

## Supplementary Online Content

Ebbing M, Bønaa KH, Nygård O, et al. Cancer incidence and mortality after treatment with folic acid and vitamin B<sub>12</sub>. *JAMA*. 2009;302(19):2119-2126.

**eTable 1.** Circulating Levels of B Vitamins and Total Homocysteine at Baseline and During Study Treatment

**eTable 2.** Hazard Ratios for the Primary End Points by Serum Levels of Folate and Cobalamin Measured During Study Treatment

**eFigure.** Kaplan-Meier Curves for Cancer Incidence, Cancer Mortality, and All-Cause Mortality for the Main Comparisons Through Extended Follow-Up

Panel A: Cancer Incidence – Folic Acid vs Non-Folic Acid Groups

Panel B: Cancer Incidence – Vitamin B<sub>6</sub> Groups vs Non-Vitamin B<sub>6</sub> Groups

Panel C: Cancer Mortality – Folic Acid vs Non-Folic Acid Groups

Panel D: Cancer Mortality – Vitamin B<sub>6</sub> Groups vs Non-Vitamin B<sub>6</sub> Groups

Panel E: All-Cause Mortality – Folic Acid vs Non-Folic Acid Groups

Panel F: All-Cause Mortality – Vitamin B<sub>6</sub> Groups vs Non-Vitamin B<sub>6</sub> Groups

This supplementary material has been provided by the authors to give readers additional information about their work.

**eTable 1. Circulating Levels of B Vitamins and Total Homocysteine at Baseline and During Study Treatment**

	Folic Acid + Vitamins B <sub>12</sub> and B <sub>6</sub> (n = 1708)		Folic Acid + Vitamin B <sub>12</sub> (n = 1703)		Vitamin B <sub>6</sub> (n = 1705)		Placebo (n = 1721)		P Value <sup>a</sup>
Serum folate (ng/mL)									
Baseline (n = 6773)	3.9	(2.9)	3.9	(2.7)	3.8	(2.8)	3.9	(2.7)	.56
1-2 mo (n = 6126)	25.3	(14.5)	29.8	(14.8)	3.0	(1.8)	3.9	(2.5)	<.001
Final study visit <sup>b</sup> (n = 5567)	25.5	(17.9)	29.3	(19.6)	3.7	(2.6)	4.5	(3.3)	<.001
Serum cobalamin (pg/mL)									
Baseline (n = 6749)	477	(226)	477	(243)	477	(228)	470	(236)	.73
1-2 mo (n = 6138)	692	(287)	683	(290)	496	(225)	481	(227)	<.001
Final study visit <sup>b</sup> (n = 5578)	761	(341)	760	(344)	465	(217)	473	(219)	<.001
Plasma total homocysteine (μmol/L)									
Baseline (n = 6814)	11.0	(4.6)	11.1	(4.4)	11.0	(4.7)	11.2	(4.6)	.75
1-2 mo (n = 6142)	8.3	(2.9)	8.4	(2.8)	11.2	(4.4)	11.5	(4.6)	<.001
Final study visit <sup>b</sup> (n = 5582)	8.1	(2.9)	8.3	(3.1)	11.2	(4.4)	11.2	(4.7)	<.001
Plasma pyridoxal 5' phosphate (ng/mL)									
Baseline (n = 6722)	8.1	(6.0)	7.9	(5.5)	8.3	(6.3)	8.0	(5.5)	.07
1-2 mo (n = 6028)	87.4	(42.9)	9.3	(6.1)	89.3	(43.3)	9.3	(6.3)	<.001
Final study visit <sup>b</sup> (n = 5496)	75.5	(62.8)	9.2	(6.5)	75.3	(69.0)	9.5	(6.4)	<.001
Values are medians and interquartile ranges. SI conversion factors: To convert folate to nmol/L, multiply by 2.266. To convert cobalamin to pmol/L, multiply by 0.7378. To convert pyridoxal 5' phosphate to nmol/L, multiply by 4.046. <sup>a</sup> Kruskal-Wallis test across the four intervention groups. <sup>b</sup> The final study visit occurred after median (interquartile range) 39 (12) months of in-trial follow-up.									

**eTable 2. Hazard Ratios for the Primary End Points by Serum Levels of Folate and Cobalamin Measured During Study Treatment**

Serum Level, Quartiles	Total No. <sup>a</sup>	Cancer Incidence			Cancer Mortality			All-Cause Mortality				
		No. of Events	Hazard Ratio <sup>b</sup> (95% CI)	P Value <sup>c</sup>	No. of Events	Hazard Ratio <sup>b</sup> (95% CI)	P Value <sup>c</sup>	No. of Events	Hazard Ratio <sup>b</sup> (95% CI)	P Value <sup>c</sup>		
Folate <sup>d</sup>												
Quartile 1	1592	143	1.08 (0.85-1.37)	.55	49	1.17 (0.76-1.78)	.48	222	1.33 (1.08-1.64)	.007		
Quartile 2	1592	130	1		39	1		152	1			
Quartile 3	1592	141	1.10 (0.86-1.39)	.45	57	1.46 (0.97-2.19)	.07	165	1.08 (0.87-1.35)	.49		
Quartile 4	1591	169	1.30 (1.03-1.63)	.03	63	1.57 (1.05-2.34)	.03	272	1.71 (1.41-2.09)	<.001		
All	6367	583		.13	208		.10	811		<.001		
Cobalamin <sup>e</sup>												
Quartile 1	1592	142	1.15 (0.91-1.46)	.25	49	1.27 (0.84-1.93)	.25	194	1.12 (0.92-1.37)	.27		
Quartile 2	1592	128	1		41	1		188	1			
Quartile 3	1593	171	1.32 (1.05-1.66)	.02	58	1.37 (0.92-2.04)	.13	208	1.06 (0.87-1.29)	.55		
Quartile 4	1590	142	1.08 (0.85-1.37)	.54	60	1.41 (0.95-2.09)	.09	221	1.12 (0.92-1.36)	.25		
All	6367	583		.09	208		.35	811		.63		

Abbreviations: CI, confidence interval.

SI conversion factors: To convert folate to nmol/L, multiply by 2.266. To convert cobalamin to pmol/L, multiply by 0.7378.

<sup>a</sup>A total of 6367 (93.1%) of participants had one or two measurements of serum folate and of serum cobalamin during study treatment with B vitamins or placebo. Folate and cobalamin quartiles were computed by using the mean of measurements from the study visit at 1 to 2 months after randomization and at the final study visit median 39 months after randomization, or the actual value if only one sample was available after random treatment assignment.

<sup>b</sup>Hazard ratios estimated by Cox regression, unadjusted, stratified by trial. Participants in the second serum folate quartile or cobalamin quartile during study treatment had the lowest cancer incidence, cancer mortality and all-cause mortality through extended follow-up, thus quartile 2 was used as the reference category.

<sup>c</sup>P value for difference between quartiles with quartile 2 as reference category, or for heterogeneity across all quartiles.

<sup>d</sup>For serum folate, quartile 1, ≤3.80 ng/mL; quartile 2, 3.81-10.56 ng/mL; quartile 3, 10.57-27.66 ng/mL; quartile 4, >27.66 ng/mL.

<sup>e</sup>For serum cobalamin, quartile 1, ≤456.3 pg/mL; quartile 2, 456.4-595.9 pg/mL; quartile 3, 596.0-769.0 pg/mL; quartile 4, >769.0 pg/mL.

**eFigure. Kaplan-Meier Curves for Cancer Incidence, Cancer Mortality, and All-Cause Mortality for the Main Comparisons Through Extended Follow-Up**

**Panel A:** Cancer Incidence – Folic Acid vs Non-Folic Acid Groups

**Panel B:** Cancer incidence – Vitamin B<sub>6</sub> Groups vs Non-Vitamin B<sub>6</sub> Groups

**Panel C:** Cancer Mortality – Folic Acid vs Non-Folic Acid Groups

**Panel D:** Cancer Mortality – Vitamin B<sub>6</sub> Groups vs Non-Vitamin B<sub>6</sub> Groups

**Panel E:** All-Cause Mortality – Folic Acid vs Non-Folic Acid Groups

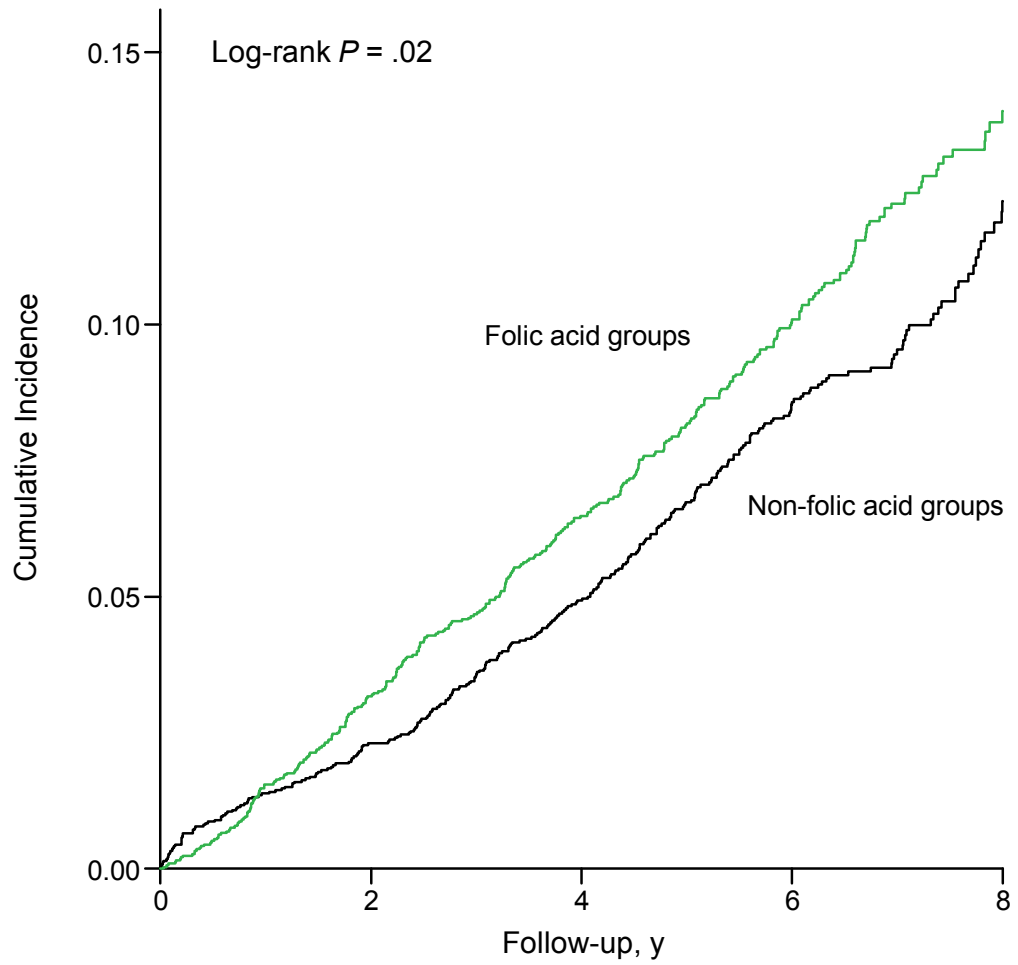
**Panel F:** All-Cause Mortality – Vitamin B<sub>6</sub> Groups vs Non-Vitamin B<sub>6</sub> Groups

The primary end points were cancer incidence (Panels A and B), cancer mortality (Panels C and D) and all-cause mortality (Panels E and F) from randomization throughout the year 2007. Non-melanoma skin cancers were not included in cancer incidence. The comparisons were between folic acid groups and non-folic acid groups (Panels A, C and E) and between vitamin B<sub>6</sub> groups and non-vitamin B<sub>6</sub> groups (Panels B, D and F).

Folic acid groups were assigned to treatment with folic acid, 0.8 mg, and vitamin B<sub>12</sub>, 0.4 mg, or folic acid and vitamin B<sub>12</sub> in combination with vitamin B<sub>6</sub>, 40 mg. Non-folic acid groups were assigned to treatment with vitamin B<sub>6</sub> alone, or placebo.

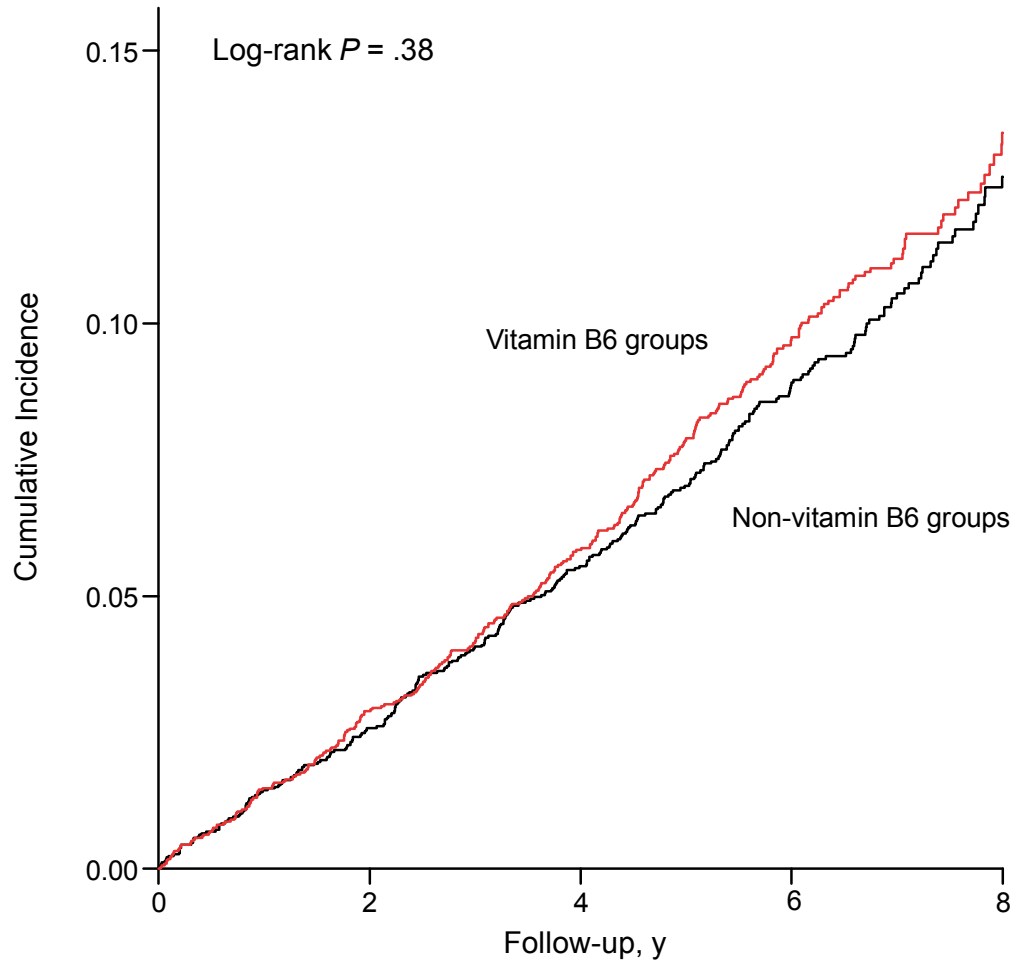
Vitamin B<sub>6</sub> groups were assigned to treatment with vitamin B<sub>6</sub>, 40 mg alone or in combination with folic acid, 0.8 mg, and vitamin B<sub>12</sub>, 0.4 mg. Non-vitamin B<sub>6</sub> groups were assigned to treatment with folic acid and vitamin B<sub>12</sub>, or placebo.

Panel A - Cancer Incidence



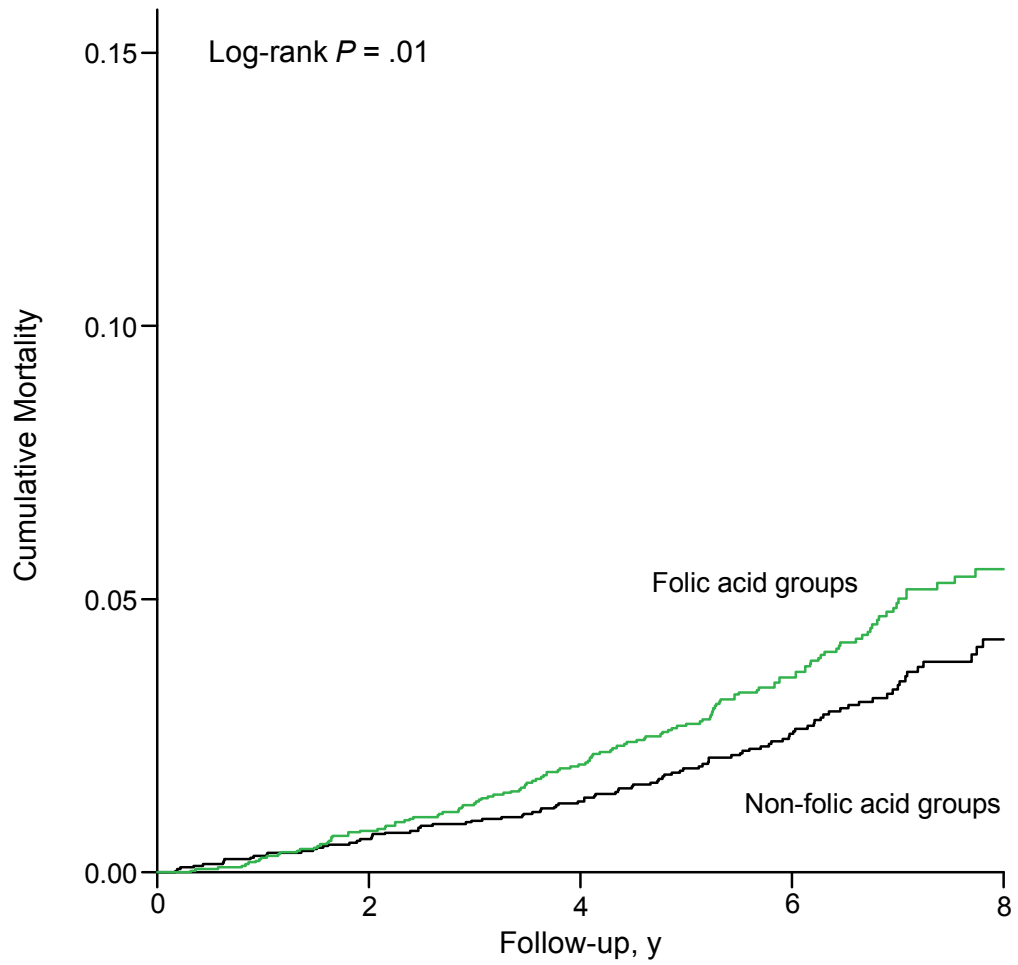
No. at risk					
Folic acid groups	3411	3163	2887	1924	481
Non-folic acid groups	3426	3201	2948	1985	509

Panel B - Cancer Incidence



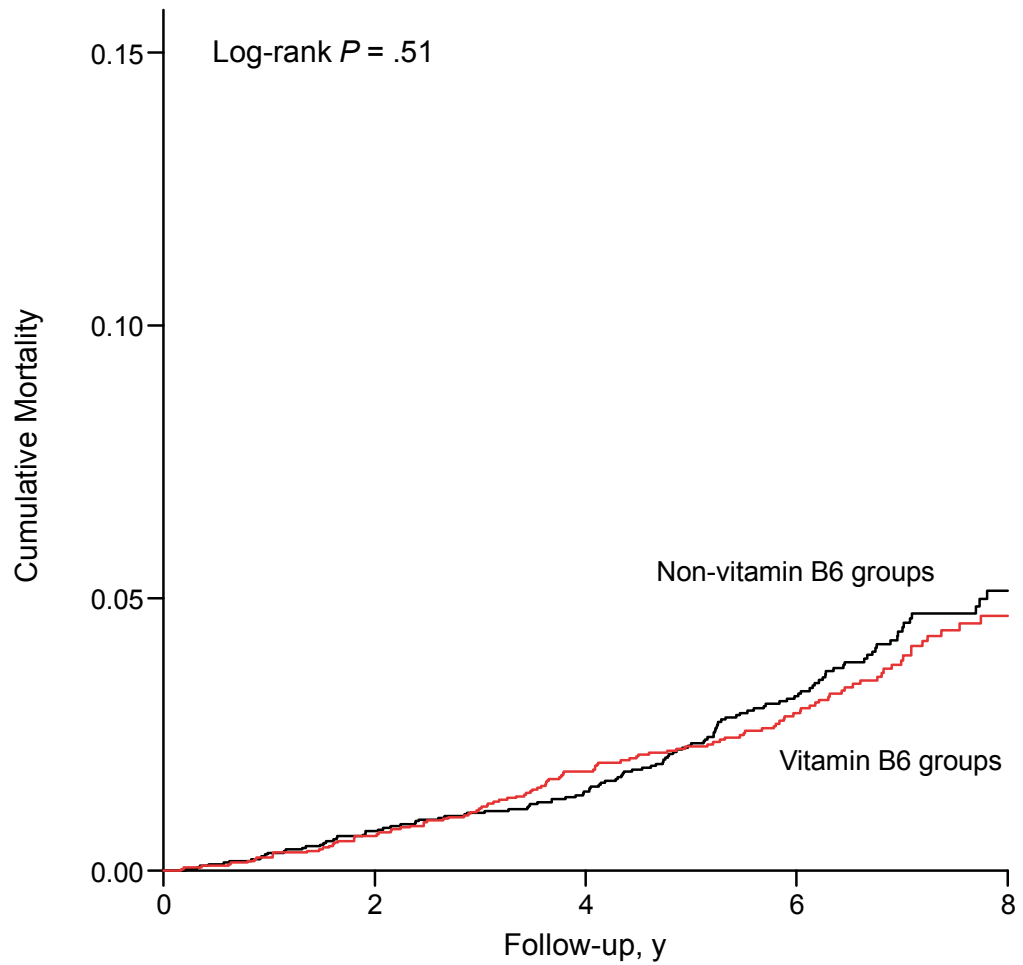
No. at risk					
Vitamin B6 groups	3413	3160	2884	1936	488
Non-vitamin B6 groups	3424	3204	2951	1973	502

Panel C - Cancer Mortality



No. at risk					
Folic acid groups	3411	3237	3009	2054	520
Non-folic acid groups	3426	3253	3053	2108	554

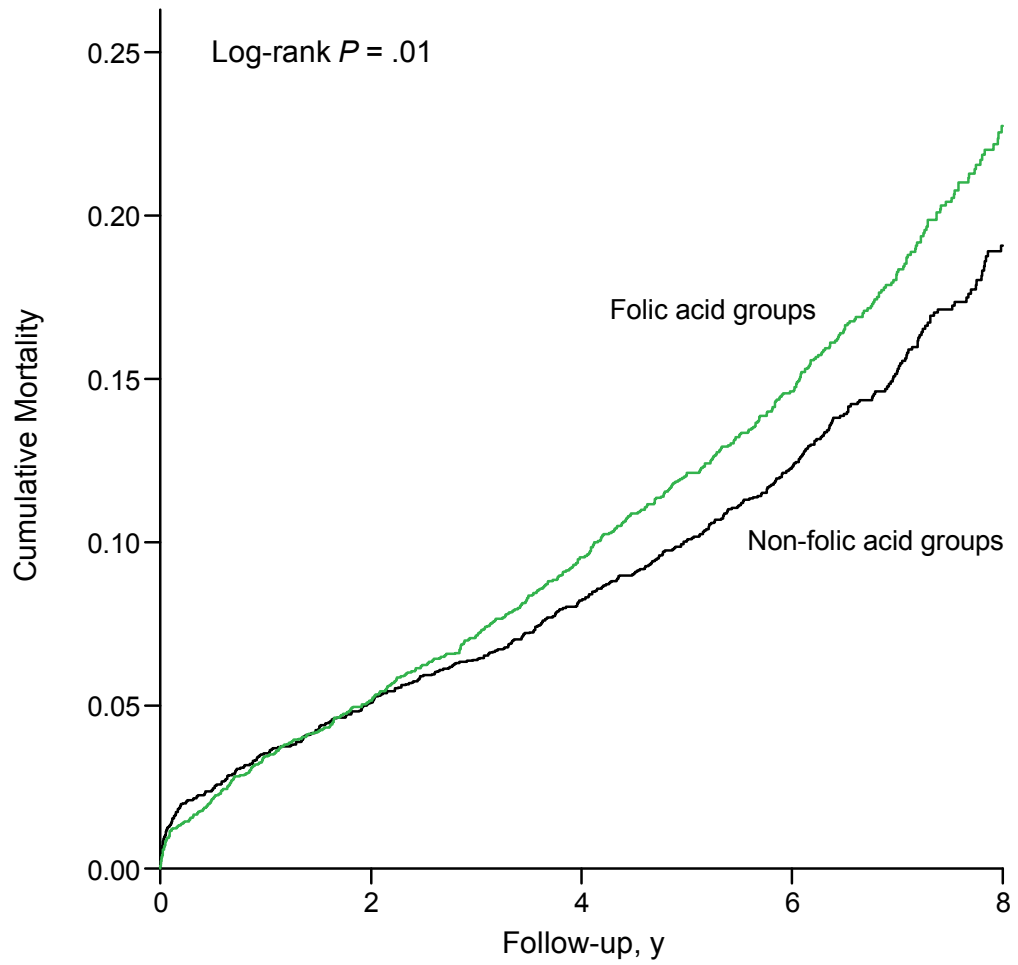
Panel D - Cancer Mortality



No. at risk	0	2	4	6	8
Vitamin B6 groups	3413	3230	2995	2069	535
Non-vitamin B6 groups	3424	3260	3067	2093	539

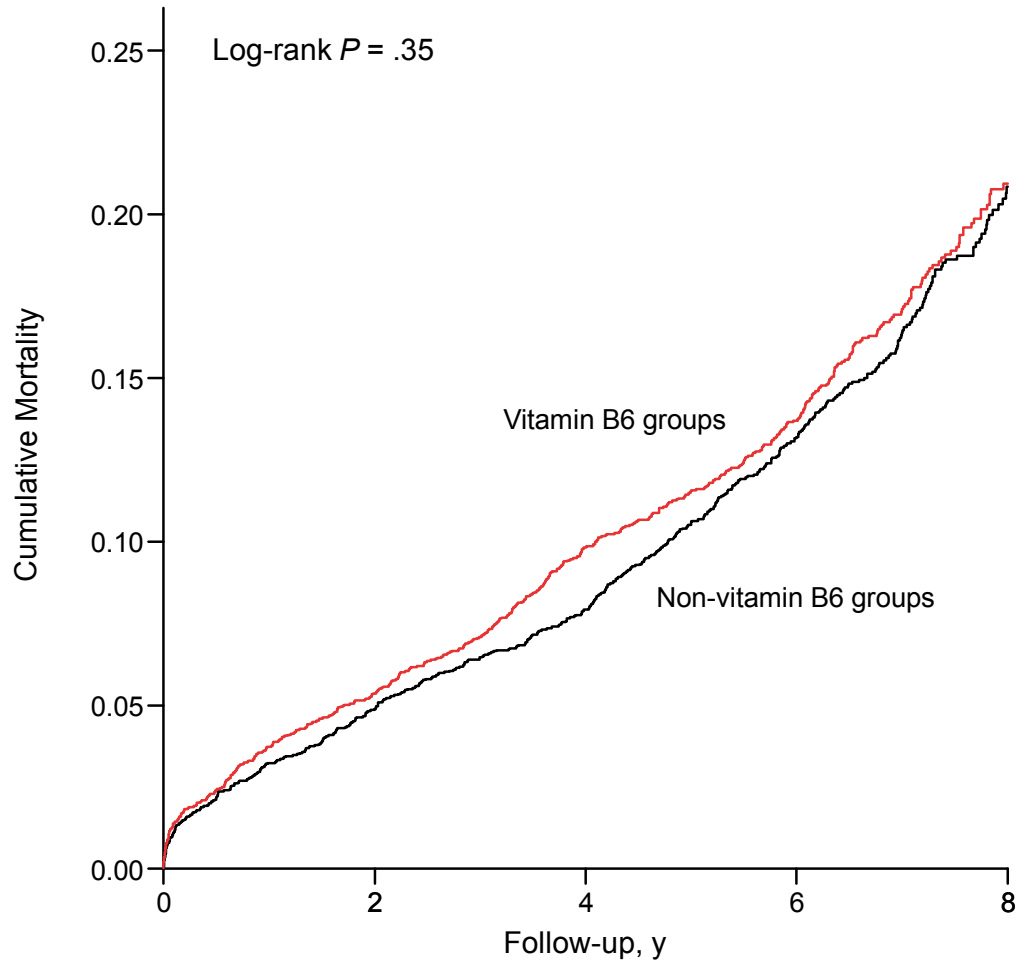


Panel E - All-Cause Mortality



No. at risk						
Folic acid groups	34	11	3237	3009	2054	520
Non-folic acid groups	3426	3253	3053	2108	554	

# Panel F - All-Cause Mortality



No. at risk					
Vitamin B6 groups	3413	3230	2995	2069	535
Non-vitamin B6 groups	3424	3260	3067	2093	539