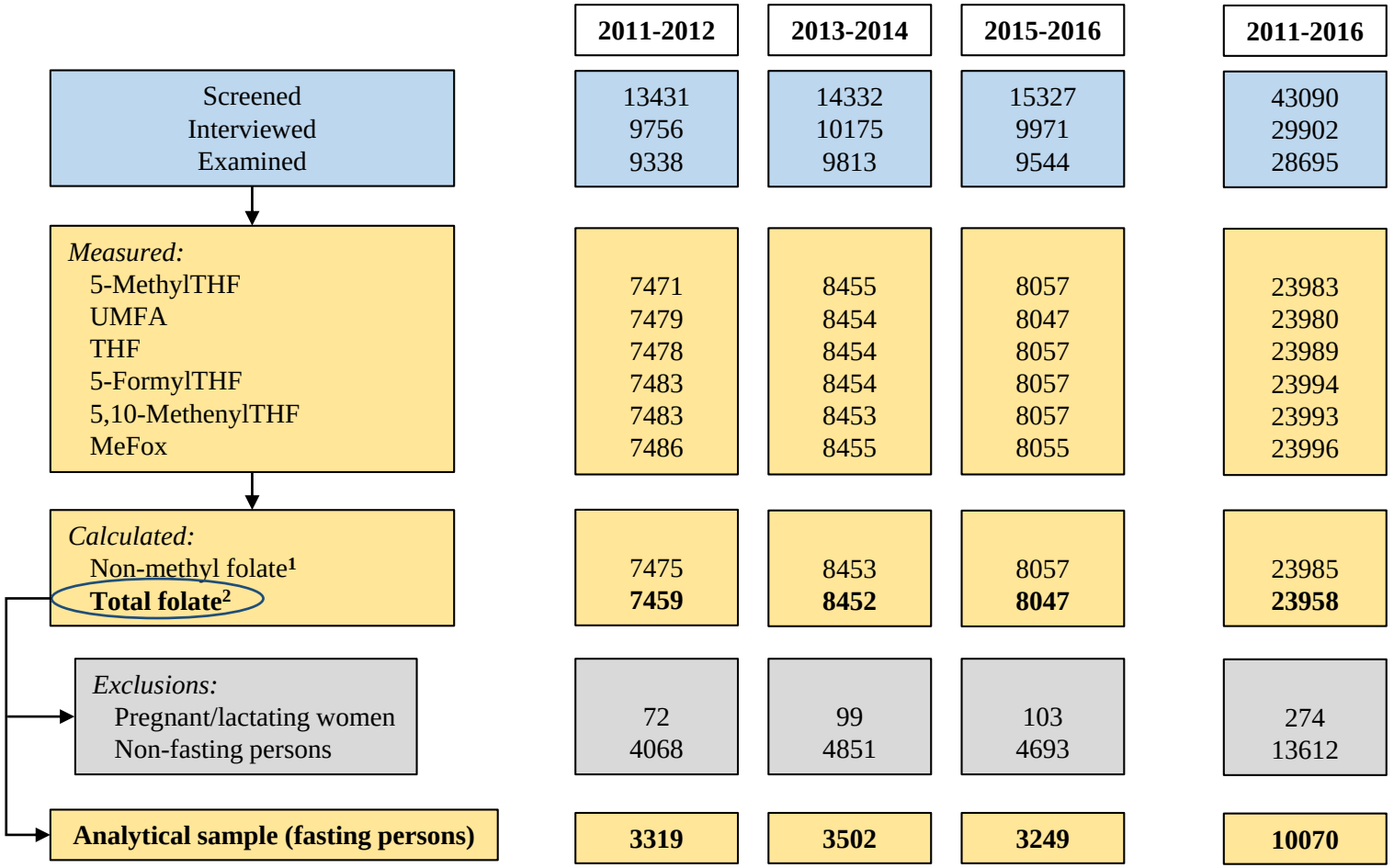
Supplementary Figure 1. Flowchart with number of US persons who were screened, interviewed, examined, for whom serum folate forms were measured, and who were excluded due to pregnancy/lactation and non-fasting status, NHANES 2011-2016



[1] Non-methyl folate is the sum of 3 minor folate forms (THF, 5-formylTHF, and 5,10-methenylTHF).
[2] Total folate is the sum of biologically active folate forms (5-methylTHF, UMFA, and non-methyl folate), not including MeFox. During 2015-2016, there were 2 persons with missing MeFox data (i.e., included in total folate, but not included in analytical sample).
5-FormylTHF, 5-formyltetrahydrofolate; 5,10-methenylTHF, 5,10-methenyltetrahydrofolate; 5-methylTHF, 5-methyltetrahydrofolate; MeFox, pyrazino-s-triazine derivative of 4α-hydroxy-5-methylTHF; THF, tetrahydrofolate; UMFA, unmetabolized folic acid.

Supplementary Methods. Additional information for methods section.

Biomarker measurements

The analytical variability of serum UMFA, a minor contributor to total folate (~2%), was higher during 2011–2014 (≥10%) when UMFA measurements were biased high (~25%) due to folic acid solubility issues in the calibrator stock solution and during the preparation of the calibration curve, resulting in overestimation of UMFA concentrations. The CDC laboratory conducted a crossover study to generate a correction equation (11). The data adjustment resulted in ~25% lower concentrations across the entire UMFA distribution, while serum total folate decreased by only ~1–2%. The National Center for Health Statistics adjusted the 2011–2014 UMFA data prior to data release using the correction equation, while 2015–2016 samples were analyzed with the new and correct folic acid calibrator requiring no adjustment. The correction of the folic acid calibration bias also reduced the analytical variability in 2015–2016 (<10%).

Statistical analyses

Concentrations by NHANES survey cycle. For analytes where >40% of results were <LOD (5-formylTHF and 5,10-methenylTHF), we compared the proportion of results ≥LOD across survey cycles using a chi-square test, while for the remaining analytes we compared the geometric means across survey cycles using a Wald *F* test. Using all available serum folate forms data from NHANES 2011–2012, 2013–2014, and 2015–2016, we found comparable median concentrations (nmol/L) for total folate (41.3, 42.9, and 39.9, respectively; $P = 0.12$) and 5-methylTHF (38.4, 40.5, and 37.9, respectively; $P = 0.12$) and small yet significant differences for minor forms: UMFA (0.74, 0.80, and 0.63, respectively; $P < 0.0001$); non-methyl folate (1.43, 1.10, and 1.11, respectively; $P = 0.0008$); and MeFox (1.49, 1.53, and 1.44, respectively; $P = 0.0307$) across survey cycles (**Supplementary Table 2**). While most samples had detectable concentrations of 5-methylTHF (100%), UMFA (>99%), MeFox (>98%), and THF (>85%) (data not shown), only a small portion of samples had detectable concentrations of 5-formylTHF (<10%) and 5,10-methenylTHF (≤11%) (Table 1). Because the minor folate forms are mostly present at low concentrations and slight variability in their concentrations over time is not unusual, we combined the data for the 3 survey cycles.

Supplementary Table 1. Quality parameters of the HPLC-tandem mass spectrometry method used to measure serum folate forms in the US population ≥1 y, NHANES 2011–2016[1]

Biomarker	QC material[2]	Concentration range[3]	Imprecision range[4]	Independent analytical runs	LOD		
					2011–2012	2013–2014	2015–2016
		nmol/L	*%*	*n*		*nmol/L*	
5-MethylTHF	Level 1	8.51–19.4	1.1–2.9	421	0.31	0.06	0.13
	Level 2	24.6–35.8	1.5–2.7	418			
	Level 3	43.1–51.7	1.5–2.8	421			
UMFA	Level 1	0.62–1.06	5.0–16.8	413	0.09	0.20	0.14
	Level 2	5.18–6.06	3.1–12.3	412			
	Level 3	9.49–11.8	3.2–12.5	413			
THF	Level 1	1.20–2.59	3.4–11.7	401	0.37	0.20	0.25
	Level 2	4.17–5.36	2.7–7.1	404			
5-FormylTHF	Level 1	0.61–2.76	3.0–10.9	396	0.30	0.20	0.20
	Level 2	2.33–5.48	2.6–7.3	399			
5,10-MethenylTHF	Level 1	1.42–2.57	3.1–8.8	395	0.34	0.31	0.20
	Level 2	4.35–5.05	3.1–5.0	398			
MeFox	Level 1	1.24–1.48	2.2–6.2	400	0.34	0.08	0.10
	Level 2	1.44–2.53	2.7–5.7	397			
	Level 3	2.81–4.64	1.9–5.3	400			

[1] 5-FormylTHF, 5-formyltetrahydrofolate; 5,10-methenylTHF, 5,10-methenyl-tetrahydrofolate; 5-methylTHF, 5-methyltetrahydro-folate; LOD, limit of detection; MeFox, pyrazino-s-triazine derivative of 4α-hydroxy-5-methylTHF; QC, quality control; THF, tetrahydrofolate; UMFA, unmetabolized folic acid.

[2] Multiple sets of serum QC materials were used over the 6-y time period; for each set, a minimum of 2 concentration levels were used.

[3] Range of concentrations for each level across multiple sets of serum QC materials.

[4] Range of imprecision for each level across multiple sets of serum QC materials (expressed as total CV over the number of analytical runs)

Supplementary Table 2. Concentrations of serum total folate and folate forms in the US population ≥1 y by survey period, NHANES 2011–2016[1]

	NHANES survey period			
Analyte	**2011–2012**	**2013–2014**	**2015–2016**	***P*[2]**
***Total folate*[3]**				
Sample size, *n*	7459	8452	8047	
Range, nmol/L	3.06–316	4.12–1260	4.07–1390	
Median (Q1, Q3), nmol/L	41.3 (28.6, 58.7)	42.9 (29.1, 61.3)	39.9 (27.5, 57.6)	0.12
5-MethylTHF				
Sample size, *n*	7471	8455	8057	
Range, nmol/L	1.88–295	3.03–331	3.16–1370	
Median (Q1, Q3), nmol/L	38.4 (26.1, 54.7)	40.5 (27.2, 57.7)	37.9 (25.7, 54.4)	0.12
UMFA				
Sample size, *n*	7479	8454	8047	
Range, nmol/L	<LOD(0.09)–214	<LOD(0.20)–1010	<LOD(0.14)–418	
Median (Q1, Q3), nmol/L	0.74 (0.55, 1.07)	0.80 (0.57, 1.25)	0.63 (0.46, 1.00)	<0.0001
***Non-methyl folate*[4]**				
Sample size, *n*	7475	8453	8057	
Range, nmol/L	<LOD(1.01)–42.8	<LOD(0.71)–53.5	<LOD(0.65)–45.1	
Median (Q1, Q3), nmol/L	1.43 (1.08, 2.05)	1.10 (0.82, 1.48)	1.11 (0.88, 1.46)	0.0008
THF				
Sample size, *n*	7478	8454	8057	
Range, nmol/L	<LOD(0.37)–11.5	<LOD(0.20)–39.3	<LOD(0.25)–23.1	
Median (Q1, Q3), nmol/L	0.96 (0.62, 1.55)	0.72 (0.45, 1.10)	0.82 (0.59, 1.15)	<0.0001
5-FormylTHF				
Sample size, *n*	7483	8454	8057	
Range, nmol/L	<LOD(0.30)–31.6	<LOD(0.20)–17.3	<LOD(0.20)–38.9	
Median (Q1, Q3), nmol/L	<LOD	<LOD	<LOD	
% ≥LOD (*n* ≥LOD)	3.56 (242)	9.62 (756)	2.75 (185)	0.0004
Median (Q1, Q3), nmol/L	0.36 (0.34, 0.40)	0.25 (0.23, 0.30)	0.28 (0.23, 0.47)	
5,10-MethenylTHF				
Sample size, *n*	7483	8453	8057	
Range, nmol/L	<LOD(0.34)–4.38	<LOD(0.31)–13.4	<LOD(0.20)–7.13	
Median (Q1, Q3), nmol/L	<LOD	<LOD	<LOD	
% ≥LOD (*n* ≥LOD)	4.43 (379)	4.33 (379)	11.0 (875)	<0.0001
Median (Q1, Q3), nmol/L	0.51 (0.41, 0.76)	0.52 (0.40, 0.78)	0.29 (0.24, 0.48)	
MeFox				
Sample size, *n*	7486	8455	8055	
Range, nmol/L	<LOD(0.34)–20.4	<LOD(0.08)–56.3	<LOD(0.10)–59.7	
Median (Q1, Q3), nmol/L	1.49 (0.93, 2.43)	1.53 (0.97, 2.54)	1.44 (0.87, 2.39)	0.0307

[1] 5-FormylTHF, 5-formyltetrahydrofolate; 5,10-methenylTHF, 5,10-methenyltetrahydrofolate; 5-methylTHF, 5-methyltetrahydrofolate; MeFox, pyrazino-s-triazine derivative of 4α-hydroxy-5-methylTHF; THF, tetrahydrofolate; UMFA, unmetabolized folic acid.

[2] For total folate, 5-methylTHF, UMFA, MeFox, non-methyl folate, and THF, the Wald *F P* value tests the null hypothesis of no difference in geometric means among survey periods; for 5-formylTHF and 5,10-methenylTHF, the chi-square Wald *F P* value tests the null hypothesis of no difference in % >LOD among survey periods.

[3] Total folate is the sum of biologically active folate forms (5-methylTHF, UMFA, and non-methyl folate), not including MeFox.

[4] Non-methyl folate is the sum of 3 minor folate forms (THF, 5-formylTHF, and 5,10-methenylTHF); non-methyl folate was <LOD if all 3 minor forms were <LOD and ≥LOD if at least 1 minor form was ≥LOD.

Supplementary Table 3. Geometric mean and selected percentile concentrations of serum total folate and folate forms by demographic variables in the fasting US population ≥1 y, NHANES 2011–2016[1]

Analyte Variable	Sample size	Geometric mean	Percentile 2.5	5	25	50	75	95	97.5
	n				*nmol/L*				
***Total folate*[2]**									
Overall	23682	38.7 (37.9, 39.6)	13.3 (12.7–13.9)	15.9 (15.5–16.4)	26.8 (26.0–27.6)	39.2 (38.1–40.1)	56.9 (55.2–58.7)	89.8 (87.1–92.5)	103 (99.0–106)
Age, y									
1–5	2118	62.2 (58.4, 66.3)	22.7* (19.4–28.4)	28.7 (22.7–32.7)	49.9 (43.0–54.6)	64.9 (60.5–71.1)	80.3 (76.9–84.1)	108 (97.6–149)	122* (105–286)
6–11	3021	59.9 (57.4, 62.4)	26.7 (24.4–29.4)	31.5 (28.4–33.7)	48.5 (45.3–50.8)	62.8 (60.3–65.7)	76.6 (73.6–80.4)	97.4 (92.4–108)	103 (99.5–131)
12–19	3435	39.8 (38.7, 41.1)	16.4 (15.2–18.1)	18.7 (18.1–19.6)	29.8 (28.3–30.8)	40.8 (39.2–42.1)	53.5 (51.9–55.5)	80.3 (77.0–86.2)	89.4 (85.8–92.4)
20–39	5017	33.6 (32.8, 34.4)	12.6 (12.1–13.6)	15.4 (14.4–16.2)	24.7 (23.7–25.6)	33.9 (32.9–35.0)	46.3 (45.0–47.6)	67.6 (66.1–70.4)	78.1 (73.6–81.9)
40–59	5145	36.5 (35.3, 37.8)	13.0 (11.3–13.2)	15.1 (13.9–15.8)	25.1 (24.1–26.3)	37.6 (35.7–39.3)	54.7 (52.3–56.7)	79.4 (76.7–84.4)	90.7 (85.7–99.9)
60–69	2552	42.2 (39.8, 44.9)	12.5 (10.7–14.4)	15.5 (13.7–17.1)	26.8 (24.8–29.7)	44.1 (39.9–46.8)	66.7 (62.3–71.8)	100 (97.5–108)	117 (104–140)
≥70	2394	49.8 (47.6, 52.2)	15.2 (12.9–16.7)	18.0 (15.9–19.5)	32.8 (30.6–34.9)	50.6 (46.5–53.8)	77.4 (74.3–82.1)	118 (112–130)	136 (125–176)
Sex									
Males	11840	36.7 (35.7, 37.7)	12.7 (12.0–13.6)	15.8 (15.2–16.5)	26.0 (25.0–26.7)	36.8 (35.4–38.3)	53.0 (51.2–54.8)	82.3 (79.5–86.3)	92.8 (90.1–98.8)
Females	11842	40.9 (39.8, 42.0)	13.8 (13.1–14.3)	16.1 (15.4–16.8)	28.0 (26.9–29.0)	41.5 (40.1–42.9)	61.3 (58.8–63.1)	95.4 (92.3–99.6)	108 (104–116)
Race-Hispanic origin									
Hispanic	6646	37.5 (36.5, 38.5)	14.8 (13.2–15.7)	17.3 (16.4–18.2)	27.6 (26.5–28.6)	37.4 (36.2–38.8)	51.5 (49.9–53.7)	78.9 (77.0–80.5)	88.9 (87.2–91.6)
NHA	2599	38.6 (37.1, 40.2)	12.8 (11.8–13.8)	15.3 (13.8–16.1)	27.1 (25.5–28.4)	38.5 (36.9–40.3)	57.3 (55.1–60.3)	86.0 (81.9–89.8)	97.8 (90.5–108)
NHB	5475	32.0 (30.9, 33.2)	12.1 (11.2–12.5)	13.9 (13.0–14.8)	22.6 (21.7–23.7)	31.5 (30.1–32.8)	45.7 (43.6–47.6)	74.8 (71.3–79.3)	86.3 (80.6–96.0)
NHW	7948	40.5 (39.4, 41.6)	13.7 (12.9–14.3)	16.2 (15.5–17.1)	27.7 (26.7–28.9)	41.3 (39.8–42.8)	60.9 (58.8–62.7)	94.4 (91.5–98.2)	106 (103–112)

Analyte Variable	Sample size	Geometric mean	Percentile 2.5	5	25	50	75	95	97.5
	n				*nmol/L*				
5-MethylTHF									
Overall	23682	36.2 (35.4, 37.1)	11.7 (11.1–12.2)	14.2 (13.8–14.6)	24.8 (23.9–25.7)	37.0 (35.9–38.1)	54.3 (52.7–55.9)	85.5 (82.6–88.6)	96.6 (93.8–101)
Age, y									
1–5	2118	58.7 (55.1, 62.6)	21.4* (16.4–25.0)	26.7 (20.0–32.2)	47.8 (41.8–52.5)	62.5 (57.3–67.8)	77.1 (74.0–79.7)	102 (91.4–127)	111* (101–164)
6–11	3021	57.1 (54.7, 59.6)	24.9 (21.5–27.8)	29.2 (26.7–31.6)	45.8 (43.1–48.3)	60.0 (57.5–63.3)	74.0 (70.5–77.1)	94.1 (89.1–102)	100 (96.6–130)
12–19	3435	37.6 (36.5, 38.9)	14.7 (13.7–16.1)	17.1 (16.2–17.8)	28.0 (26.3–28.9)	38.7 (37.2–40.2)	51.5 (49.5–53.1)	78.0 (74.2–83.6)	86.3 (83.3–90.1)
20–39	5017	31.3 (30.5, 32.2)	11.2 (10.5–11.9)	13.7 (12.6–14.4)	22.7 (21.7–23.6)	31.9 (30.9–32.9)	43.9 (42.9–45.2)	63.9 (62.7–67.3)	73.3 (68.7–77.6)
40–59	5145	34.1 (32.9, 35.4)	11.0 (9.94–12.0)	13.4 (12.3–14.2)	23.1 (21.9–24.4)	35.5 (33.7–37.1)	52.3 (50.0–54.3)	75.3 (73.8–80.4)	85.5 (81.3–91.3)
60–69	2552	39.5 (37.0, 42.0)	11.0 (9.24–12.3)	13.8 (11.6–15.2)	25.2 (23.1–27.8)	41.3 (37.3–43.8)	64.2 (59.2–68.1)	95.6 (92.3–104)	105 (97.2–137)
≥70	2394	46.7 (44.5, 49.0)	13.5 (11.4–14.3)	16.4 (14.1–17.3)	30.7 (28.1–32.7)	47.7 (44.2–51.7)	74.0 (71.4–79.0)	113 (105–123)	131 (120–158)
Sex									
Males	11840	34.3 (33.3, 35.3)	11.1 (10.4–12.0)	14.1 (13.2–14.8)	24.0 (23.1–24.9)	34.7 (33.2–36.0)	50.6 (48.7–52.1)	78.1 (75.8–81.1)	88.2 (84.8–92.1)
Females	11842	38.3 (37.2, 39.4)	12.2 (11.5–12.7)	14.4 (13.7–15.0)	26.1 (24.9–27.0)	39.3 (38.0–40.6)	58.6 (56.3–60.6)	91.2 (87.8–95.7)	103 (99.5–108)
Race-Hispanic origin									
Hispanic	6646	35.1 (34.1, 36.1)	12.8 (11.4–13.9)	15.8 (14.6–16.5)	25.5 (24.4–26.7)	35.2 (34.1–36.7)	49.0 (47.5–51.1)	75.3 (73.4–77.7)	85.2 (83.2–87.3)
NHA	2599	36.3 (34.8, 37.9)	11.2 (10.4–12.2)	13.7 (12.2–14.7)	25.1 (23.4–26.9)	36.5 (34.8–38.7)	55.1 (53.1–57.9)	83.0 (78.8–86.1)	92.5 (87.1–101)
NHB	5475	29.4 (28.4, 30.4)	10.5 (9.92–10.8)	12.1 (11.4–12.7)	20.4 (19.4–21.4)	29.1 (27.7–30.6)	43.2 (41.3–44.9)	70.1 (66.3–72.0)	77.7 (74.9–83.7)
NHW	7948	38.0 (36.9, 39.1)	12.1 (11.0–12.7)	14.4 (13.9–15.2)	25.9 (24.7–27.0)	39.2 (37.9–40.6)	58.2 (56.1–59.9)	90.2 (86.8–93.8)	101 (97.2–105)
UMFA									
Overall	23682	0.70 (0.68, 0.72)	0.27 (0.25–0.28)	0.32 (0.30–0.33)	0.48 (0.47–0.50)	0.65 (0.63–0.67)	0.91 (0.89–0.93)	1.84 (1.72–1.95)	3.24 (2.54–4.21)

Analyte Variable	Sample size	Geometric mean	Percentile 2.5	5	25	50	75	95	97.5
	n				*nmol/L*				
Age, y									
1–5	2118	0.71 (0.62, 0.79)	0.30* (0.25–0.31)	0.32 (0.29–0.38)	0.49 (0.44–0.52)	0.63 (0.58–0.68)	0.80 (0.74–0.93)	1.73 (1.38–16.7)	2.99* (1.75–85.9)
6–11	3021	0.71 (0.67, 0.76)	0.35 (0.29–0.37)	0.38 (0.36–0 .40)	0.52 (0.49–0.54)	0.62 (0.60–0.66)	0.83 (0.78–0.90)	1.78 (1.37–2.32)	3.07 (1.86–24.8)
12–19	3435	0.66 (0.64, 0.68)	0.30 (0.26–0.33)	0.34 (0.32–0.37)	0.49 (0.47–0.51)	0.64 (0.62–0.68)	0.85 (0.82–0.91)	1.38 (1.35–1.43)	1.68 (1.53–1.85)
20–39	5017	0.64 (0.61, 0.67)	0.24 (0.22–0.25)	0.30 (0.27–0.32)	0.45 (0.43–0.47)	0.60 (0.58–0.63)	0.82 (0.78–0.87)	1.58 (1.44–1.71)	2.25 (1.85–3.53)
40–59	5145	0.68 (0.65, 0.71)	0.26 (0.22–0.28)	0.30 (0.28–0.32)	0.47 (0.45–0.48)	0.62 (0.60–0.64)	0.86 (0.83–0.90)	1.78 (1.56–2.11)	3.57 (2.19–5.87)
60–69	2552	0.78 (0.74, 0.83)	0.30 (0.24–0.31)	0.32 (0.30–0.35)	0.51 (0.49–0.54)	0.72 (0.68–0.75)	1.04 (0.96–1.11)	2.36 (1.78–6.13)	7.75 (3.60–15.7)
≥70	2394	0.94 (0.88, 1.00)	0.35 (0.33–0.37)	0.41 (0.37–0.43)	0.62 (0.58–0.65)	0.83 (0.79–0.87)	1.16 (1.10–1.29)	2.74 (2.24–4.44)	6.51 (4.12–11.4)
Sex									
Males	11840	0.67 (0.65, 0.70)	0.25 (0.23–0.27)	0.30 (0.29–0.32)	0.46 (0.45–0.48)	0.62 (0.60–0.64)	0.86 (0.83–0.90)	1.85 (1.66–2.09)	3.94 (2.64–5.66)
Females	11842	0.73 (0.71, 0.75)	0.28 (0.27–0.30)	0.34 (0.32–0.35)	0.51 (0.49–0.52)	0.68 (0.66–0.70)	0.93 (0.91–0.97)	1.84 (1.71–1.94)	2.75 (2.22–4.00)
Race-Hispanic origin									
Hispanic	6646	0.62 (0.59, 0.65)	0.25 (0.22–0.27)	0.29 (0.26–0.31)	0.45 (0.42–0.47)	0.60 (0.57–0.62)	0.79 (0.76–0.83)	1.37 (1.27–1.51)	1.73 (1.59–1.92)
NHA	2599	0.52 (0.50, 0.54)	<LOD	<LOD	0.36 (0.34–0.38)	0.50 (0.48–0.52)	0.70 (0.65–0.74)	1.35 (1.22–1.55)	1.94 (1.57–2.74)
NHB	5475	0.74 (0.71, 0.78)	0.28 (0.26–0.30)	0.33 (0.30–0.34)	0.50 (0.48–0.52)	0.68 (0.65–0.71)	0.96 (0.92–1.02)	1.89 (1.71–2.13)	3.06 (2.46–5.45)
NHW	7948	0.74 (0.71, 0.76)	0.30 (0.27–0.31)	0.34 (0.33–0.35)	0.50 (0.48–0.52)	0.67 (0.65–0.69)	0.93 (0.91–0.97)	2.00 (1.87–2.19)	4.03 (3.16–5.92)
***Non-methyl folate*[3]**									
Overall	23682	1.22 (1.15, 1.30)	<LOD	<LOD	0.89 (0.85–0.93)	1.18 (1.13–1.23)	1.56 (1.48–1.65)	3.70 (2.58–5.09)	4.71 (4.06–6.02)
Age, y									
1–5	2118	1.68 (1.53, 1.85)	<LOD*	0.86 (<LOD–0.93)	1.27 (1.14–1.33)	1.55 (1.43–1.70)	2.13 (1.85–2.43)	4.76 (3.84–7.09)	6.26 (4.69–12.6)
6–11	3021	1.39	<LOD	0.71	1.04	1.31	1.67	3.53	4.77

Analyte Variable	Sample size	Geometric mean	Percentile 2.5	5	25	50	75	95	97.5
	n				*nmol/L*				
		(1.28, 1.50)		(0.71–0.71)	(0.99–1.10)	(1.24–1.39)	(1.57–1.85)	(2.65–5.73)	(3.76–7.10)
12–19	3435	1.15 (1.06, 1.25)	<LOD	<LOD	0.88 (0.81–0.91)	1.11 (1.05–1.17)	1.43 (1.33–1.55)	3.27 (1.98–5.32)	4.11 (3.35–6.12)
20–39	5017	1.16 (1.06, 1.26)	<LOD	<LOD	0.82 (0.77–0.88)	1.09 (1.05–1.14)	1.43 (1.35–1.55)	3.84 (2.46–5.45)	4.65 (3.97–6.22)
40–59	5145	1.20 (1.12, 1.28)	<LOD	<LOD	0.89 (0.85–0.92)	1.16 (1.10–1.23)	1.52 (1.44–1.60)	3.67 (2.39–5.12)	4.72 (3.97–5.94)
60–69	2552	1.29 (1.20, 1.38)	<LOD	<LOD	0.92 (0.82–0.99)	1.25 (1.20–1.35)	1.65 (1.57–1.80)	3.55 (2.59–5.06)	4.68 (4.13–7.52)
≥70	2394	1.40 (1.32, 1.49)	<LOD	<LOD	1.02 (0.97–1.08)	1.42 (1.35–1.49)	1.81 (1.73–1.92)	3.90 (2.78–6.02)	5.41 (4.30–7.98)
Sex									
Males	11840	1.22 (1.14, 1.30)	<LOD	<LOD	0.88 (0.84–0.92)	1.17 (1.11–1.23)	1.55 (1.48–1.64)	3.77 (2.64–4.94)	4.62 (4.00–5.96)
Females	11842	1.23 (1.15, 1.31)	<LOD	<LOD	0.90 (0.86–0.93)	1.18 (1.14–1.23)	1.56 (1.48–1.67)	3.67 (2.50–5.48)	4.79 (4.07–6.05)
Race-Hispanic origin									
Hispanic	6646	1.24 (1.10, 1.39)	<LOD	<LOD	0.89 (0.83–0.95)	1.15 (1.08–1.22)	1.51 (1.38–1.73)	4.34 (3.08–5.80)	4.89 (4.47–6.49)
NHA	2599	1.25 (1.13, 1.38)	<LOD	<LOD	0.91 (0.84–0.97)	1.18 (1.11–1.25)	1.59 (1.44–1.79)	3.82 (2.34–12.0)	4.92 (3.46–12.0)
NHB	5475	1.23 (1.11, 1.36)	<LOD	<LOD	0.84 (0.78–0.89)	1.12 (1.06–1.21)	1.56 (1.42–1.82)	4.31 (3.23–5.89)	5.16 (4.40–6.93)
NHW	7948	1.22 (1.15, 1.29)	<LOD	<LOD	0.90 (0.85–0.94)	1.20 (1.14–1.25)	1.56 (1.49–1.64)	3.24 (2.39–4.98)	4.45 (3.57–6.23)
MeFox									
Overall	23682	1.14 (1.11, 1.17)	0.38 (0.36–0.40)	0.46 (0.44–0.47)	0.74 (0.71–0.76)	1.09 (1.05–1.13)	1.64 (1.59–1.71)	3.38 (3.21–3.54)	4.39 (4.14–4.72)
Age, y									
1–5	2118	0.64 (0.59, 0.69)	<LOD*	<LOD	0.45 (0.43– 0.49)	0.60 (0.55–0.64)	0.84 (0.76–0.95)	1.86 (1.51–2.67)	2.38* (1.83–5.70)
6–11	3021	0.66 (0.62, 0.70)	<LOD	<LOD	0.48 (0.46–0.50)	0.61 (0.58–0.65)	0.83 (0.76–0.89)	1.68 (1.45–2.37)	2.40 (1.76–4.87)
12–19	3435	0.93 (0.89, 0.97)	0.37 (<LOD–0.39)	0.43 (0.40–0.45)	0.64 (0.60–0.67)	0.89 (0.84–0.95)	1.30 (1.19–1.37)	2.45 (2.27–2.63)	2.98 (2.63–3.38)
20–39	5017	1.06	0.36	0.43	0.70	1.00	1.51	3.01	3.79

Analyte Variable	Sample size	Geometric mean	Percentile 2.5	5	25	50	75	95	97.5
	n				*nmol/L*				
		(1.01, 1.10)	(<LOD–0.38)	(0.40–0.45)	(0.68–0.73)	(0.95–1.05)	(1.43–1.60)	(2.73–3.39)	(3.51–4.56)
40–59	5145	1.14 (1.10, 1.19)	0.40 (0.36–0.44)	0.50 (0.46–0.52)	0.77 (0.72–0.81)	1.11 (1.03–1.17)	1.61 (1.52–1.72)	3.20 (2.94–3.49)	4.02 (3.63–4.37)
60–69	2552	1.35 (1.30, 1.41)	0.46 (0.37–0.51)	0.58 (0.50–0.62)	0.93 (0.86–1.01)	1.34 (1.25–1.40)	1.86 (1.75–1.99)	3.55 (3.32–4.29)	4.95 (4.28–6.04)
≥70	2394	1.74 (1.65, 1.83)	0.56 (0.50–0.60)	0.66 (0.58–0.70)	1.13 (1.05–1.18)	1.66 (1.58–1.74)	2.52 (2.37–2.75)	5.40 (5.08–6.03)	6.77 (5.80–8.07)
Sex									
Males	11840	1.12 (1.08, 1.16)	0.37 (<LOD–0.40)	0.45 (0.43–0.47)	0.72 (0.70–0.75)	1.06 (1.01–1.12)	1.60 (1.52–1.68)	3.31 (3.08–3.54)	4.22 (4.05–4.64)
Females	11842	1.16 (1.12, 1.21)	0.39 (0.37–0.40)	0.46 (0.44–0.48)	0.75 (0.72–0.78)	1.11 (1.07–1.16)	1.70 (1.62–1.79)	3.42 (3.26–3.61)	4.58 (4.12–5.07)
Race-Hispanic origin									
Hispanic	6646	0.88 (0.85, 0.91)	<LOD	0.37 (<LOD–0.39)	0.60 (0.58–0.61)	0.82 (0.80–0.84)	1.22 (1.17–1.28)	2.73 (2.49–2.96)	3.42 (3.20–3.62)
NHA	2599	1.00 (0.96, 1.05)	0.37 (<LOD–0.40)	0.43 (0.38–0.47)	0.68 (0.65–0.71)	0.96 (0.91–1.02)	1.38 (1.30–1.48)	2.75 (2.50–3.09)	3.27 (3.12–4.23)
NHB	5475	0.96 (0.92, 0.99)	<LOD	0.37 (0.36–0.39)	0.62 (0.59–0.64)	0.88 (0.85–0.92)	1.38 (1.32–1.45)	3.05 (2.75–3.42)	4.25 (3.63–4.87)
NHW	7948	1.27 (1.23, 1.31)	0.46 (0.43–0.48)	0.53 (0.51–0.55)	0.84 (0.82–0.87)	1.23 (1.18–1.26)	1.81 (1.73–1.87)	3.60 (3.39–3.95)	4.73 (4.31–5.09)

[1] Values are weighted geometric means (95% CI) and weighted percentiles (95% CI). Fasting refers to no food intake for past ≥8 hours prior to blood draw. 5-MethylTHF, 5-methyltetrahydrofolate; MeFox, pyrazino-s-triazine derivative of 4α-hydroxy-5-methylTHF; NHA, non-Hispanic Asian; NHB, non-Hispanic Black; NHW, non-Hispanic White; UMFA, unmetabolized folic acid.

[2] Total folate is the sum of biologically active folate forms (5-methylTHF, UMFA, and non-methyl folate), not including MeFox.

[3] Non-methyl folate represents sum of 3 minor forms (tetrahydrofolate, 5-formyltetrahydrofolate, and 5,10-methenyltetrahydrofolate).

* Estimate is subject to greater uncertainty due to small sample size.

Supplementary Table 4. Concentrations of serum total folate and folate forms by selected physiologic and lifestyle variables for the fasting US population ≥1 year, NHANES 2011–2016[1]

Variable categories	Sample size	Total folate	5-MethylTHF	UMFA	Non-methyl folate	MeFox
	n	*(nmol/L)*				
Inflammation[2]						
CRP <5	2583	37.9 (36.2, 39.7)	35.9 (34.2, 37.7)	0.62 (0.59, 0.65)	1.08 (1.03, 1.13)	1.03 (0.98, 1.08)
CRP ≥5	625	33.9 (31.3, 36.8)	31.6 (29.0, 34.4)	0.65 (0.58, 0.72)	1.14 (1.08, 1.20)	1.28 (1.16, 1.42)
P value		0.0146	0.0077	0.4441	0.001	<0.0001
Spearman correlation *r*		-0.11	-0.11	0.02	0.13	0.21
Kidney function[3]						
eGFR 0–<60	579	46.0 (42.2, 49.8)	42.5 (39.2, 46.0)	1.09 (0.99, 1.19)	1.38 (1.27, 1.51)	2.59 (2.40, 2.80)
eGFR 60–<90	2,229	40.4 (39.1, 41.9)	37.8 (36.5, 39.2)	0.76 (0.73, 0.80)	1.27 (1.19, 1.34)	1.31 (1.27, 1.35)
eGFR ≥90	6,213	36.3 (35.4, 37.2)	34.0 (33.1, 34.9)	0.65 (0.63, 0.67)	1.18 (1.09, 1.27)	1.03 (1.00, 1.07)
P value		<0.0001	<0.0001	<0.0001	0.0005	<0.0001
Spearman correlation *r*		-0.10	-0.10	-0.19	-0.11	-0.31
BMI[4]						
Underweight	913	50.2 (48.0, 52.6)	47.8 (45.6, 50.1)	0.68 (0.66, 0.72)	1.31 (1.23, 1.41)	0.80 (0.76, 0.85)
Normal weight	3,168	40.8 (39.6, 42.0)	38.2 (37.0, 39.5)	0.70 (.672, .734)	1.19 (1.11, 1.28)	1.05 (1.01, 1.11)
Overweight	2,745	38.8 (37.4, 40.3)	36.4 (35.0, 37.8)	0.71 (0.68, 0.73)	1.22 (1.13, 1.31)	1.16 (1.11, 1.20)
Obese	3,118	35.4 (34.5, 36.4)	33.0 (32.1, 33.9)	0.70 (0.67, 0.72)	1.24 (1.16, 1.33)	1.27 (1.22, 1.31)
P value		<0.0001	<0.0001	0.4197	0.0016	<0.0001
Spearman correlation *r*		-0.17	-0.09	0.02	0.03	0.19
BSA[5], cm×kg						
<1.5	1,499	51.5 (49.9, 53.2)	48.9 (47.3, 50.6)	0.68 (0.65, 0.72)	1.34 (1.24, 1.44)	0.82 (0.78, 0.86)
1.5–1.8	2,958	41.2 (39.9, 42.6)	38.6 (37.3, 40.1)	0.72 (0.70, 0.75)	1.20 (1.12, 1.28)	1.12 (1.07, 1.16)
1.8–2	2,433	38.4 (37.0, 39.8)	35.9 (34.6, 37.3)	0.69 (0.67, 0.72)	1.20 (1.12, 1.29)	1.15 (1.11, 1.20)
≥2	3,054	34.8 (33.8, 35.8)	32.4 (31.4, 33.4)	0.69 (0.67, 0.72)	1.23 (1.15, 1.31)	1.24 (1.19, 1.28)
P value		<0.0001	<0.0001	0.1236	<0.0001	<0.0001
Spearman correlation *r*		-0.22	-0.22	-0.01	-0.01	0.10
Smoking status[6]						
Cotinine ≤10	7,916	41.1 (40.1, 42.1)	38.6 (37.7, 39.6)	0.71 (0.68, 0.73)	1.24 (1.16, 1.32)	1.12 (1.09, 1.16)

Cotinine >10	2,001	31.2 (30.3, 32.2)	28.8 (27.8, 29.9)	0.69 (0.65, 0.72)	1.17 (1.08, 1.26)	1.21 (1.15, 1.26)
P value		<0.0001	<0.0001	0.3428	0.0203	0.0027
Spearman correlation *r*		-0.23	-0.14	-0.07	-0.06	0.00
Alcohol intake[7]						
No drinks	2,362	39.9 (38.6, 41.2)	37.3 (36.0, 38.5)	0.78 (0.75, 0.81)	1.23 (1.15, 1.31)	1.35 (1.30, 1.40)
<1 (not 0)	3,969	37.4 (36.4, 38.3)	34.9 (34.0, 35.9)	0.69 (0.67, 0.72)	1.19 (1.12, 1.28)	1.17 (1.13, 1.21)
1–<2	490	34.3 (32.3, 36.4)	31.8 (29.9, 33.9)	0.63 (0.58, 0.69)	1.23 (1.13, 1.34)	1.10 (1.04, 1.15)
≥2	352	34.7 (31.6, 38.1)	32.3 (29.3, 35.6)	0.62 (0.56, 0.68)	1.26 (1.12, 1.41)	1.13 (1.04, 1.23)
P value		0.0002	0.0003	<0.0001	0.4037	<0.0001
Spearman correlation *r*		-0.08	-0.08	-0.15	-0.03	-0.12

[1] Values are geometric means (95% CI); non-methyl folate represents sum of 3 minor forms: tetrahydrofolate, 5-formyltetrahydrofolate, and 5,10-methenyltetrahydrofolate; total folate is the sum of biologically active folate forms (5-methylTHF, UMFA, and non-methyl folate), not including MeFox; *P* value is the unadjusted Wald F *P* value. 5-methylTHF, 5-methyltetrahydrofolate; BSA, body surface area; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; MeFox, pyrazino-s-triazine derivative of 4α-hydroxy-5-methylTHF; UMFA, unmetabolized folic acid.

[2] CRP (mg/L) used to assess inflammation; measured as serum high sensitivity CRP in participants ≥1 y; only available in NHANES 2015–1016; CRP <5 (no inflammation), CRP ≥5 (inflammation).

[3] eGFR [mL/(min×1.73 m^2)] used to assess kidney function; available for persons ≥12 y; eGFR 0–<60 (chronic kidney disease), eGFR 60–90 (mild decrease in kidney function); eGFR ≥90 (normal kidney function).

[4] BMI (kg/m^2): calculated for participants ≥2 y; <18.5 (underweight); 18.5–>25 (normal weight); 25–<30 (overweight); and ≥30 (obese).

[5] BSA used to assess body size; calculated as square root of [(height in cm × weight in kg)/3600] or square root of [(height in inches × weight in pounds)/3131]; calculated for participants ≥2 y.

[6] Serum cotinine (μg/L) used as biomarker of tobacco smoke exposure; calculated for participants ≥3 y; cotinine ≤10 (nonsmoker), cotinine >10 (smoker).

[7] Calculated for participants ≥18 y as average daily number of "standard" drinks [(quantity × frequency) / 365.25]; 1 drink ≈ 15 g ethanol.

Supplementary Table 5. Relative contribution of folate forms to serum total folate by demographic variables for the fasting US population ≥1 y, NHANES 2011–2016[1]

Variable	Sample size	5-MethylTHF	UMFA	Non-methyl folate	MeFox[2]
	n		*%*		
Overall	10070	93.7 (93.3, 94.1)	2.4 (2.3, 2.5)	3.9 (3.5, 4.3)	3.6 (3.5, 3.7)
Age group, y					
1–5	265	94.8 (93.7, 95.9)	2.0 (1.1, 3.0)	3.1 (2.8, 3.5)	1.3 (1.1, 1.4)
6–11	706	95.6 (95.0, 96.1)	1.7 (1.3, 2.2)	2.7 (2.4, 3.0)	1.3 (1.2, 1.5)
12–19	1660	94.6 (94.0, 95.1)	1.9 (1.8, 2.0)	3.5 (3.0, 4.0)	2.7 (2.6, 2.9)
20–39	2475	93.3 (92.7, 94.0)	2.4 (2.2, 2.5)	4.3 (3.7, 4.9)	3.7 (3.5, 3.9)
40–59	2545	93.5 (93.0, 94.0)	2.5 (2.3, 2.7)	4.0 (3.6, 4.4)	3.7 (3.5, 3.9)
60–69	1293	93.6 (93.0, 94.1)	2.6 (2.3, 2.9)	3.8 (3.4, 4.2)	4.0 (3.7, 4.2)
≥70	1126	94.0 (93.6, 94.4)	2.6 (2.3, 2.9)	3.4 (3.2, 3.7)	4.2 (4.0, 4.4)
P value[3]		<0.0001	<0.0001	<0.0001	<0.0001
Sex					
Male	5028	93.5 (93.1, 94.0)	2.4 (2.3, 2.6)	4.0 (3.6, 4.5)	3.7 (3.5, 3.8)
Female	5042	93.9 (93.5, 94.4)	2.3 (2.2, 2.5)	3.7 (3.3, 4.1)	3.5 (3.4, 3.7)
P value[3]		0.0069	0.42	<0.0001	0.09
Race-Hispanic origin					
Hispanic	2725	93.8 (93.1, 94.5)	2.1 (2.0, 2.2)	4.1 (3.4, 4.8)	2.9 (2.8, 3.0)
Non-Hispanic Asian	1174	94.2 (93.4, 94.9)	1.8 (1.6, 2.0)	4.0 (3.3, 4.7)	3.1 (3.0, 3.3)
Non-Hispanic Black	2359	92.0 (91.3, 92.8)	3.1 (2.9, 3.3)	4.9 (4.1, 5.6)	3.7 (3.5, 3.9)
Non-Hispanic White	3467	93.9 (93.6, 94.3)	2.4 (2.3, 2.5)	3.7 (3.3, 4.0)	3.8 (3.6, 3.9)
P value[3]		<0.0001	<0.0001	0.0032	<0.0001

[1] Values are weighted mean percent (95% CI) contribution to serum total folate (sum of 5-methylTHF, UMFA, and non-methyl folate, not including MeFox). Fasting refers to no food intake for the past ≥8 h prior to blood draw. Non-methyl folate is the sum of 3 minor

folate forms (tetrahydrofolate, 5-formyltetrahydrofolate, and 5,10-methenyltetrahydrofolate). 5-MethylTHF, 5-methyltetrahydrofolate; MeFox, pyrazino-s-triazine derivative of 4α-hydroxy-5-methylTHF; UMFA, unmetabolized folic acid.

[2] Values are weighted mean percent (95% CI) contribution to serum total folate plus MeFox.

[3] Wald *F P* value tests the null hypothesis of no difference in the means across the categories for each demographic variable.

Supplementary Table 6. Absolute concentrations of folate forms and relative contribution of folate forms to serum total folate by weighted decile of serum total folate in the fasting US population ≥1 y, NHANES 2011–2016[1]

Decile category	Sample size	5-MethylTHF	UMFA	Non-methyl folate	MeFox[2]
	n		*nmol/L*		
1st (<19.2 nmol/L)	961	13.6 (13.3, 13.9)	0.54 (0.51, 0.56)	1.04 (0.94, 1.14)	1.11 (0.89, 1.34)
2nd (19.2–<24.3 nmol/L)	1041	20.0 (19.8, 20.2)	0.63 (0.60, 0.66)	1.21 (1.05, 1.37)	1.23 (1.16, 1.31)
3rd (24.3–<29.2 nmol/L)	1034	24.8 (24.6, 25.0)	0.63 (0.60, 0.66)	1.30 (1.08, 1.53)	1.20 (1.10, 1.29)
4th (29.2–<33.9 nmol/L)	1008	29.5 (29.3, 29.7)	0.67 (0.64, 0.71)	1.32 (1.16, 1.47)	1.27 (1.19, 1.35)
5th (33.9–<39.2 nmol/L)	1017	34.5 (34.3, 34.7)	0.71 (0.65, 0.77)	1.30 (1.13, 1.46)	1.35 (1.26, 1.43)
6th (39.2–<45.1 nmol/L)	985	39.8 (39.6, 40.1)	0.77 (0.71, 0.84)	1.35 (1.20, 1.50)	1.39 (1.31, 1.47)
7th (45.1–<52.4 nmol/L)	1033	46.0 (45.7, 46.4)	0.94 (0.74, 1.14)	1.53 (1.29, 1.78)	1.44 (1.28, 1.61)
8th (52.4–<62.3 nmol/L)	1034	54.5 (54.1, 54.9)	1.02 (0.79, 1.24)	1.48 (1.33, 1.63)	1.47 (1.39, 1.55)
9th (62.3–<76.2 nmol/L)	970	65.5 (65.1, 65.8)	1.39 (1.16, 1.61)	1.61 (1.42, 1.81)	1.60 (1.49, 1.71)
10th (≥76.2 nmol/L)	987	94.8 (86.8, 103)	3.94 (2.87, 5.00)	2.29 (1.99, 2.60)	2.23 (1.83, 2.64)
	n		*%*		
1st (<19.2 nmol/L)	961	89.2 (88.5, 89.9)	3.70 (3.50–3.90)	7.10 (6.50–7.80)	n/a
2nd (19.2–<24.3 nmol/L)	1041	91.5 (90.8–92.3)	2.90 (2.80–3.00)	5.50 (4.80–6.30)	n/a
3rd (24.3–<29.2 nmol/L)	1034	92.7 (91.9–93.5)	2.40 (2.30–2.50)	4.90 (4.00–5.70)	n/a
4th (29.2–<33.9 nmol/L)	1008	93.7 (93.2–94.1)	2.10 (2.00–2.30)	4.20 (3.70–4.70)	n/a
5th (33.9–<39.2 nmol/L)	1017	94.5 (94.0–95.0)	1.90 (1.80–2.10)	3.60 (3.10–4.00)	n/a
6th (39.2–<45.1 nmol/L)	985	94.9 (94.5–95.3)	1.80 (1.70–2.00)	3.20 (2.90–3.60)	n/a
7th (45.1–<52.4 nmol/L)	1033	94.9 (94.3–95.5)	1.90 (1.50–2.30)	3.20 (2.70–3.70)	n/a
8th (52.4–<62.3 nmol/L)	1034	95.6 (95.2–96.1)	1.80 (1.40–2.20)	2.60 (2.30–2.90)	n/a
9th (62.3–<76.2 nmol/L)	970	95.6 (95.2–96.1)	2.00 (1.70–2.30)	2.40 (2.10–2.60)	n/a
10th (≥76.2 nmol/L)	987	94.4 (93.5–95.2)	3.30 (2.60–4.00)	2.30 (2.00–2.70)	n/a

[1] Values are weighted mean concentrations (95% CI) in upper half and weighted mean percent contribution (95% CI) to serum total folate (sum of 5-methylTHF, UMFA, and non-methyl folate) in lower half of table. Fasting refers to no food intake for the past ≥8 h prior to blood draw. Non-methyl folate is the sum of 3 minor folate forms (tetrahydrofolate, 5-formyltetrahydrofolate, and 5,10-methenyltetrahydrofolate). 5-MethylTHF, 5-methyltetrahydrofolate; MeFox, pyrazino-s-triazine derivative of 4α-hydroxy-5-methylTHF; UMFA, unmetabolized folic acid.

[2] No relative contribution provided for MeFox, because this folate form is not part of serum total folate.

Supplementary Figure 2. Demographic characteristics per decile of serum total folate, fasting persons ≥1 y, NHANES 2011-2014. Supplement use refers to dietary supplements containing folic acid. NHA, non-Hispanic Asian; NHB, non-Hispanic Black; NHW, non-Hispanic White.