**Supplemental Table 1** Plasma and red blood cell (RBC) folate concentrations and correlation coefficients for the intervention group (by baseline and 6 months), stratified by folic acid dosage, genotype and BMI1

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Baseline (Month 0)** | | | | | | | | **Month 6** | | | |
|  | n | | Plasma folate concentration (nmol/L)2,3 | | RBC folate concentration (nmol/L)2,3 | | Correlation coefficient4 | | n | Plasma folate concentration (nmol/L)2,3 | RBC folate concentration (nmol/L)2,3 | Correlation coefficient4 |
| All | 1108 | | 9.7  (4.7, 24.1) | | 600  (272, 1272) | | 0.46 | | 977 | 29.4  (8.0, 107.5) | 968  (388, 2585) | 0.67 |
| Folic acid dosage |  |  | |  |  |  | |  |  |  |  |  |
| 100 μg/day | 368 | | 9.6  (4.6, 24.3) | | 589  (249, 1208) | | 0.39 | | 330 | 20.3  (7.4, 51.8) | 755  (355, 1998) | 0.44 |
| 400 μg/day | 371 | | 9.7  (4.9, 25.3) | | 609  (279, 1275) | | 0.49 | | 332 | 34.5  (8.0, 88.0) | 1047.3  (405, 2295) | 0.70 |
| 4000 μg/day | 183 | | 9.7  (4.8, 22.8) | | 594  (244, 1175) | | 0.52 | | 162 | 54.4  (10.2, 511.9) | 1442  (556, 2952) | 0.60 |
| 4000 μg/week | 186 | | 9.8  (4.6, 22.6) | | 613  (293, 1298) | | 0.51 | | 153 | 24.1  (6.8, 63.9) | 912  (371, 1957) | 0.50 |
| *MTHFR* 677 genotype |  |  | |  |  |  | |  |  |  |  |  |
| *CC* | 163 | | 10.3  (5.1, 26.2) | | 667  (300, 1567) | | 0.45 | | 159 | 33.9  (11.7, 102.0) | 1044  (439, 2495) | 0.60 |
| *CT* | 448 | | 9.9  (5.0, 22.8) | | 617  (289, 1350) | | 0.43 | | 434 | 32.2  (8.9, 131.6) | 1005  (405, 2593) | 0.67 |
| *TT* | 330 | | 9.1  (4.6, 25.0) | | 554  (252, 1028) | | 0.49 | | 316 | 24.8  (5.9, 99.5) | 900.4  (367, 2752) | 0.66 |
| BMI (kg/m2) |  |  | |  |  |  | |  |  |  |  |  |
| <25 | 670 | | 9.7  (4.6, 23.7) | | 591  (277, 1076) | | 0.48 | | 584 | 30.0  (8.0, 106.6) | 955  (388, 2166) | 0.68 |
| 25−<30 | 287 | | 9.9  (4.7, 24.9) | | 619  (261, 1124) | | 0.44 | | 260 | 28.8  (7.1, 101.6) | 999  (414, 2263) | 0.68 |
| ≥30 | 69 | | 9.2  (4.6, 24.3) | | 652  (318, 1251) | | 0.41 | | 60 | 27.2  (5.7, 131.6) | 988  (351, 2306) | 0.59 |

1 Includes data of intervention group at baseline and 6 months (n = 2085). BMI, body mass index; *MTHFR*, *methylenetetrahydrofolate reductase*; RBC, red blood cell.

2 Measured by Molloy method (microbiologic assay with chloramphenicol-resistant strain and folic acid calibrator) (Molley AM et al, 1997).

3 All values are geometric means and 2.5th and 97.5th percentiles in parentheses.

4 Correlation coefficients between natural logarithm of RBC folate concentrations and natural logarithm of plasma folate concentrations.

**Supplemental Table 2** Plasma and red blood cell (RBC) folate concentrations and correlation coefficients for all women at baseline (n = 1673), stratified by genotype, BMI, vitamin B12 and hemoglobin1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Plasma folate concentration (nmol/L)2, 3 | | RBC folate concentration (nmol/L)2, 3 | | Correlation coefficient4 |
| Subgroup |  |  |  |  |  |
| Intervention (n = 1108) | 9.7 | (4.7, 24.1) | 600 | (272, 1272) | 0.46 |
| Non-intervention (n = 565) | 8.7 | (4.2, 22.8) | 543 | (253, 1244) | 0.41 |
| *MTHFR* 677 genotype |  |  |  |  |  |
| *CC* (n = 196) | 10.4 | (5.1, 27.9) | 648 | (253, 1567) | 0.46 |
| *CT* (n = 559) | 9.8 | (4.9, 24.1) | 599 | (275, 1298) | 0.43 |
| *TT* (n = 439) | 8.9 | (4.3, 25.1) | 529 | (229, 1208) | 0.53 |
| BMI (kg/m2) |  |  |  |  |  |
| <25 (n = 1020) | 9.3 | (4.6, 22.1) | 575 | (270, 1240) | 0.45 |
| 25−<30 (n = 427) | 9.5 | (4.7, 25.1) | 595 | (261, 1356) | 0.46 |
| ≥30 (n = 109) | 9.0 | (4.2, 24.3) | 610 | (254, 1631) | 0.36 |
| Vitamin B12 (pmol/L) |  |  |  |  |  |
| <148 (n = 358) | 8.8 | (4.1, 25.1) | 496 | (239, 1177) | 0.49 |
| 148–221 (n = 498) | 8.8 | (4.6, 20.0) | 553 | (253, 1143) | 0.41 |
| >221 (n = 817) | 9.9 | (4.7, 24.9) | 640 | (292, 134) | 0.42 |
| Hemoglobin (g/L) |  |  |  |  |  |
| <120 (n = 259) | 8.7 | (3.7, 22.8) | 627 | (296, 1391) | 0.35 |
| ≥120 (n = 1413) | 9.4 | (4.6, 24.1) | 572 | (255, 1244) | 0.48 |

1 Includes data of intervention group and non-intervention group at baseline. BMI, body mass index; *MTHFR*, *methylenetetrahydrofolate reductase*; RBC, red blood cell.

2 Measure by Molloy method (microbiologic assay with chloramphenicol-resistant strain and folic acid calibrator) (Molley AM et al, 1997).

3 All values are geometric means and 2.5th and 97.5th percentiles in parentheses.

4 Correlation coefficients between natural logarithm of RBC folate concentrations and natural logarithm of plasma folate concentrations.

**Supplemental Table 3** Estimated plasma folate concentrations corresponding to the red blood cell folate concentration of 906 nmol/L, stratified by subgroups and different follow-up time1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Baseline and month 6 (n = 2085) | | Baseline (n = 1108) | | Month 6 (n = 977) | |
|  | Median plasma folate concentration in nmol/L (95% CI) | Median difference (95% CI) | Median plasma folate concentration in nmol/L (95% CI) | Median difference (95% CI) | Median plasma folate concentration in nmol/L (95% CI) | Median difference (95% CI) |
| Folic acid dosage |  |  |  |  |  |  |
| 100 μg/day | 38.4 (33.3, 45.8) | 13.9 (8.7, 21.5) | 33.1 (25.8, 49.5) | 9.8 (0.6, 26.4) | 36.7 (31.4, 45.9) | 11.3 (-5.4, 50.6) |
| 400 μg/day | 24.4 (23.1, 25.9) | Reference | 23.2 (19.9, 28.9) | Reference | 25.5 (23.2, 27.7) | Reference |
| 4000 μg/day | 22.0 (20.5, 23.6) | -2.4 (-4.4, -0.4) | 22.8 (18.7, 31.5) | -0.4 (-7.4, 8.8) | 13.2 (9.2, 17.3) | -12.2 (-16.8, -7.7) |
| 4000 μg/week | 25.6 (23.0, 28.8) | 1.0 (-1.8, 4.5) | 23.4 (18.9, 33.6) | 0.2 (-7.0, 10.5) | 23.6 (19.6, 28.1) | -1.9 (-6.5, 3.2) |
| *MTHFR* 677 genotype |  |  |  |  |  |  |
| *CC* | 23.2 (21.2, 25.5) | Reference | 21.5 (17.5, 30.3) | Reference | 24.9 (21.2, 28.3) | Reference |
| *CT* | 25.1 (23.8, 26.6) | 1.9 (-0.7, 4.4) | 26.4 (22.0, 34.5) | 4.7 (-4.9, 13.8) | 25.2 (23.1, 27.4) | 0.4 (-3.8, 4.6) |
| *TT* | 26.3 (24.6, 28.2) | 3.1 (0.2, 5.9) | 27.7 (22.7, 37.2) | 6.1 (-4.0, 16.3) | 25.2 (22.9, 27.8) | 0.4 (-3.7, 4.8) |
| BMI (kg/m2) |  |  |  |  |  |  |
| <25 | 26.7 (25.5, 28.1) | Reference | 25.6 (22.3, 30.6) | Reference | 26.5 (24.7, 28.3) | Reference |
| 25−<30 | 23.3 (21.9, 25.0) | -3.3 (-5.3, -1.4) | 27.8 (22.2, 39.7) | 2.3 (-5.5, 14.6) | 23.4 (21.2, 25.8) | -3.1 (-6.0, -0.1) |
| ≥30 | 21.9 (19.0, 26.1) | -4.5 (-8.0, -0.5) | 19.1 (14.4, 37.7) | -6.4 (-13.5, 12.2) | 21.7 (16.1, 27.7) | -4.8 (-10.6, 1.4) |

1 Includes data of intervention group at baseline and 6 months (n = 2085). BMI, body mass index; CI, credible interval, *MTHFR*, *methylenetetrahydrofolate reductase*.

**Supplemental Table 4** Estimated plasma folate concentrations corresponding to the red blood cell folate concentration of 906 nmol/L for the intervention group (includes baseline and 6 months), using Bayesian unadjusted and adjusted linear models1

|  |  |  |  |
| --- | --- | --- | --- |
|  | Median plasma folate concentration in nmol/L (95% CI) | | |
|  | Unadjusted | Adjusted without missing values imputed | Adjusted with missing values imputed2 |
| All | 25.5 (24.6, 26.4) | 25.2 (24.3, 26.3) | 25.5 (24.6, 26.5) |
| *MTHFR* 677 genotype | |  |  |
| *CC* | 23.2 (21.2, 25.5) | 22.9 (21.1, 24.9) | 22.8 (21.2, 24.7) |
| *CT* | 25.1 (23.8, 26.6) | 24.8 (23.5, 26.1) | 25.0 (23.8, 26.2) |
| *TT* | 26.3 (24.6, 28.2) | 27.0 (25.4, 28.9) | 27.7 (26.1, 29.5) |
| BMI (kg/m2) |  |  |  |
| <25 | 26.7 (25.5, 28.1) | 26.5 (25.3, 27.8) | 26.7 (25.5, 27.9) |
| 25-<30 | 23.3 (21.9, 25.0) | 23.4 (21.8, 25.0) | 24.0 (22.6, 25.5) |
| ≥30 | 21.9 (19.0, 26.1) | 21.4 (18.8, 24.5) | 21.5 (19.1, 24.4) |

1 Includes data of intervention group at baseline and 6 months (n = 2085). Adjusted for age, BMI, and genotype. BMI, body mass index; CI, Bayesian credible interval; *MTHFR*, *methylenetetrahydrofolate reductase*.

2 Missing data on *MTHFR* genotype and BMI were replaced with imputed values.

**Supplemental Table 5** Estimated plasma folate concentrations corresponding to the red blood cell folate concentration of 906 nmol/L for all women at baseline, using Bayesian unadjusted and adjusted linear models1

|  |  |  |  |
| --- | --- | --- | --- |
|  | Median plasma folate concentration in nmol/L (95% CI) | | |
|  | Unadjusted | Adjusted model A2 | Adjusted model B3 |
| All | 27.2 (24.6, 30.9) | 27.0 (24.0, 31.2) | 28.8 (25.2, 33.8) |
| *MTHFR* 677 genotype | |  |  |
| *CC* | 22.2 (18.4, 29.9) | 23.1 (20.1, 27.2) | 24.3 (20.9, 28.9) |
| *CT* | 28.3 (23.8, 36.2) | 25.3 (22.6, 29.7) | 27.2 (23.8, 32.0) |
| *TT* | 26.5 (22.6, 32.8) | 31.0 (26.7, 37.1) | 33.4 (28.3, 40.9) |
| BMI (kg/m2) |  |  |  |
| <25 | 27.5 (24.1, 32.3) | 27.9 (24.7, 32.6) | 29.9 (25.9, 35.4) |
| 25-<30 | 27.0 (22.5, 35.1) | 26.0 (22.4, 30.3) | 27.3 (23.7, 32.6) |
| ≥30 | 26.5 (18.4, 64.2) | 24.1 (19.7, 30.0) | 25.5 (20.7, 32.4) |
| Vitamin B12 (pmol/L) |  |  |  |
| <148 | 34.6 (27.6, 48.2) | - | 38.3 (31.6, 48.4) |
|  |  |  |  |
| 148–221 | 29.8 (24.1, 40.8) |  | 31.0 (26.4, 37.9) |
| >221 | 25.6 (22.3, 30.7) |  | 24.5 (21.8, 28.3) |
| Hemoglobin (g/L) |  |  |  |
| <120 | 28.5 (20.9, 50.1) | - | 25.8 (19.3, 35.1) |
| ≥120 | 26.4 (23.8, 29.8) | - | 28.9 (25.3, 34.0) |

1 Includes data of intervention group and non-intervention group at baseline (n = 1673). Missing data were excluded from the models. BMI, body mass index; CI, credible interval; *MTHFR*, *methylenetetrahydrofolate reductase*.

2 Adjusted for age, BMI, and genotype.

3 Adjusted for age, BMI, genotype, vitamin B12, hemoglobin (anemia).

Contacted

n = 2084

Ineligible for folic acid intervention

* Anemia only: n= 207
* Vitamin B12 deficiency only: n = 305
* Both anemia and vitamin B12 deficiency: n = 53

Enrolled with blood sampling

n = 1673

Randomized

n = 1108

100 µg

1 time/day

n = 182

25 µg

4 times/day

n =186

100 µg

4 times/day

n = 185

400 µg

1 time/day

n = 186

4000 µg

1 time/day

n = 183

4000 µg

1 time/week

n = 186

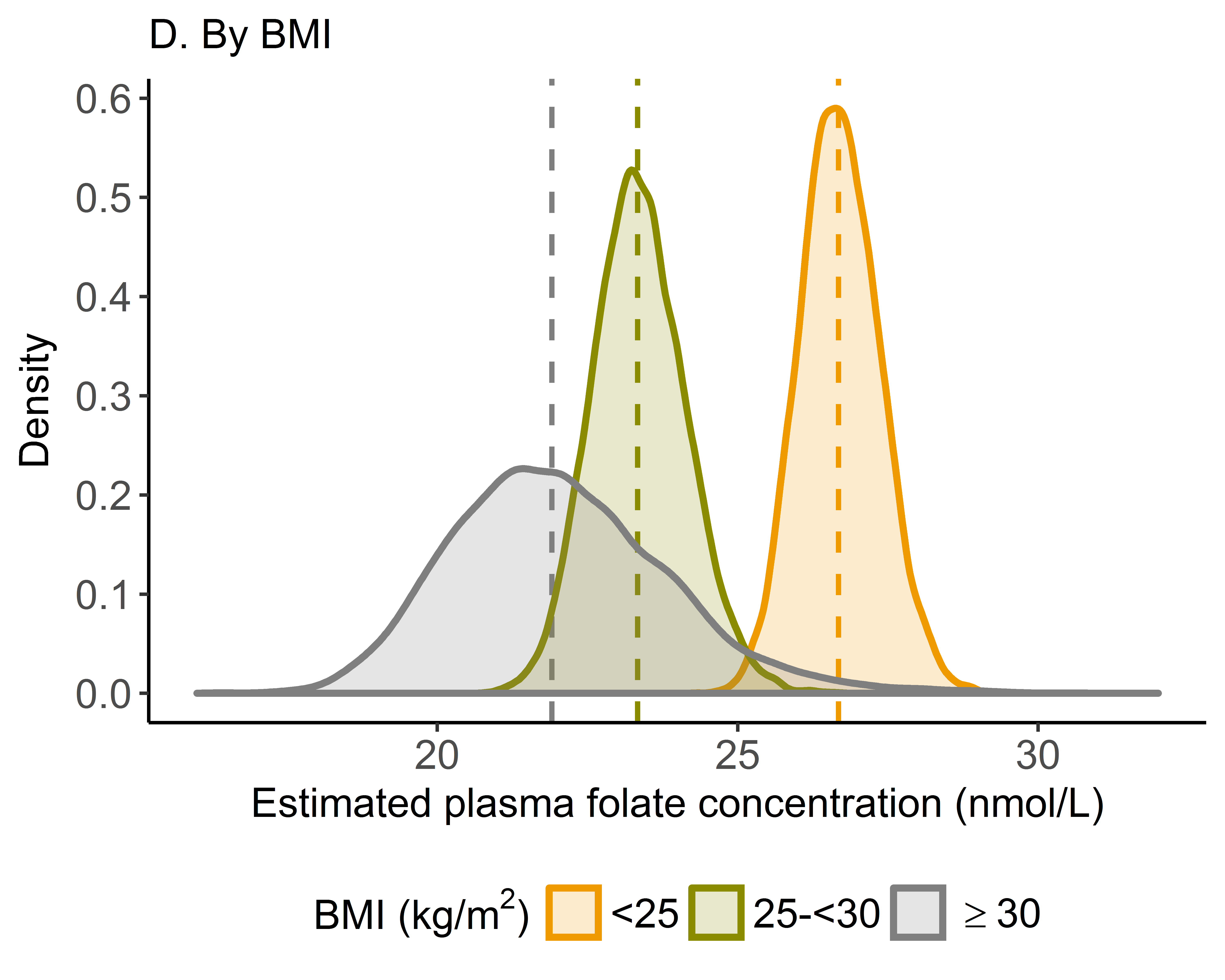
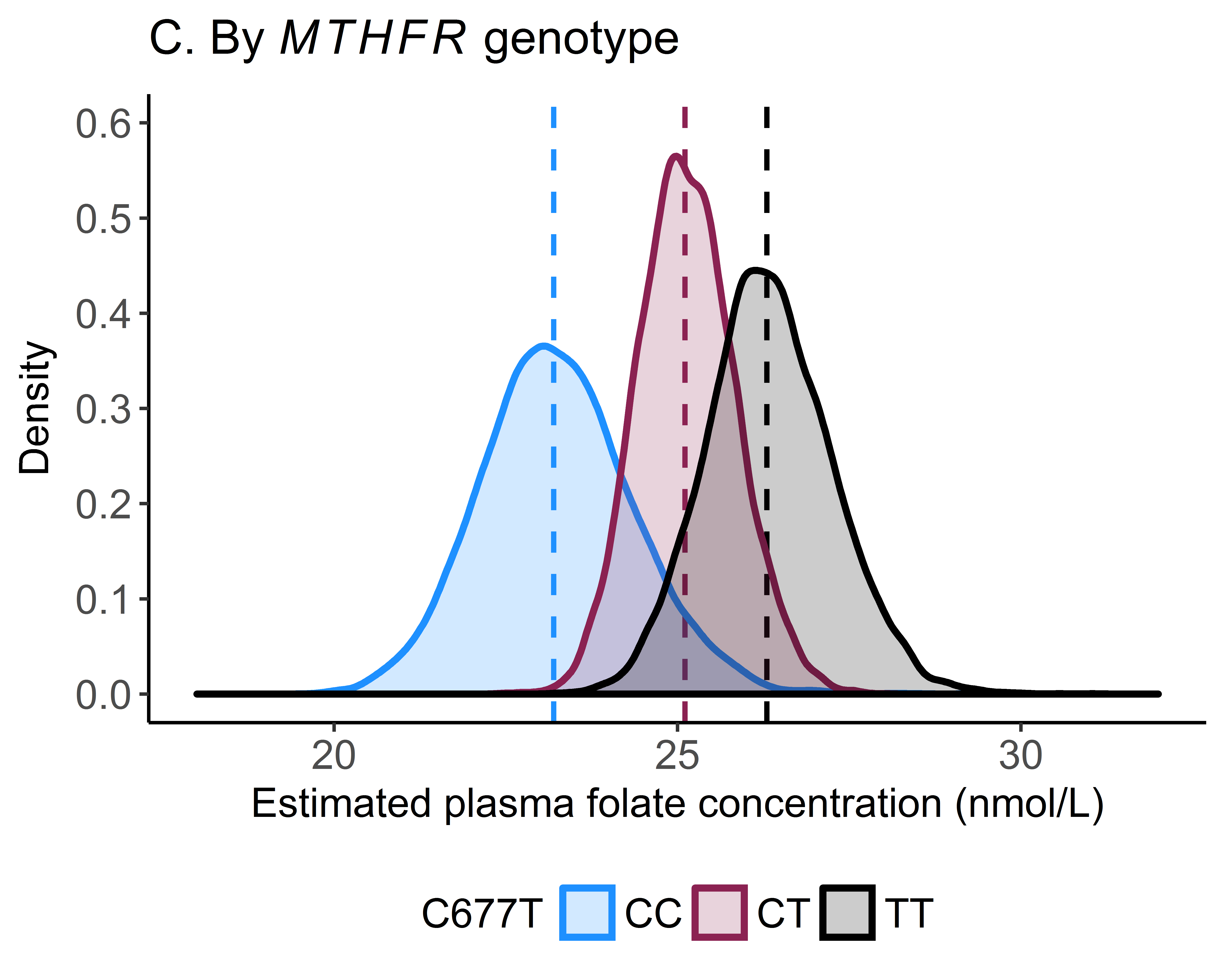
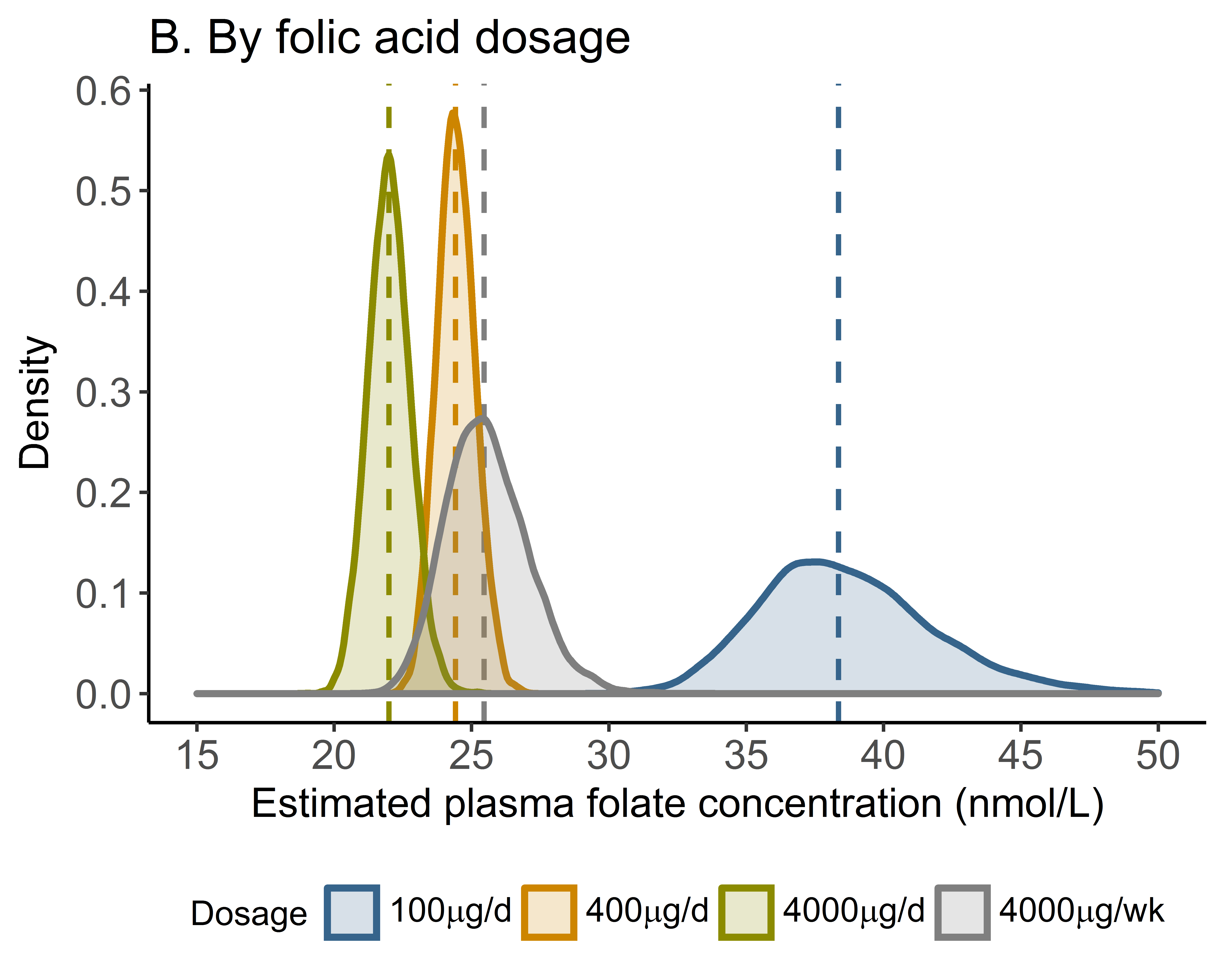
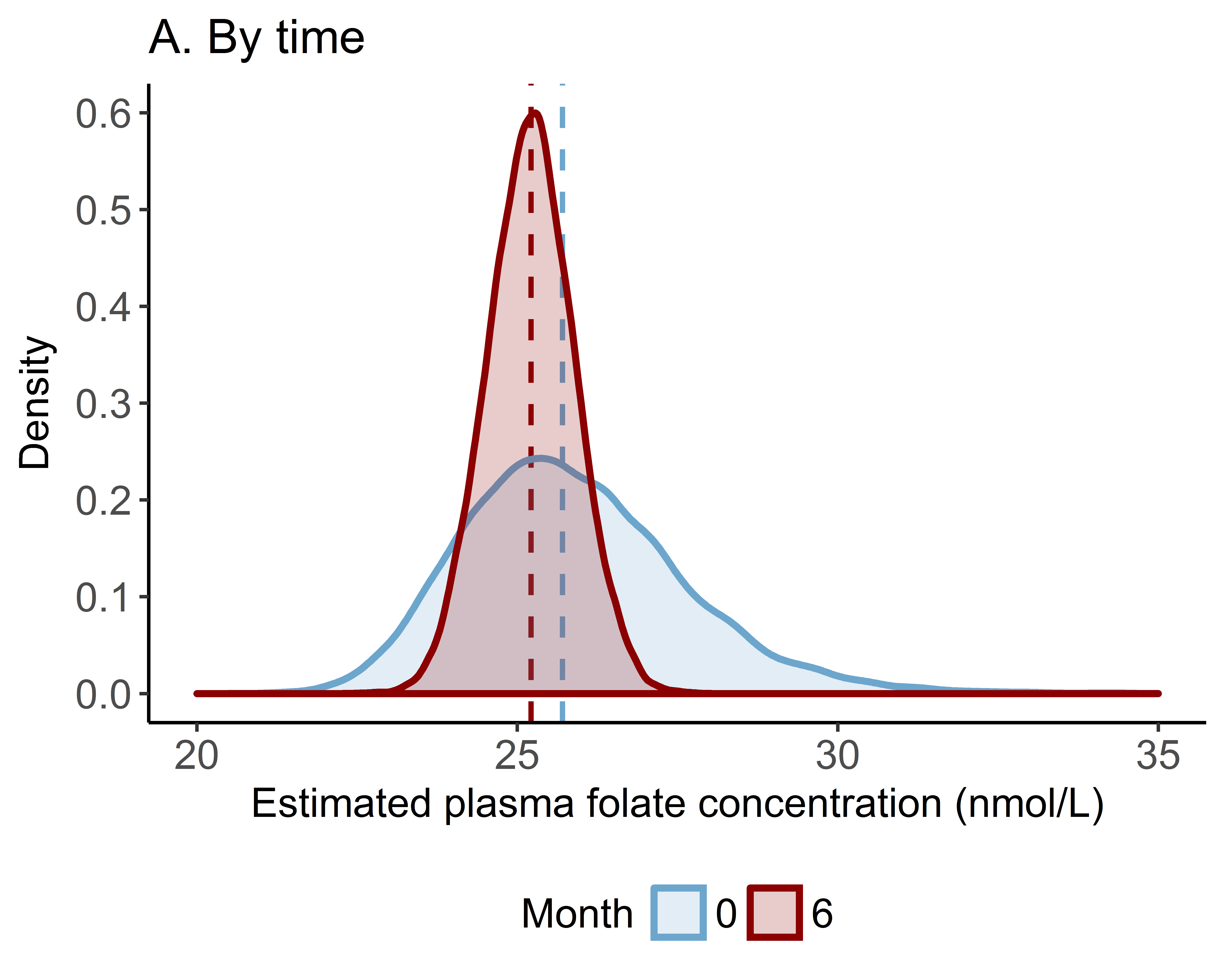
(100 µg/day)

n = 368

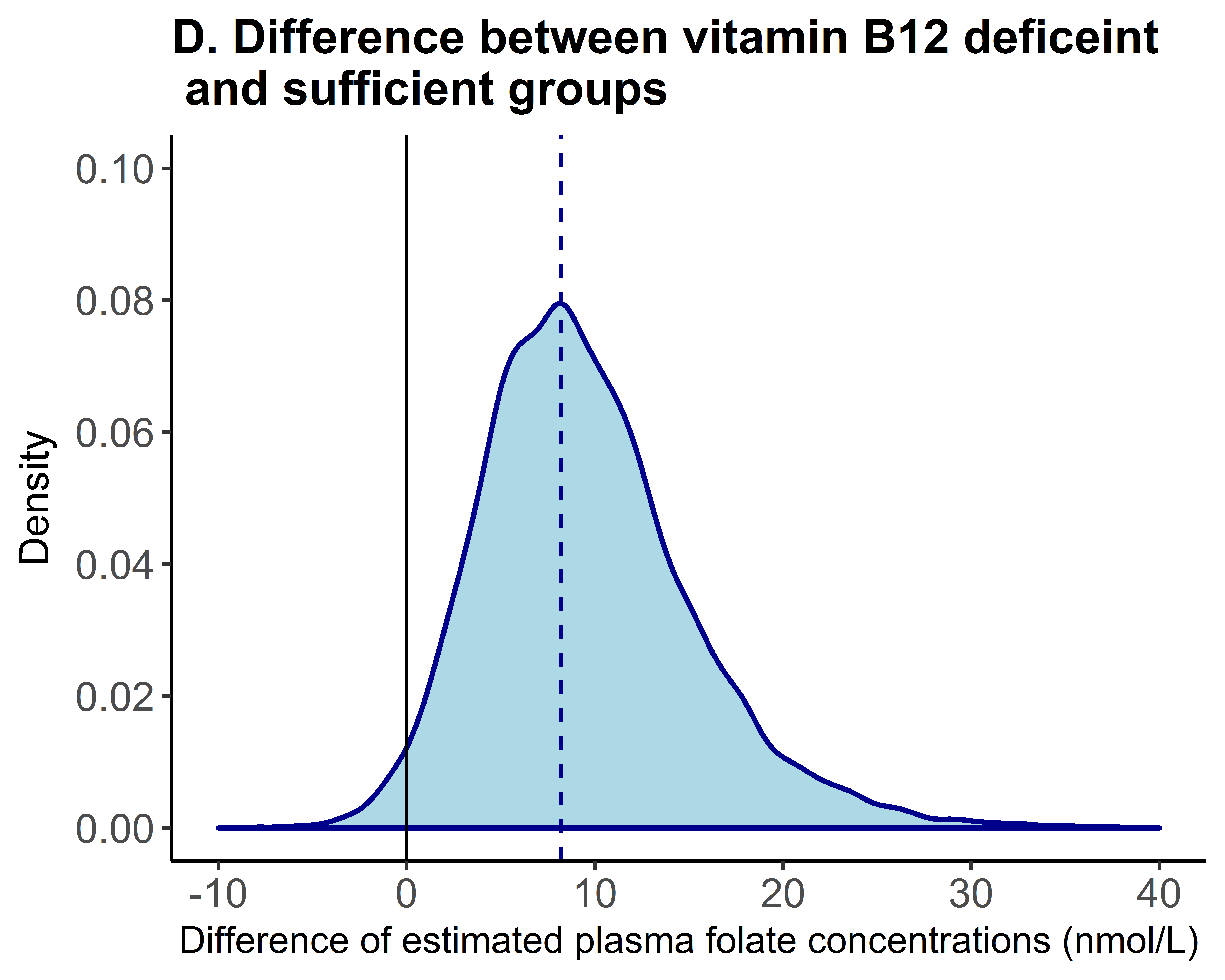
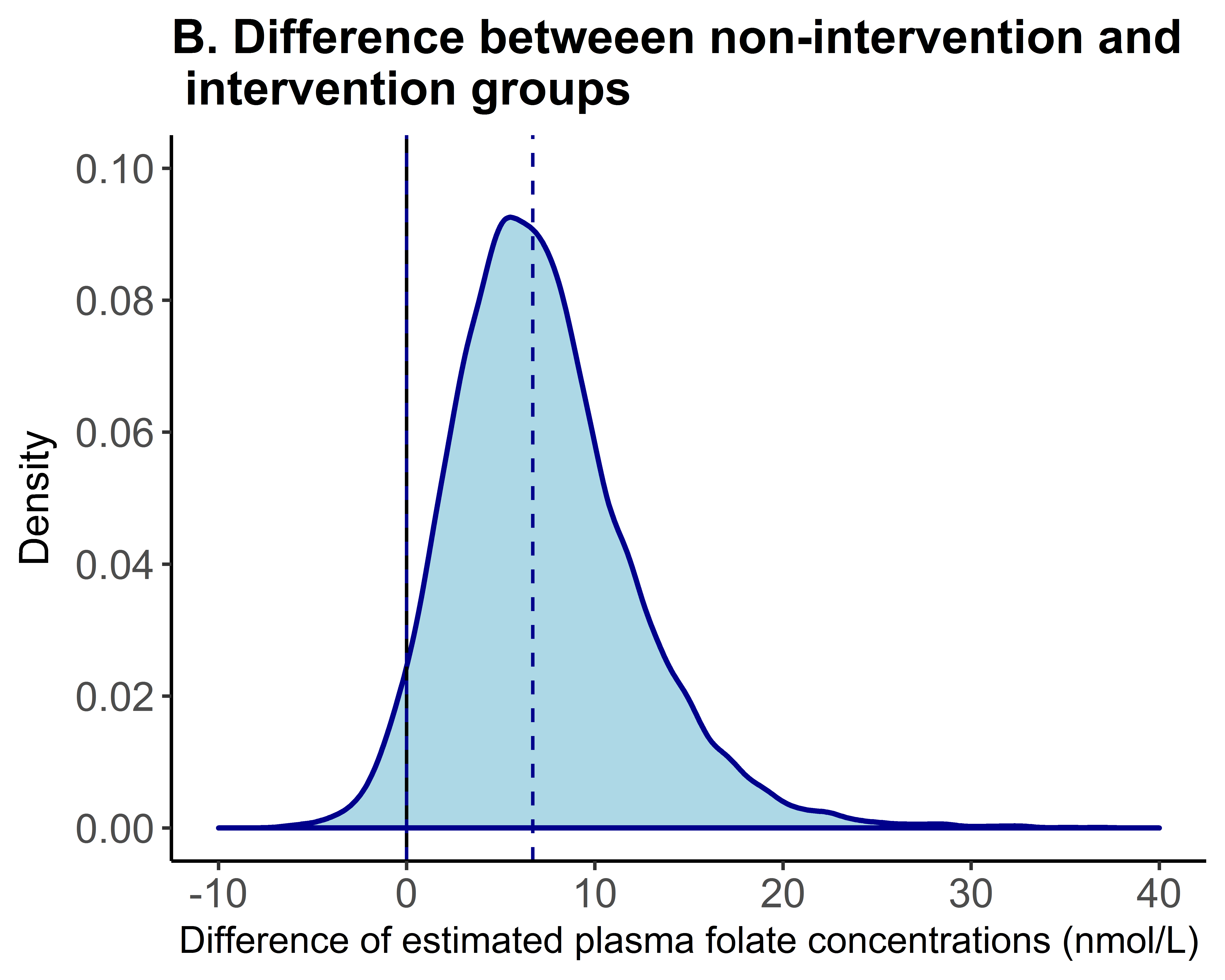
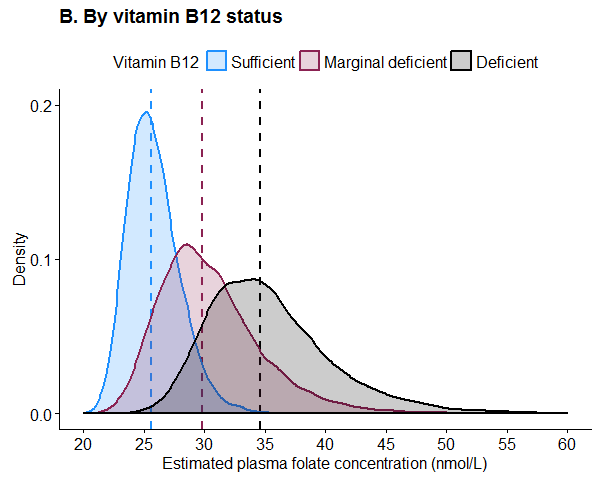
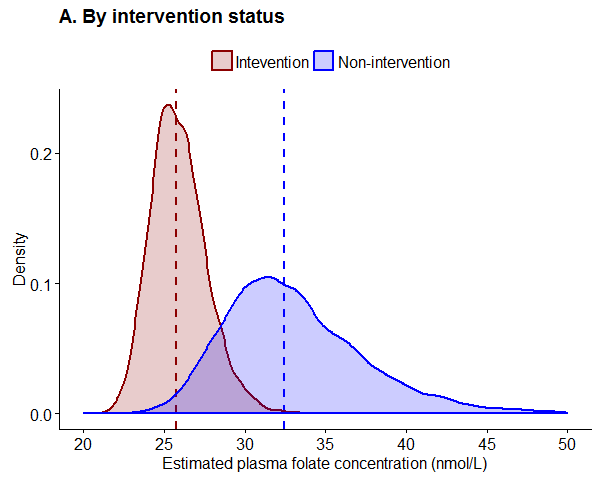
(400 µg/day)

n = 371

**Supplemental Figure 1** Selection of women for the folic acid supplement randomized trial. Anemia: hemoglobin (Hb) <120 g/L. Vitamin B12 deficiency: <148 pmol/L.



**Supplemental Figure 2** Estimated plasma folate concentration corresponding to the red blood cell folate concentration of 906 nmol/L using data from the intervention group, stratified by (A) baseline and 6 months after supplementation, (B) folic acid dosage (100 µg/day, 400 µg/day, 4000 µg/day, or 4000 µg/week), (C) *MTHFR C677T* genotype (*CC*, *CT*, or *TT*), and (D) BMI (<25, 25–<30, or ≥30 kg/m2). Dashed lines represent the medians of the distributions. BMI, body mass index; *MTHFR*, methylenetetrahydrofolate reductase.



**C**

**Supplemental Figure 3** Estimated plasma folate concentrations corresponding to the red blood cell folate concentration of 906 nmol/L using data from all women at baseline, stratified by (A) the intervention and non-intervention groups, and (B) the vitamin B12 deficient (<148 pmol/L), marginal deficient (148–221 pmol/L) and sufficient (>221 nmol/L) groups. Additional panels show differences between (C) the non-intervention and intervention group, and (D) the vitamin B12 deficiency and sufficiency. Dashed lines represent the medians of the distributions. Solid lines represent the mean difference of zero (C, D).